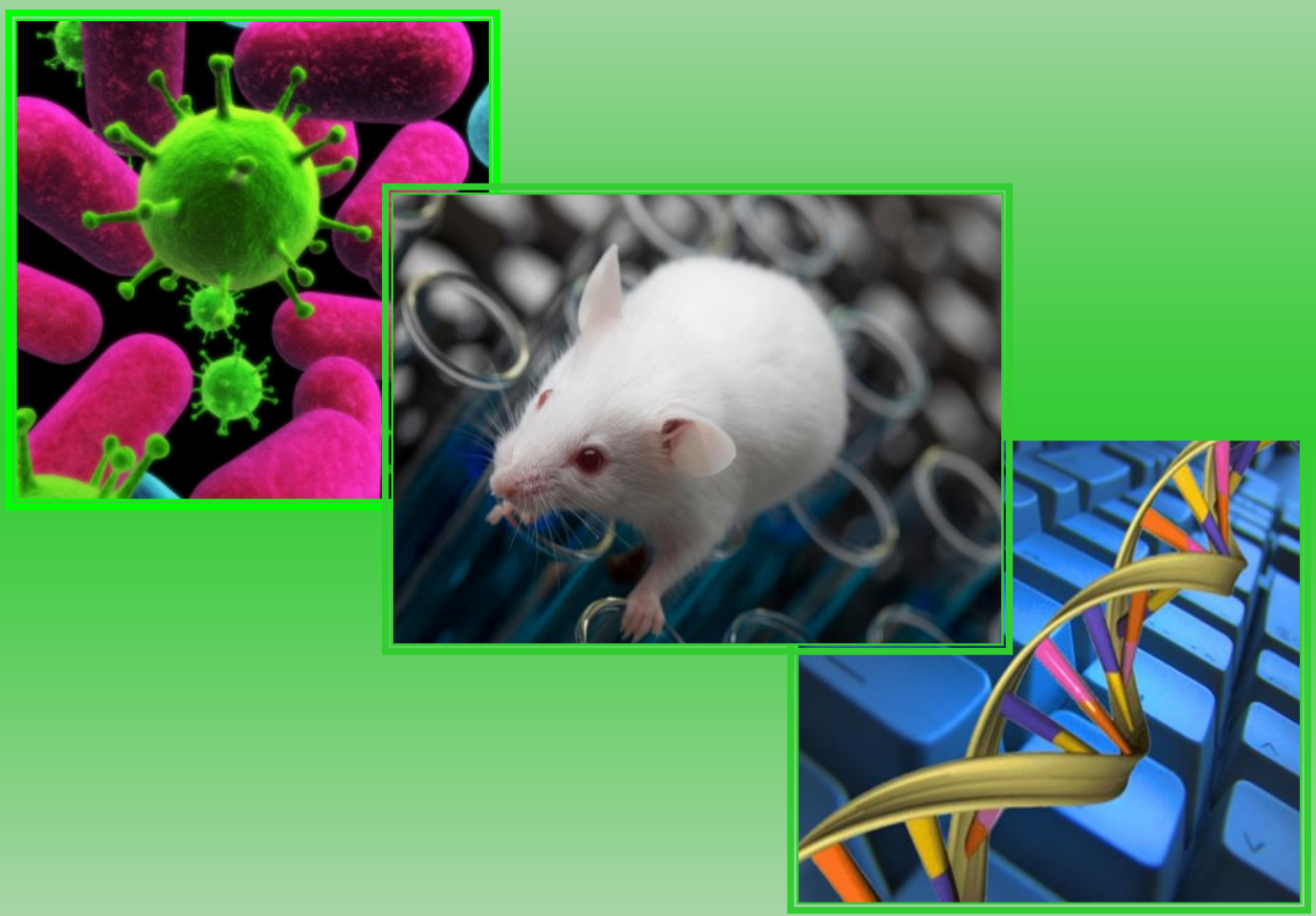
Experimental Models and Methods in Biomedical Research



PROGRAM AND ABSTRACTS

16-17 March, 2010

SOFIA, BULGARIA

THE WORKSHOP IS ORGANIZED
BY THE INSTITUTE OF EXPERIMENTAL PATOLOGY AND PARASITOLOGY
UNDER THE AUSPICES OF
THE BULGARIAN ACADEMY OF SCIENCES

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EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH

WORKSHOP FOR STUDENTS AND YOUNG SCIENTISTS

16th March, 2010

10.00 - 10.15 h OPENING REMARKS

Session A.

Chairpersons:

Assist. Prof. Radostina Alexandrova, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assoc. Prof. Georgi Miloshev, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

10.15 – 10.45 h

AO1. EXPERIMENTAL MODELS IN CANCER RESEARCH

Radostina Alexandrova, Tanya Jivkova, Lora Dyakova, Eleonora-Leventieva-Necheva, Reni Kalfin, Gergana Sabruteva, Plamena Borisova, Constanta Timcheva, Gabriela Marinescu, Daniela Cristina Culita, Luminita Patron

10.45 – 11.15 h

AO2. *SACCHAROMYCES CEREVISIAE* – MODEL ORGANISM FOR STUDY OF HUMAN DISEASES

Milena Georgieva, George Miloshev

11.15-11.30 h COFFEE BREAK

Session B.

Chairpersons:

Assist. Prof. Reneta Toshkova, MD, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

11.30 – 12.30 h POSTER SESSIONS A AND B

Session C.

Chairpersons:

Assoc. Prof. Anna Tolekova, MD, PhD
Faculty of Medicine, Trakya Universit, Stara Zagora

Assist. Prof. Dimitar Ivanov, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

13.30 -14.00 h

CO1. SIALYLTRANSFERASE ACTIVITIES OF ZAJDELA HEPATOMA CELLS

D. Ivanov, R. Gavazova

14.00 – 14.30 h

CO2. APPLICATION OF THE METHOD OF HEMODIALYSIS FOR EXPERIMENTAL PURPOSES

Rosen Iliev, Petya Hadzhibozheva, Mariya Kamburova, Tsvetelin Georgiev

14.30 – 15.00 h

CO3. TRICHINELLOSIS – A UNIQUE CHALENGE TO THE ADAPTIVE PROPERTIES OF STRIATED MUSCLE CELL

Rositsa Milcheva, Svetlozara Petkova, Zuzana Hurniková, Pavel Babál

15.00 – 15.15 h COFFEE BREAK

15.15-16.00 h POSTER SESSION C

17th March, 2010

Session D.

Chairpersons:

Prof. Elena Nikolova, PhD, DSc
Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences

Irena Andonova, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

10.00 – 10.30 h

DO2. MALDI-TOF MASS-SPECTROMETRY IN HIGH-THROUGHPUT SNP GENOTYPING

Irena Andonova, Varban Ganev

10.30 – 11.00 h

DO3. MASS-SPECTROMETRY IN CANCER PROTEOMICS AND DRUG DISCOVERY

Irena Andonova, Varban Ganev

11.00-11.15 COFFEE BREAK

11.15-11.45 h

DO1. ASSOCIATION OF FOKI POLYMORPHISM OF THE VITAMIN D RECEPTOR (VDR) GENE WITH BONE MINERAL DENSITY (BMD) IN A BULGARIAN POPULATION SAMPLE

Zhivka Ivanova, Andon Toshev, Petia Genova- Kalou

11.45 – 12.15 h

DO4. CELL CYCLE

Pavel Mitrenga

12.15 – 13.30 h POSTER SESSION D

Session E.

Assoc. Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Assist. Prof. Petia Genova–Kalou, PhD
National Centre for Infectious and Parasitic Diseases

14.30-15.00 h

EO1. INVESTIGATIONS ON JC POLYOMAVIRUS INFECTION AMONG BLOOD DONORS

Vera Devenska, Ilia Tzekov, Zlatko Kalvachev

15.00 – 15.30 h

EO2. REACTIVATION WITH HUMAN HERPES VIRUS TYPE 6 (HHV-6) IN PATIENTS WITH DIFFERENT DISORDERS IN CENTRAL NERVOUS SYSTEM AT 2009 IN BULGARIA

Petia Genova-Kalou, Magdalena Teoharova, Svetlana Kioseva, Krasimira Idakieva

15.30 -15.45 h CLOSING REMARKS

Session A.

Chairpersons:

Assist. Prof. Radostina Alexandrova, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assoc. Prof. Georgi Miloshev, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

AO1. EXPERIMENTAL MODELS IN CANCER RESEARCH

Radostina Alexandrova[1], Tanya Jivkova[1], Lora Dyakova[2], Eleonora Leventieva-Necheva[2], Reni Kalfin[2], Gergana Sabruteva[1], Plamena Borisova[2], Constanta Timcheva[3], Gabriela Marinescu[4], Daniela Cristina Culita[4], Luminita Patron[4]

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AP1. MODEL SYSTEMS OF PROSTATE CANCER: CHALLENGES AND PROGRESS

Radostina Alexandrova[1], Tanya Jivkova[1], Lora Dyakova[2], Eleonora Leventieva-Necheva[2], Reni Kalfin[2]

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Prostate cancer (PCa) continues to be a major cause of morbidity and mortality in men around the world. At the same time, the field of prostate cancer research continues to be hindered by the lack of relevant preclinical models to study tumorigenesis and to further development of effective prevention and therapeutic strategies. Recent reports have revealed differences in the molecular basis of PCa among people of differing racial or ethnic backgrounds (Navone, 1998; Pienta et al., 2008; Kimura et al., 2009). For a long time, only three cell lines, namely LNCaP, PC3, and DU145, were routinely used to study prostate cancer in the lab. The success rate to establish cell lines from human prostate cancer tissues is low, in the 1% range. Currently, only about 10 prostate cancer cell lines are available, and many of them do not reproduce typical features of the human disease, like androgen receptor expression or prostate specific antigen (PSA) secretion (Fizazi, Navone, 2005). Several in vivo models were artificially established by transforming prostate cells by potent oncogenes. Other models were developed by injecting prostate cancer cell lines into the prostate (orthotopic model), the vessel, or the bones of immuno-deficient mice, to mimic localized and metastatic prostate cancer (Fizazi, Navone, 2005). Up to now, approximately 25 xenograft models of human

prostate cancer have been established and reported in the literature. The available xenografts seem to represent the various stages of clinical prostate cancer, such as early progression and transition from androgen-dependent to androgen-independent growth (van Weerden, Romijn, 2000).

To improve the therapy of advanced prostate cancer (CaP), it is critical to develop animal models that mimic CaP bone metastases. Unlike the human disease, CaP xenograft models rarely metastasize spontaneously to bone from the orthotopic site of primary tumor growth (Corey et al., 2002). The MDA PCa2b cell line was used to generate an in vitro model of bone metastases by a co-culture system with osteoblasts (Fizazi, Navone, 2005). A sensitive real-time polymerase chain reaction (QPCR) assay have been developed for metastasis assay of human prostate cancer (PCa) growth in severe combined immunodeficient (SCID) mice (Havens et al., 2008).

Advances in science and technology have allowed us to manipulate the mouse genome and analyse the effect of specific genetic alterations on the development of prostate cancer in vivo. It is possible now to analyse the molecular basis of initiation, invasion and progression to metastatic disease. The current mouse models utilise knockout, knock-in or conditional regulation of expression using Cre-loxP technology. Genes that have been targeted include homeobox genes, tumour suppressors and oncogenes, growth factors (and their receptors), steroid hormones and cell-cycle regulators, as well as pro- and anti-apoptotic proteins. Bigenic models indicate that that two 'hits' are required for progression from intra-epithelial neoplasia (PIN) to invasion carcinoma, and two to five hits are needed for metastasis. Currently the PB-Cre4 x PTEN(loxP/loxP) mouse is the only model that spans the entire continuum from initiation to local invasion and metastasis (Ahmad et al., 2008).

The canine prostate gland shares many morphological and functional similarities with the human prostate and dogs are the only other large mammals that commonly develop spontaneous prostate cancer. However, the incidence of prostate cancer is much lower in dogs and the precise cell of origin is not known. Dogs with naturally-occurring prostate cancer are relevant models for the disease in humans and pre-clinical studies of new diagnostics and therapies in dogs may benefit both humans and dogs with prostate cancer (Leroy, Northrup, 2009).

The Prostate Cancer Models Working Group (PCMWG, Prostate Cancer Foundation) reviewed the state of prostate cancer preclinical models and identified the current limitations of cell line, xenograft and genetically engineered mouse models that have hampered the transition of scientific findings from these models to human clinical trials. In addition, the PCMWG identified administrative issues that inhibit the exchange of models and impede greater interactions between academic centers and these centers with industry. The PCMWG identified potential solutions for discovery bottlenecks that include: 1) insufficient number of models with insufficient molecular and biologic diversity to reflect human cancer; 2) a lack of understanding of the molecular events that define tumorigenesis; 3) a lack of tools for studying tumor-host interactions; 4) difficulty in accessing model systems across institutions, and 5) addressing why preclinical studies appear not to be predictive of human clinical trials (Pienta et al., 2008

In summary, model systems of prostate cancer that accurately reflect the different disease stages are necessary to ensure a proper experimental design aimed at increasing our understanding of the biology of the disease and such models are essential tools to accelerate development of new therapies for prostate cancer (Navone et al., 1998-1999).

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Watanabe, M., A. Takagi. Yakugaku Zasshi, 128, 2008, 37-44.

AP2. EXPERIMENTAL MODELS IN LUNG CANCER RESEARCH

Radostina Alexandrova[1], Tanya Jivkova[1], Eleonora Leventieva-Necheva[2], Lora Dyakova[2], Reni Kalfin[2], Constanta Timcheva[3]

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Lung cancer is the leading cause of cancer-related mortality for both man men and women worldwide (Jewal et al., 2004). The exceptionally high morbidity of lung cancer is due in part to our inability to diagnose the disease at an early stage: the majority of patients with lung cancer present with symptoms related to the primary tumor or to metastases and are diagnosed with advanced –stage disease. As stage of the disease correlates inversely with survival, the prognosis of these patients is extremely poor despite aggressive local and systemic therapies (Mountain, 1997; Shaw et al., 2006). The pathogenesis of lung cancer remains highly elusive due to its aggressive biologic nature and considerable heterogeneity, as compared to other cancers. These circumstances substantially impede study of the disease in humansand necessitate the use of experimental models that can be used under more uniform, controlled conditions than that achievable in the clinical settings (Lin, Johnston, 2002). Humans are one of the only few species susceptible to develop spontaneous lung cancer. Lung tumors in domestic animals were periodically observed by veterinarians, but Livingood's histologic description of a papillary tumor in a mouse initiated the idea of using animals as experimental tools (Livingood, 1986; Liu and Johnson, 2002). Currently several types of animal models are widely used for experimental lung cancer research. These include chemically induced lung tumors, transgenic mouse models and human tumor xenografts (Liu, Johnson, 2002). Chemical or carcinogen induced lung tumors have been described in a variety of species, including dogs, cats, hamsters, mice and ferrets (Waattenberg and Leong, 1970; Shimkin and Stoner, 1975; Benfield et al., 1986; Stoner, 1991; Kim and Lee, 1996). Different generation of mouse models have evolved employing diverse innovative strategies to model lung cancer. Although they have been informative and further propel our understanding of human lung cancer, they still do not fully recapitulate the complexities of human lung cancer. Recently, through the use of gene targeting strategies, genetically engineered mouse models of lung cancer have been generated that closely mimic the human condition. These models are based on targeted mutations and tumor suppressor genes known to play a role in human lung tumorigenesis (Liu and Johnson, 2002; Shaw et al., 2005; Dutt and Wong, 2006).

Orthotopic lung cancer are described using endobronchial, intrathoracic or intravenous injection of tumor cell suspension (McLemore et al., 1987; Wang et al., 1992 a,b; Howard et al., 1991, 1999) and by surgical implantation of fresh tumor tissue (McLemore et al., 1988; Wang et al., 1992 c; Rashidi et al., 2000).

Permanent cell lines established from non-small cell (A549, NCI-H460, NCI-H125) and small cell (NCI-H345) lung tumors are also used in lung cancer research.

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AP3. GLIOMA MODELS

Radostina Alexandrova[1], Eleonora Leventieva-Necheva[2], Tanya Jivkova[1], Lora Dyakova[2], Reni Kalfin[2], Constanta Timcheva[3]

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Gliomas of astrocytic, oligodendroglial and ependymal origin account for more than 70% of all brain tumors. They are classified into four clinical grades, grade 4 or glioblastoma multiforme (GBM) is the most common (65%) and aggressive of these tumors. GBM either arise de novo (primary) or progress to GBM from lower-grade gliomas (secondary). Survival of patients affected by GBM has remained virtually unchanged during the last decades (i.e. 6-12 months post-diagnosis) despite advances in surgery, radiation and chemotherapy. Less than 3% of glioblatoma patients are still alive at 5 years after diagnosis, higher age being the most significant predictor of poor outcome (Dai, Holland, 2001; Candolfi et al., 2007).

Brain tumors are a component of several inherited tumor syndromes, but the prevalence of these syndromes is very low. Several occupational, environmental carcinogens, and diet (N-nitroso compounds) have been reported to be associated with an elevated glioma risk, but the only environmental factor unequivocally associated with an increased risk of brain tumors, including gliomas, is therapeutic X-irradiation. In particular, children treated with X-irradiation for acute lymphoblastic leukaemia show a significantly elevated risk of developing gliomas and neuroectodermal tumor, often within 10 years after therapy (Ohgaki, Kleihues, 2005).

Many researchers have been successful in treating GBM models in animals, but the success has been limited when new treatment principles have been translated into the clinic. One reason for this failure is the lack of appropriate animal models that reflect the behaviour of human GBMs (Terzis et al., 2006).

An ideal glioma model have to recapitulate the genetic alterations of human gliomas and show histological resemblance to human tumors. The model could then be used to further investigate tumorigenic factors in the specific pathways and be used to test potential therapeutic targets (Hu, Holland, 2005).

For decades people have been modelling gliomas using various techniques and animal species. To data, four major strategies have been successfully employed to reach the goal: chemical mutagen-induced models, xeno- or allograft transplantation-induced models, germline genetic modification-induced models, and somatic genetic modification-induced models (Dai, Holland, 2001).

The rat has been one of the most widely used experimental animals, and rat brain tumor models have been used extensively since the mid 1970s. The ability to produce genetically engineered lines has increased the use of murine models over the past few years. Feline and canine models have been used less frequently, but nevertheless, still provide an intermediate between rodent models and humans (Barth, Kaur, 2009).

Gliomas can be induced by treating pregnant rats with mutagenic alkylating agents such as N-methylnitrosourea or N-ethyl-N-nitrosourea, which cause point mutations in the cells of the developing brain. The tumors show histological similarity to human gliomas. However, because it is difficult to identify the primary causal mutations for the lesions and the cell-of-origin of the tumors is unknown, the modelling systems are non-reproducible. Furthermore, since the model provides little insight as to the actual genetic etiology of the tumor in humans, it is also difficult to use such models to test specific molecular therapeutic blockade (Hu, Holland, 2005; Barth, Kaur, 2009). Germline modification with a transgene or gene targeting in mice allows determination of the effects of gain-of-function and loss-of-function mutations. Another modelling system is gene transfer to somatic cell by retroviral infection of neonatal mice (Hu, Hilland, 2005).

Xenograft or aloograft models are generated by transplanting cultured glioma cells of human or rodent origin to immunodefiicient mice or rats. These models generate tumors with reproducible high incidence, growth rate and survival pattern. However, the tumors do not recapitulate the histological feature characteristic of human glioma and there is a lack of immunological interactions between tumor and host. Moreover, the genetics of the original tumor cells are likely to be altered by selective pressure during extended cell culture, so that the xenograft and allograft tumors may not correctly represent the human or rodent gliomas. Nevertheless, because these models can easily generate reproducible tumors, they have been widely used for therapeutic testing. Unfortunately, they have not been good predictors of response in humans (Dai, Holland, 2001).

The induction of experimental brain tumors by the injection of Rous sarcoma virus has been described in canines, rats, and monkeys. Tumors were induced by inoculating neonatal Fischer rats i.c. with purified avian sarcoma virus (ASV) suspensions. All of the animals develop tumors within 2 weeks following ASV injection, 94% of which were anaplastic astrocytoma, and the remainder were low grade gliomas or sarcomas. This model has been used to study the effects of chemo- and radiotherapy, Blood Brain Barrier disruption, and tumor permeability. The response to immunotherapy indicated that these tumors were immunogenic, and expressed a variety of virally encoded tumor specific antigens (Copeland et al., 1976; Barth, 1998; Prabhu et al., 2000).

Permanent glioma cell lines are invaluable tools in understanding the biology of glioblastomas. Besides, cultured cells still can be serially transplanted into nude mice supplying

a trustworthy model for the study of behaviour of human tumors, such as metabolism, relapse, drug sensitivity, and resistance (Wang et al., 2007).

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AO2. *SACCHAROMYCES CEREVISIAE* – MODEL ORGANISM FOR STUDY OF HUMAN DISEASES

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Saccharomyces cerevisie is a unicellular organism used in the bread-making industry. Yeast cells are eukaryotic and many of their basic biological properties are shared with the human beings. In comparison with human genome ~ $4x10^8$ base pairs, yeast genome is just over 12 million base pairs in length and contains about 6000 genes. Surprisingly, about 20 per cent of human genes, proved to be involved in certain disease development, have counterparts in yeast. This suggests that most of the diseases result from the disruption of very basic cellular processes, such as DNA repair and cell division. Therefore, studying their mechanisms in yeast cells is a promising alternative.

Yeasts *Saccharomyces cerevisiae* are used as model organisms for human diseases for more than 10 years, including neurodegenerative diseases, aging, tumor development, heart failures and even psychological problems. The results from this bright approach allow scientists to apply the revealed mechanisms in the search for novel therapeutic strategies.

Here, we report our attempts to transform *S. cerevisiae* into a model for studying of human glioma development. Human gliomas account for a large number of medical cases in Bulgaria. Annually, 250-300 patients are diagnosed with gliomas. The failure of glioma treatment is determined by the invasiveness, irresponsiveness to any kind of therapy and tendency for recurrence of these tumors. In spite of the technological advances leading to a better surgical resection and the improvement of radio- and chemotherapy, still there is no significant amelioration of patients' quality of life, free survival and prognosis. Obviously, the explanation for this poor outcome lies in the cellular mechanisms of glioma development. We decided to use yeast cells as a model organism for a detailed molecular study of human gliomas expansion. The obtained results will be discussed together with development of strategies for future gliomas treatment.

AP4. YEASTS AS A MODEL SYSTEM

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Yeasts are eukaryotic micro-organisms classified in the kingdom Fungi, with about 1,500 species currently described; they dominate fungal diversity in the oceans. Most reproduce asexually by budding, although a few do so by binary fission. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as *pseudohyphae*, or *false hyphae* as seen in most molds. Yeast size can vary greatly depending on the species, typically measuring 3–4 μm in diameter, although some yeasts can reach over 40 μm.

The useful physiological properties of yeast have led to their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest and largest application of this technology. Many types of yeasts are used for making many foods: Baker's yeast in bread production, brewer's yeast in beer fermentation, yeast in wine fermentation and for xylitol production. Yeasts are also one of the most widely used model organisms for genetics and cell biology. Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes.

The sequencing of the human genome promised the identification of disease-causing genes and, subsequently, therapies for those diseases. However, when identifying the genetic basis of a disease, it is not uncommon to discover an abnormal protein whose normal function is unknown. The genetic manipulations required to assign function to genes is often extremely difficult, if not impossible, in human cells. Model organisms have been used to facilitate understanding of gene function because of the ease of genetic manipulations and because many features of eukaryotic physiology have been conserved across phyla. Yeast is a simple eukaryote with a tractable genome, a short generation time, and a large network of researchers who have generated a vast arsenal of research tools. These traits make yeast ideally suited to help reveal the function of genes implicated in human disease.

The yeast *Saccharomyces cerevisiae* is also an excellent model for gaining insights into the molecular basis of human mitochondrial disorders, particularly those resulting from impaired mitochondrial metabolism. Most of our current knowledge about mitochondrial biogenesis in humans derives from yeast genetics and biochemistry. Systematic yeast genome-wide approaches have allowed for the identification of human disease genes. In addition, the functional characterization of a large number of yeast gene products resident in mitochondria has been instrumental for the later identification and characterization of their human orthologs. The usefulness of yeast as a model system for human mitochondrial disorders is evaluated.

The similarity between yeast and human genom make yeasts perfect model system for studying biochemical processes and gene function giving us fast and accurate information.

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AP5. EXPERIMENTAL MODEL OF CANCER STEM CELLS

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The biology of human cancers can be studied with the help of human tumor samples. Such pathological samples present the real state of the tumor in vivo. They can be used to define its pathology, gene expression and metabolism. The interpretation of the findings must take into account the complexity and heterogeneity of solid tumors. These samples represent only one late time point in the evolution of the lesion.

Experimental models are necessary for the following reasons:
- To obtain a dynamic view of a cancer from its origin to the later stages, observed in patients, i.e. its physiopathological evolution;
- To test hypotheses about the origin, pathogenesis and physiopathology of human tumors;
- To investigate in detail the physiopathological pathways;
- To screen and test chemical compounds as potential drugs;
- To define potential diagnostic signatures.

Experimental models of cancers

Human cancer cell lines have been by far the most used among various possible experimental models. They have retained hallmarks of cancer cells. Human cell lines are preferred because the same oncogene may give different phenotypes in human and in transgenic mice. The cells cultured in serum containing medium were genetically unstable. The cells, survived the 'crisis' stage of primary cultures, had accumulated new genetic lesions that were not present in the original tumor. However, there are tumor-initiating cells in many of the existing cell lines that are used in xenograft assays.

Surface antigens such as SSAs and CD 133 protein, proposed to enrich the number of stem sell, that are often used for stem cell isolation. While some studies report the presence of CD133+ cells in established human brain cancer cell lines provide compelling evidence that primary glioblastoma cells, cultured in serum containing medium rapidly differentiate and have limited potential to proliferate or initiate tumors in vitro. By contrast, the same cells cultured in serum free, stem cell medium retained their ability to initiate a tumor even after many passages in culture. These results suggest that many established cell lines, that have been routinely cultured in serum-containing medium for decades, may have similarly evolved, no longer representing the original tumor.

In vitro cell culture models do provide the advantage of a tightly controlled experimental pattern. Use of a primary cell culture system to examine whether agents known to differentiate normal neural stem cells could be used to induce differentiation of glioma-derived cancer stem cells. They showed that bone morphogenetic proteins (BMP) could induce loss of stem cell characteristics in cancer stem cells in vitro. They validated their in vitro findings, using an in vivo xenograft model.

In vivo models of cancer stem cells - Xenograft of human cells in mice

The existence of human cancer stem cells was first verified by xenografting human leukemic cells into NOD-Prkdc mice and demonstrating that only a small percentage of engrafted cells with markers characteristic of HSCs were capable of initiating leukemia in recipient mice. In that study, xenografting into immune-deficient mice has become the standard protocol for demonstrating tumor-initiating capacity of putative cancer stem cells. Subsequently,

human cancers from many major organ sites have been shown to contain cancer stem cells, with varying degrees of rigorous analysis.

More recent studies, that examined cellular characteristics of cancer stem cells, were performed by combining in vitro manipulations with in vivo testing using human cancer cells xenografted into mice. The cancer stem cell niche were examined using brain cancer cells and was found that Nestin + CD133+ cancer cells were positioned near the endothelium, similar to normal neural stem cells. It is proposed that targeting the endothelium may be an effective way to destroy cancer stem cells and was shown that anti-angiogenic drugs were indeed effective in reducing the number of Nestin + CD133+ cells.

The way in which studying stem cell biology contributed to understanding brain cancer is likely to be more complex than simple parallels to normal development. These studies present three models of increasing complexity to illustrate some of the variables. Each of the presented models has supporting and opposing evidence, but many questions are still unanswered. The actual picture of brain tumor initiation and progression and the complete description of the role of stem cells in brain tumors are probably even more complex.

Tumor heterogeneity and undifferentiated character points to the possibility, that tumors might arise from progenitor cells, which are more susceptible to oncogenic transformation, due to their ability to selfperpetuate, and thus accumulate mutations over time. It is still unclear whether brain tumors are able to arise from any given cell within the brain, or if histologically different tumors arise from the same or different precursor cells.

Normal cells can scarcely dedifferentiate under conditions of extreme stress, and the only way to prevent differentiation in culture is by adding inhibitory factors. While some directionality has been shown in the case of tumor stem cell differentiation, brain tumor modeling experiments indicate that differentiation status of tumor cells can be easily changed by affecting signaling pathways and support an alternative explanation. Tumor heterogeneity may represent inherent instability in gene expression patterns, that confers undifferentiated cell character as opposed to stage in the stem cell lineage defining patterns of gene expression. Genetic alterations could make differentiation status of brain tumor cells unstable, floating up and down the lineage - perhaps in fact dynamic to the extent that it may be impossible to be assigned to any differentiation status at all.

Brain tumor biology is very complex.

It is not possible to describe fully and sufficiently all events during tumorigenesis by a limited cancer stem cell model. Brain tumors are characterized by unstable differentiation status, judging from expression of certain markers. Regional signaling patterns may be sufficient to change apparent differentiation and lineage stage. Additional mutations affecting various cells within the tumor cause further shifts in signaling patterns, apparent differentiation status, and lineage, which in addition to the autocrine and paracrine effects within the tumor, result in perpetually changing tumor phenotype. Using of cell culture methods, xenotransplantation in immune deficient mice, and transgenic mouse models must be used judiciously to test various aspects of the hypothesis. While each model has its own limitations, combining different models to validate key observations will be critical in understanding the biology of cancer stem cells.

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AP6. IN VITRO CYTOTOXIC EFFECT OF A PLANT POLYPHENOL EXTRACT FROM *GERANIUM SANGUINEUM* L., ON PRIMARY AND PERMANENT TUMOR CELL LINES

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Natural products and their derivatives have been shown to be valuable resource of novel anti-cancer drugs and play a fundamental role in cancer chemotherapy. Over 60 % of approved anticancer drugs are obtained from natural sources. Examples are the *Vinca* alkaloids, the taxanes, and the camptothecins, derived from plants.

Breast cancer is the most common cancer and the second leading cause of cancer-related death for women in the world. For this reason breast cancer cell lines are widely used in laboratory practice as *in vitro* models in cancer research.

The current study was designed to investigate the *in vitro* antitumor activity of the polyphenolic complex (PC), obtained from the medicinal plant *Geranium sanguineum* L., on two human permanent (MDA-MB-231 and MDA-MB- 468) and two primary breast cancer cell lines as well as on primary Graffi myeloid cell culture. The cytotoxicity of PC was determined either by cell counting using trypan blue exclusion method or by the colorimetric MTT assay.

The results presented show growth inhibition *in vitro* of human breast permanent and primary cell lines, as well as of the Graffi myeloid carcinoma cells, treated with PC. *[PC is most effective in the inhibition of human permanent breast cancer cell lines (IC_{50} - 209 ± 8 μg/ml and 205 ± 9 μg/ml for MDA-MB-231 and MDA-MB-468, respectively) as compared to primary breast cancer cell lines (114 ± 8 μg/ml and 119 ± 9 μg/ml respectively)]*. Human permanent breast cancer cell lines (IC_{50} - 209 ± 8 μg/ml and 205 ± 9 μg/ml for MDA-MB-231 and MDA-MB-468, respectively) are more susceptible to the cell growth inhibitory effect of PC as compared to primary breast cancer cell lines (114 ± 8 μg/ml and 119 ± 9 μg/ml respectively). Paclitaxel (used as a positive control) inhibited the growth of MDA-MB-231 and MDA-MB-468 cells with a median inhibition concentration (IC_{50}) of 10 ± 3 ng/ml and 13 ± 3 ng/ml, respectively. Treatment of primary Graffi myeloid cell culture with PC, applied in the dose of IC_{50} induced inhibition of cell growth with index of inhibition of 1.17, compared to the control.

Conclusion: This study reports the first preliminary evidence on the antiproliferative properties of the semi-standardized plant polyphenol extract PC on human breast carcinoma cells. The presented results suggest that the preparation could be a promising chemotherapeutic agent and should be examined further *in vivo*.

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AP7. INHIBITION OF CELL PROLIFERATION AND INDUCTION OF APOPTOSIS BY RED MICROALGAL POLYSACCHARIDES

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Introduction: The spectrum of a physiological activity of the carbohydrates that build algal cell walls is quite extended in relation to the characteristics of the environment for the development of algae /aquatic or soil/ with which they maintain a constant exchange through their cell surface. They exhibit a significant biological activity /antiviral, antimicrobial, immunostimulating, anti-cancer, antioxidant, anticoagulant, antiangiogenic, osmosis-regulating/ and valuable physicochemical properties, high reactivity, ability to form complexes. The efforts of scientists from all over the world are aimed at creating anti-tumor products of natural origin, which have high biological activity, low toxicity and possess a broad spectrum of therapeutic activity.
Aim: This study was designed to determine the anti-proliferative and apoptotic properties of algal polysaccharides as well as to elucidate the mechanism of their action, using human permanent and animal tumor cell lines as a model system.
Material and methods: The effect of polysaccharides from red microalgae Dixoniella grisea and Porphyridium cruentum (Rhodophyta) was investigated on three permanent human tumor lines - HEp-2 (human laryngeal epithelial carcinoma), MCF-7 (breast adenomacarcinoma) and HeLa (cervical cancer) and on primary culture from Graffi myeloid tumor in hamsters in doses of 50 and 100 γ/ml. Inhibition of cell proliferation was determined by MTT assay. Cell apoptosis was examined with double staining method with acridine orange (AO) and propidium iodide (PI). The mechanism of action of polysaccharides was investigated by DNA fragmentation assay.
Results: Both polysaccharides decreased the tumor cell proliferation in a concentration-dependent manner in vitro. The observed significant anti-proliferative effect was more pronounced at a dose of 100 γ/ml. The polysaccharides applied at a dose equivalent of cytotoxic strongly stimulate the proliferation of bone marrow cells derived from Graffi tumor-bearing animals. Characteristic manifestations of apoptosis including morphological features (cell shrinkage, chromatin fragmentation, plasma membrane blebbing and apoptotic bodies) were observed when the cells were treated with polysaccharides. Further analysis using agarose gel electrophoresis showed that both polysaccharides from algal strains caused nuclear DNA fragmentation. It was observed a clear DNA ladder after treatment of Graffi tumor cells with 50 and 100 γ/ml polysaccharides, which is a hallmark of apoptosis.
It is known that sulfated polysaccharides, such as those derived from Dixoniella grisea and Porphyridium cruentum bind a broad range of proteins such as growth factors and adhesion molecules on cell surface. As a result sulfated polysaccharides can influence the proliferation, differentiation, apoptosis and metastasis of tumor cells. Our studies indicate that both algal polysaccharides may be a promising alternative to synthetic substances as a natural compound with high immunostimulating and antitumor activities.

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AP8. AN USEFUL ANIMAL MODEL FOR STUDYING DOXORUBICIN TOXICITY AND A STRATEGY FOR ITS REDUCTION

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The anthracycline doxorubicin (DOX) remains among the most effective anticancer drugs ever developed, with high antitumor efficacy in breast cancer, aggressive lymphomas, childhood solid tumors and soft tissue sarcomas. The therapeutic value of DOX, however, is limited by its dose dependent cardiotoxicity. The most widely accepted mechanism accounting for anthracycline–induced heart injury involves reactive oxygen species (ROS) generation. We investigated DOX-induced cardiotoxicity after a single intraperitoneal injection of 20 mg/kg b.wt. to Balb/c mice. Significant decrease in the cardiac level of the major intracellular antioxidant-glutathione (GSH), as well as severe histopathological changes in the hearts of experimental mice were observed under light and transmission electron microscope.

This useful animal model for studying doxorubicin toxicity was further exploited for creation of antioxidant strategy to attenuate DOX cardiac toxicity. We investigated the protective effects of whey proteins as functional food ingredients, which have been proved to exert antioxidant and anticarcinogenic action (1, 2, 3). The majority of whey proteins are cysteine-rich and take part in regulation of GSH concentrations in vivo (4). Our results indicate that the significant reduction in GSH cardiac content after DOX treatment was partly restored in mice with whey supplemented chow diet. The severe histopathological changes in heart samples of DOX treated mice were partially reduced in whey supplemented group.

In conclusion, in this study we show that oxidative damage to the heart promotes the myocardial toxicity induced by DOX in Balb/c mice. This effect may be limited by whey protein feeding. The protective properties of whey could be attributed to its antioxidant capacity in connection with promoting GSH synthesis.

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AP9. DAUNORUBICIN-LOADED POLY(BUTYLCYANOACRYLATE) NANOPARTICLES AND: EFFECT ON VIABILITY AND PROLIFERATION OF CULTURED HUMAN TUMOR CELL LINES

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Poly(alkylcyanoacrylates) (PACA) are remarkable polymers considered as building material for different nanoparticulate structures. PACA nanoparticles have been developed 25 years ago by Couvreur et al. [1] taking advantage of the polymer possibility for in vivo degradation by lysosomal enzymes and its good acceptance of living tissues. The increasing therapeutic interests of PACA nanoparticles as drug carriers, due to their capacity for targeting and transport of many types of drugs, promotes them as the most promising polymer colloidal drug delivery system [2]. They are able to transport dugs across barriers allowing delivery of therapeutic doses in difficult tissues to reach including in the brain [3,4] or in multidrug resistant cells [5].
The aim of the study presented here was to evaluate the influence of daunorubicin loaded poly(butylcyanoacrylaate) nanoparticles (Dau/NPs) on viability and proliferation of cultured tumor cell lines established from some of the most common, invasive and socially important human malignancies: glioblastoma multiforme (8 MG BA), cervical carcinoma (HeLa), liver cancer (HepG2) and lung carcinoma (A549). Dau/NPs were applied at a concentration range of 0.0156 – 2.0/0.156 -20 μg/mL for 24, 48 and 72h. The investigations were performed using MTT test and Neutral red uptake cytotoxicity assay.

Key words: Poly(butylcyanoacrylate) nanoparticles; drug delivery system; Daunorubicin; cytotoxic/antiproliferative activity; tumor cell line

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АР10. ВЛИЯНИЕ НА ПИРОКСИКАМ ВЪРХУ ПРЕЖИВЯЕМОСТТА И ПРОЛИФЕРАТИВНАТА АКТИВНОСТ НА ТУМОРНИ И НЕТУМОРНИ КЛЕТКИ

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Въведение: Пироксикамът прдставлява нестероидно противовъзпалително лекарствено средство, намиращо приложение в медицината главно като болкоуспокояващ агент. Във ветеринарната медицина пироксикамът се използва при лечението на определени неоплазии, експресиращи циклооксигеназен рецептор. Разтворимостта му в DMSO прави възможно прилагането му чрез липозоми. **Целта** на представеното изследване беше да се изследва влиянието на пироксикама върху преживяемостта и пролиферативната активност на култивирани в лабораторни условия човешки туморни и нетуморни клетки.
Материали и методи: В експериментите като моделни системи бяха използвани следните три човешки клетъчни линии: Lap3 (нетуморни клетки), HeLa (карцином на шийката на матката), A549 (рак на белия дроб). Проучванията бяха проведени чрез MTT тест, като пироксикамът беше приложен в концентрации от 20 до 200 μg/ml за 24 и 72 часа. С получените данни бяха построени криви „концентрация – отговор".
Резултати: Установено беше, че пироксикамът намалява преживяемостта и пролиферативната активност на третираните клетки, като ефектът му нараства с увеличаването на концентрацията и времето на въздействие. От използваните като експериментални модели три клетъчни линии относително най-висока чувствителност към действието на пироксикама проявиха клетките от линия HeLa, докато белодробните ракови клетки от линия A549 показаха сравнително най-висока устойчивост.
Изводи: Пироксикамът повлиява преживяемостта и растежния потенцал на изследваните клетъчни линии. В ход са проучвания насочени към изясняване на потенциалните цитотоксични и антипролиферативни активности на метални комплекси на пироксикам върху култивирани в лабораторни условия туморни и нетуморни клетки с човешки и животински произход.
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AP11. EFFECTS OF MONENSIN ON VIABILITY AND PROLIFERATION OF CULTURED TUMOR AND NONTUMOR CELLS

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Introduction Monensin is a an antiprotozoal agent produced by Streptomyces cinnamonensis. It is known to exert antifungal, antiprotozoal, antiviral and ionophoric effect. From chemical point of view monensin is a furan with molecular weight approximately 671g/mol. The substance is soluble in organic compounds, incl. solubility in the lipid component of biological membranes, but poorly soluble in water.

Recent studies show monensin`s effect as pharmacological inhibitor of macroautophagy and suggested its role in cell cycle arrest and apoptosis. It has been tested in various cancer cell lines with promising results in terms of inhibiting tumor cell growth.

Aim

The aim of our study was to examine the effect of monensin on viability and proliferation of cultured human tumor and nontumor cells.

Materials and Methods

The following human cell lines were used in our experiments: HELA (cervical cancer), Lap3 (nontumor diploid cells) and A549 (lung cancer). The effect of monensin on cell viability and proliferation was evaluated by MTT (3-(4, 5-dimethylthiazol-2-y1)2, 5-diphenyltetrazolium bromide)-based colorimetric assay. Monensin was initially dissolved in Dimethyl sulfoxide (up to concentration of 1 mg/ml) and then diluted in culture medium. The compound was applied at concentrations of 5, 10 and 25 μg/ml for 24h, 48h and 72h. Cells treated only with cell culture medium, as well as with the solvent DMSO were used as controls.

Conclusions

Our study showed that monensin decreased significantly viability and proliferation of the treated cells in a time- and concentration-dependent manner. Intensive experiments are unredway to clarify better the potential antitumor activity of this compound and its metal complexes as well as their mechanism(s) of action.

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AP12. EXPERIMENTAL MODELS OF NEURODEGENERATIVE DISORDERS: ALZHEIMER'S AND PARKINSON'S DISEASES ANIMAL MODELS

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Alzheimer's and Parkinson's diseases are among the most spread and leading cause of death in people over 65 years of age. Interesting is the fact that some patients have clinical and pathological features of both diseases, raising the possibility of overlapping pathogenetic pathways (Masliah E. *et* al., 2001). Hence, the development of rodent models for Alzheimer's and Parkinson's degenerative disorders is a critical step for both understanding the disease and developing therapeutic drugs.

An option is mice to be bred that carry many of the abnormal genes causing the various problems associated with Alzheimer's and Parkinson's diseases. These mice, called transgenic mice, manifest the signs and symptoms of the diseases as age-related memory and learning impairment, loss of brain cells, cerebral accumulation of β-amyloid peptides and tau proteins in the case of Alzheimer's disease and α-synuclein for Parkinson's disease. Another option is to induce non-transgenic (i.e. pharmacological) models of neurodegenerative disorders in rodents by injecting different substances – for example, intra-ventricular infusion of Aβ1-40, phosphorylated tau proteins (A68 or PHF-tau) or lipopolysaccharide in rat brain (Frautschy S. *et* al., 1995; Shin R. *et* al., 1993; Hauss-Wegrzyniak B. & Wenk G., 2002).

In our experiments a total of 30 male Wistar rats, weighing 150-200 g at the time of surgery, were randomly divided in groups and housed in cages with free access to rat chow and water. All experiments have been performed according to the "Principles of laboratory animal care" (NIH publication No. 85-23), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 by the US Department of Health and Human Services). The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), had their heads shaved, and placed in a stereotaxic apparatus (Fig. 1). The scalp was cleaned with a jodine solution, incised on the midline and a burr hole was drilled through the skull at the appropriate location (Fig. 2). The target coordinates were: AP = +0.2; LR = -3.0; H = -5.6 according to the stereotaxic atlas (Pellegrino T. & Cushman G., 1967). In order to prove the correct coordinates, few rats received an injection of 2 μl methylene blue ink. The experimental group received an injection of 20 μg/2 μl of 6-OHDA (Sigma-Aldrich, St. Louis, MO, USA; calculated as free base, dissolved in ice-cold saline with 0.02 % ascorbic acid) while the control group received an injection of 2 μl saline. All injections were made into the right striatum area by a Hamilton microsyringe at a rate of 1 μl/min. The needle was left in place an additional 2 min before being slowly withdrawn. The wound was closed with stainless steel clips and the rat was allowed to recover before being returned to its cage.

Fig. 1

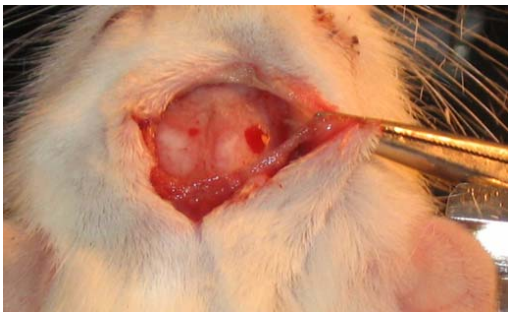

Fig. 2

The experimental model of Parkinson's disease was proved by the rotational behavior of rats induced by apomorphine (0.5 mg/kg, s.c.) two weeks after surgery (Przedborski *et* al., 1995; Kirik *et* al., 2000). The rats who received a methylene blue injection were anesthetized with urethane (1.2 g/kg, i.p.) and killed by decapitation. The brain was rapidly removed (Fig. 3). The control slices were cut and observed in order to verify the position of the injection (Fig. 4).

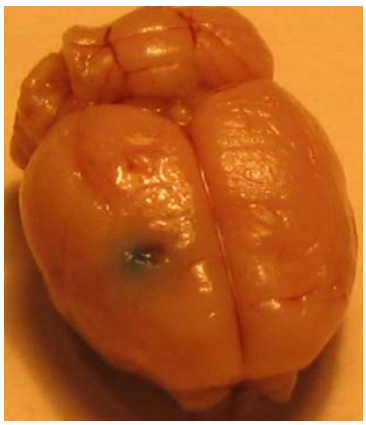

Fig. 3

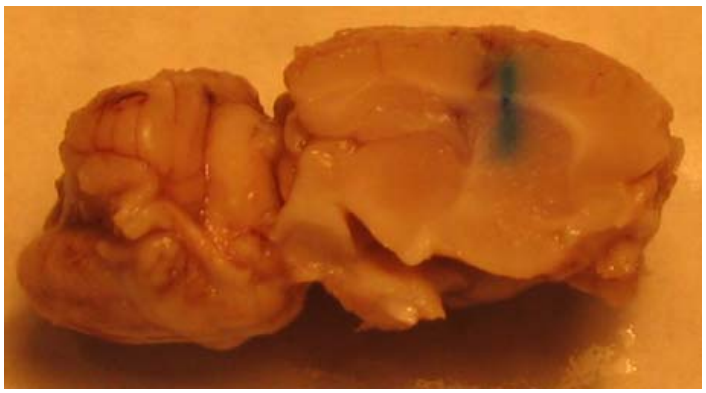

Fig. 4

Acknowledgement: This work was supported by a Grant MU-L-1502 from the National Science Fund, Sofia, Bulgaria.

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AP13. FUNCTIONS OF THE HUMAN BRAIN AND ITS CONNECTIONS WITH LANGUAGE REFLEXES

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For a long time, scientists have tried to understand the functions and the abilities of the human brain from a linguistics perspective and also have explored how humans recognize and pick up a particular language. All humans have some kind of inborn mechanisms and capacities to acquire a language. In addition, this mechanism, according to Chomsky's hypothesis, is called a Language Acquisition Device (LAD). According to the LAD hypothesis, the brain is considered as a congenital device for learning a language. Chomsky believed that language experience is not only an important part to activate the LAD mechanism, but also an essential aspect is nature versus nurture. Moreover, the human nervous system plays also an important role as a mediator between the receptors and the organs of the human body. As a matter of fact, humans react to outside influences not only through complex motive and other reactions, but also they communicate through gestures, mimics, language, and emotions. One of the most important reactions of the nervous system is the reflex – conditional and unconditional. Reflexes are a result of the actions of the central nervous system of the human beings and characterize the individual experiences of every human being. The conditional reflexes are not considered as innate but as acquired. All humans gain their own conditional reflexes. Specifically, one of these reflexes is language, which as a matter of fact, is a combination of grammatical rules, norms, and various symbols that are used by humans for communication either verbally or in a written form. Apart from Chomsky's LAD hypothesis, another scientist who has been dealing with language acquisition and cognition is Vygotsky, whose theory is called the zone of proximal development. Vygotsky found the relation between language and thought, and his research was based on how humans think. He considered language as a tool for communication which supports the development of language itself and cognition.

Thus, the zone of proximal development enables educators and parents to define the learner's immediate needs and the shifting developmental status, which allows for what has already been achieved developmentally, and for what the learner will be able to master in the future.

Session B.

Chairpersons:

Assist. Prof. Reneta Toshkova, MD, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

BP1. PHI- X174 IN THE EXPERIMENTAL BIOLOGY

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Enterobacteria phage phiX174, also known as Bacteriophage phi-X174, or just phage phi-X174, is one of the most used model organisms in a variety of studies and researches in the field of genetics, genomics, medicine, biochemistry and many other biological sciences. Phi-X174 is a virus from the ssDNA group, Microviridae family, genus Microvirus. Phi-X174 is a phage on E. coli. This virus has very small genome. Its DNA molecule is single stranded, with circular topology, containing 5386 bases which are coding 11 genes. The coding frames for 7 of the proteins overlap.

In 1977 the genome of phi-X174 was fully sequenced by Fred Sanger and his team. This was the first fully sequenced DNA based genome.

The phage had also a very significant role in the experimental biology in 2003 when it was announced that the whole genome of phi-X174 was assembled from synthetic oligonucleotides, by H.O. Smith, C.A. Hutchinson and their team. They succeeded to shorten the time for the assembly of 5 – 6 kb segments of DNA. To prove their methodology, the scientists completed the infectious genome of phi-X174 for 14 days, from a single pool of chemically synthesized oligonucleotides. After the oligonucleotides were purificated from molecules with unwanted chain length, they were ligated under strict annealing conditions. The ligation products were then assembled in full-length genomes which had lower infectivity than the natural DNA. However after electroporation into E. coli, fully infectious virions were recovered.

Another study used recoverable virus vectors for measuring mammalian mutagenesis. According to the studies, mutations in the transgenic system can emerge from three origins of DNA damage and replication errors- in vivo, ex vivo, and in vitro. These three origins of mutation can be differentiated in phi-X174 am3. The in vivo mutations were fixed in the animal, the ex vivo- in bacterial cells, and the in vitro- during the first replications of nonmutant phages, under selective conditions. The result showed that the in vivo revertants reflect the mutagenic treatment. This study is a clear example of the experimental use of the phage phi-X174 for the increasing of the sensitivity of assays for in vivo mutations.

Another property of phi-X 174, which makes that virus very suitable for a model organism, as well as other bacteriophages, is the ease with which important population parameters can be manipulated from the scientists in the study. This can be of great help to the researchers trying to identify the major factors of the emergence of new viruses and the possible outcomes of their spreading, which may help predicting epidemics and therefore increase the chances for applying better epidemiological strategies.

There are also researchers proposing bacterial ghosts, made by phi-X174 E – mediated inactivation, for vaccine candidates. Bacterial ghosts are empty bacterial cells which can be made by the controlled expression of the phi-X174 lysis gene E in gram-negative bacteria.

The researcher's team is claim that these bacterial ghosts could be used to obtain vaccine candidates.

Phi-X174 DNA can also be used as exogenous reference for measuring mitochondrial DNA copy number. According to some scientists the ratio of mtDNA to nDNA varies in repeated DNA extractions. They discovered that PhiX174 DNA, added before DNA extraction, is extracted with a similar efficiency to mtDNA. This is making the DNA of phi-X174 an alternative reference for quantifying mtDNA copy number.

As a model system, phi-X174 was even used for testing virus removal by air filters. Due to its small size, this virus is eligible for this kind of test, which can this way improve the air filters efficiency, to stop even one of the smallest viral particles.

Phi-X174 has all the needed properties to be one of the most important model organisms in the experimental biology. It's broad spreading, and the ease of cultivating makes this virus very accessible for all types of studies. Its small genome allowed the scientists to make one of the most important steps in the field of the gene sequencing, revealing for the first time the full sequence of a DNA based genome in 1977. This virus was even synthetically produced in 2003 in a study which developed a new and faster methodic for oligonucleotide assembly. Evidently Phi-X174 has a great role in various types of biological studies and researches, and could rightly be nominated for one of the most interesting and studied model organisms.

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BP2. *DROSOPHILA* S2 SYSTEM FOR HETEROLOGOUS GENE EXPRESSION

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Continuous cell lines of *Drosophila* are used as hosts for the expression of heterologous gene products. There are many different cell lines including those derived from wild-type and mutant lines of *Drosophila melanogaster.* The most popular are the Schneider lines, S2 and S3 (Schneider, 1972). The S2 cell line has been used for the expression and analysis of intracellular, secreted and membrane-associated proteins. This includes cytokines, oncogenes, antibodies, receptors and viral antigens.

The S2 cell line is derived from primary cultures of late stage, 20- to 24-hour old *D.melanogaster* embryos. Cells grow out of this heterogenous population in approximately 3 months, attaining immortalized nontumorgenic growth. The resultant cell line has originally the characteristics of epithelial-like cells – growing in a loose monolayer without piling up into central foci.

S2 cells are maintained in a few types of commercially available media. This includes the original Schneider's medium – based on the approximate composition of larval hemolymph; M3 medium. Both are suitable for growth of S2 cells when supplemented with 10% heat-inactivated serum (65°C for 30 min). A modification of the M3 medium enables suspension growth in serum-free conditions.

S2 cells are amenable to both transient and stable transfections. Stable lines are produced by cotransfecting a drug resistance plasmid along with an expression plasmid containing the gene of interest under the control of a constitutive or inducible promoter. For example, a plasmid mtaL, containing the inducible (by Cu or Cd) methallothionein promoter, efficient SV40 late poly(A) signal, and polylinker region for subcloning genes for expression is cotransfected with the drug resistance plasmid, pCoHygro, containing the hygromycin acetyltransferse gene under the control of the strong constitutive *Drosophila* copia gene promoter. Selection with hygromycin B leads to the selection of stable lines in 4-6 weeks.

Standard DNA transfection methods can be used after stable *Drosophila* cell line is achieved, including calcium phosphate DNA precipitation, lipid mediated transfection (liposomes), and electroporation.

There are several promoter vectors for high-level expression in *Drosophila* S2 cells. Some of them are presented in the following table:

Vector	**Promoter**	**Note**
pMta	Metallothionein	Tightly regulated inducible *Drosophila* promoter, SV40 early poly(A)
pMtaL	Metallothionein	Contains SV40 late poly(A), which is 3 times more efficient than SV40 early poly(A)
pRmHa	Metallothionein	Contains alcohol dehydrogenase poly(A)
pMttBNS	Metallothionein	Contains human tPA signal peptide sequence for directing secretion
pMtBL2	Metallothionein	Contains *Drosophila* BiP signal peptide sequence for directing secretion
pHt4	Hsp70	Inducible expression under heat shock
pDS47SV40	DS47	Strong constitutive promoter from an abundantly expressed gene in *Drosophila* S2 cells, SV40 late poly(A)
pPac	Actin 5C	Strong constitutive *Drosophila* promoter, actin poly(A)
pA5CSV40	Actin 5C	Strong constitutive *Drosophila* promoter, SV40 late poly(A)
pCopia	Copia	Strong constitutive *Drosophila* promoter

An important advantage of the S2 system is the availability of both tightly regulated inducible expression vectors and strong constitutive vectors. Inducible expression under the

tightly regulated Metallothionein promoter (pMT) makes possible the coordinated expression of genes at a very high copy number. It even allows high level production of lethal gene products.

Another important advantage of the S2 system is the ability to secrete proteins over a long period of time. Furthermore, purification from S2 culture supernatants is facilitated greatly by growth in serum-free media conditions.

Advantages of *Drosophila* S2 expression system - an insect expression system:

- High density growth at 25°C without CO_2 supplemention;
- Growth in suspension – no need for trypsinization;
- Inexpensive media – serum-free or supplemented 10% serum;
- Stable or transient expression;
- Non-lytic expression;
- Stable line in 3-4 weeks;
- One-step gene amplification;
- Stable lines amenable to grow in bioreactors for large-scale expression;
- Stable lines can be stored indefinitely at -70°C;
- Regulated expression at high copy number;
- High level constitutive or inducible expression;
- Higher eukaryotic signals for protein folding, processing, secretion, subcellular localization, and glycosylation;
- Membrane receptor expression in stable lines.

Disadvantages of *Drosophila* S2 expression:

- Unable to grow at low density;
- Growth is slower than mammalian cultures;
- Mammalian promoters function poorly in S2 cells;
- Insect glycosylation is simpler in structure than vertebrates, containing high mannose content and lacking sialic acid.

Conclusions:

The continuous *Drosophila* cell lines are mainly attributable to the regulated high copy number expression that can be achieved in just a few weeks. The expressed proteins in this system exhibit authentic properties and functions. This includes membrane-linked, transmembrane, intracellular, and secreted proteins from insects and vertebrate species. The S2 expression system is ideal to study mechanisms of DNA transcription, RNA transport, protein folding, protein secretion, cell adhesion, and receptor function.

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ВР3. РИБА ЗЕБРА
(*DANIO RERIO)*

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Риба зебра (семейство Cyprinidae) е тропическа сладководна риба, разпространена във водите около екватора. Често обитава потоци, канали, езера и бавно движещи се водни обекти, включително оризови полета. Тялото и е пигментирано – именно наблюдаваните характерни ивици са дали наименованието й. На дължина достига 6 см. Жизненият й цикъл продължава 2-3 години, но при подходящи условия на средата може да достигне 5 години.

Риба зебра е много распространена аквариумна рибка. През последните две десетилетия обаче, тази риба не се използва само като декоративен вид - тя е и важен експериментален модел за изучаване структурата на гръбначните животни и техния геном в лабораторни условия. Смята се, че наред с останалите т. нар. организми-модели каквито са плъховете,мишките и жабите, риба зебра ще подпомогне проучванията върху етиологията и патогенезата на редица заболявания при човека и ще допринесе за разработването на адекватни профилактични, диагностични и лечебни подходи. Безспорно, паралелното използване на различни организми-модели предоставя по-пълна и точна информация на учените по интересуващите ги въпроси. .

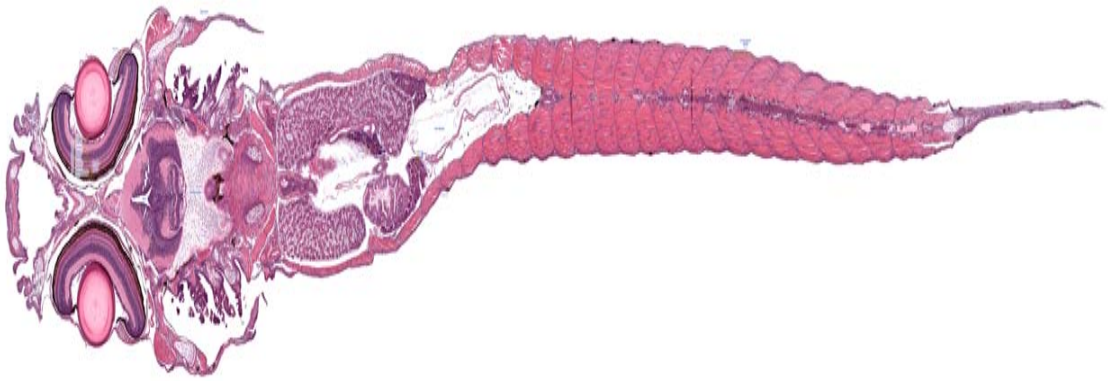
За разлика от останалите лабораторни животни (плъхове, мишки, и жаби и др.), които отдавна са наберили своето място като експериментални модели, рибата зебра е сравнително нов изследователски обект. За първи път тя е използвана като лабораторен модел от Джордж Стрейсинджър – молекулярен биолог от Университета в Орегон, през 70те години на 20ти век. Той установява, че рибата е достатъчно малка, за да позволи поддържане на голям брой индивиди, необходими за провеждането на генетични изследвания. В същото време тя е достатъчно голяма, за да се направи възможно провеждането на различни манипулации, като трансплантация и т.н. В началото д-р Стрейсинджър е подложен на острите критики на своите колеги, което забавя с почти десет години публикуването на първата му книга, озаглавена “Zebrafish”. В нея авторът описва нормалното морфологично и функционално развитие на рибата зебра. Може би най-големите предимства на този животински вид като лабораторен модел се дължат на: лесно четящата се последователност на генетичния й код; безпроблемното й наблюдаване под микроскоп; възможността за ясно отчитане на промени в развитието и поведението й;

наличието на добре характеризирани мутанти. Специално внимание заслужава фактът, че ембрионът на *Danio rerio* е прозрачен, което позволява уникален визуален достъп до вътрешната анатомия и физиология на рибката. Така например, могат да бъдат проследени движението на отделни клетки, развитието и функционирането на органите и ситемите (кожа, кости, мускули, сърце, бъбреци; кръвообращението и централната нервна система). В допълнение, не бива за забравяме, че ембриоталното развитие при тези рибки (в сравнение с жаба, гризачи и др.) е бързо, а ембрионите са по-просто устроени и, както вече споменахме, прозрачни. Женската рибка може да възпроизведе до 200 яйца на седмица, докато мишката износва до 15 ембриони. Освен това**,** за разлика от ембрионите на мишката, които се развиват в организма на майката и тя трябва да бъде убита, за да се стигне до тях, при риба зебра това не е необходимо. При нея проследяването на ембриоталното развитие може да стане във всеки един момент, без това да засегне живота нито на ембриона, нито на майката.

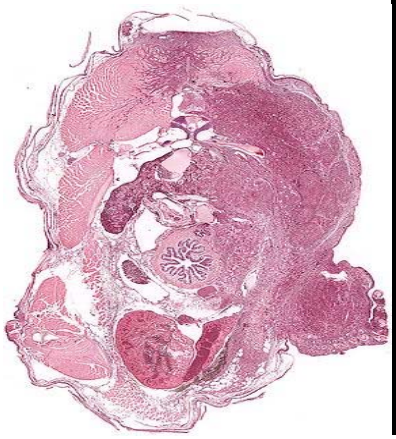
Не без значение е и възможността за лесно предизвикване на мутации в риба зебра, които могат да бъдат отразени на огромни екрани. Тази специална технология за трансфер на гени при риба зебра е силно напреднала. Всичко това е подпомогнало създаването на генетични карти, които са полезни за провеждане на сравнителни проучвания с човешките гени. Установено е наличието на значителна прилика между геномите на риба зебра и човека. Геномът при рибката обхваща 1,700 милиарда бази, което е около половината от размера на човешкия геном. Създадена е Zebrafish информационната мрежа (ZFIN) - он-лайн база данни с информация за *Danio rerio*. ZFIN предвижда интегриран интерфейс за заявки и визуализиране на голям обем от данни, получени от редица проучвания. За да се улесни използването на рибата зебра като модел на човешката биология, ZFIN съдържа информация и за други организми-модели (например мишка), както и база данни за заболяванията при човека, предлага многобройни връзки с външни бази данни. ZFIN се намира в Университета на Орегон в САЩ и е направена с финансови средства, предоставени от Националния институт по здравето (NIH - USA).

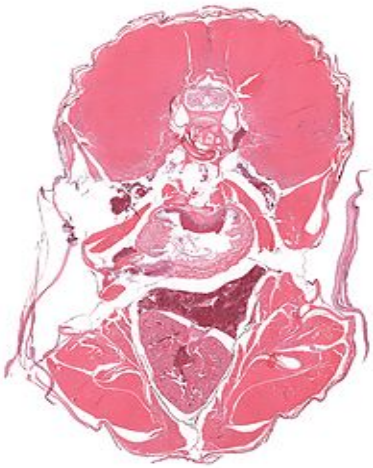
Любопитен факт за риба зебра е, че дори умирайки се грижи за потомството си - тя изпуска особени химически вещества, под действието на които ембрионите започват бързо да се развиват. Тези вещества рибата изработва не само в предсмъртна агония, но и при нараняване. Ихтиолозите вече са изучавали влияние, което тези "тревожните молекули" оказват върху млади и възрастните индивиди, но до момента никой не е анализирал въздействието на тези вещества върху ембрионите. Изследователите от Университета в Плимут провели експерименти с два вида риба-зебра. Както се оказало, под влияние на "тревожните молекули" (събрани от мъртви възрастни индивиди) ембрионите и на двата вида по-бързо. И все пак има известна раздлика - при представителите на вида *Danio albolineatus* се ускорява развитието на сърцето, а при ембрионите на *Danio rerio* започва интензивно образуване на мускули.

В следния сайт може да откриете 3D филм изобразяващ етапите на анатомично развитие при риба зебра : http://zfatlas.psu.edu/movies/4_Day_Fish.m4v



Изследването на Danio rerio позволява значителен напредък при изучаването на раковите заболявания при човека и откриването на терапевтични средства срещу тях.





Индивид с туморно образование Здрав индивид

"Това е най-комплексната система описана до сега" споделя Проф. Гари Литман от “Florida College of Medicine”. Сладководната, аквариумна рибка има гени, които могат да произвеждат молекули за борба против агресивен рак. Генетиката на съвсен безвредната тропическа рибка зебра показва, че имунната й система е силно развита и може да ни „подскаже” научи някои неща за недостатъците в човешката защита против заболявания. Учените са приятно изненадани от възможностите на рибката и се надяват с нейна помощ да намерят пътя към разрешаването на редица важни медико-биологични проблеми, сред които важно място заемат т.нар социално значими заболявания.

Изследванията с участието на *Danio rerio* позволяват постигането на напредък в различни области, като онкология, имунология, токсикология, репродуктивнна медицина, тератология, невробиология, екология, генетика, проучванията върху стволовите клетки и процесите на регенерация, еволюционната теория и др.

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BP4. SEVERE COMBINED IMMUNODEFICIENCY (SCID) MICE –AN EXPERIMENTAL MODEL USED IN VARIETY OF RESEARCHES

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In the recent years an experimental model that gained a widespread popularity among biomedical and pharmaceutical science societies is the SCID mouse. Although there are other species with forms of SCID the immune depleted mice have shown more similarities in biological functions with human. That makes them common model organisms for research in immonology, infections deseases, cancer and stem cell biology. In addition they are useful for examining the safety of new therapeutic agents and vaccines in immunocompromized individuals.

SCID mice are a result of a recessive mutation in Chromosome 16, which leads to a failure of the activity of an enzyme responsible for the DNA repair (Prkdc or „protein kinase, DNA activated, catalytic polypentiole").The lack of V(D)J recombination causes a failure in developing cellular and humoral immune systems. As a result they can not produce T and B lymphocytes or activate some component of the complement system and can not fight against any infection, tumors or transplants.

For more efficient immunocompromised strains to be created SCID mice can be crossed with mice carrying mutations in related genes, such as interleukin-2R gamma. However they can't be used in long –term experiments, because of their short life. Immunodeficient mice develope thymic lymphomas quite often.

Large number of interesting researches includes SCID mice as an experimental model, but a few have drawn our attention on them. Because they give a good example for the different kinds of application of SCID mice. They were used as an animal model of Rheumatoid Arthritis (RA) for observing inflammation, immune reactivity, and angiogenesis. After SCID mice were engrafted with rheumatoid arthritis synovium they were observed for changes in the function or the morphology of the RA synovial in the RA-SCID grafts. The result from these study showed that the RA-SCID model preserves many of the phenotypic and functional features of the inflamed RA synovium. That makes RA-SCID mice an efficient model for the trial of therapeutic agents.

To be clarified the mechanisms of wound healing and transplantation are used SCID mice, which are engrafted with human skin and functional human immune system. SCID mice with transplanted human skin had Gr1+ cells which suppress the survival of human endothelium in the graft. But with anti-Gr1+ antibody the graft endothelium is preserved, wound healing is promoted, leading to tissue development and graft remodeling. It was observed a formation of multilayered stratified human epidermis with well developed vasculature, human fibroblast and passenger leukocytes, caused by the excellent engraftment of the transplanted skin. However, the injection of CD4 or CD8 human peripheral blood mononuclear cells in these mice causes the rapid disruption of the grafted skin.

Among the various research areas where SCID mice are used are immunology and experimental oncology. One interesting example is the discovery of the lymphocyte- activating monoclonal antibody induced regression of human tumors. Monoclonal antibodies (BAT) were selected due to their ability to stimulate human lymphocyte proliferation. Then they were applied to mice with different kinds of tumor, such as B16 melanoma, 3LL carcinoma and methylcholanthrene fibrosarcoma. The observed antitumor effect was tremendous. But in order to prove the role of the BAT alone in the induced cytotoxity against tumor cells were used SCID mice. As they lack T lymphocytes and NK cells the injected BAT caused only a slight but

significant antitumor effect. However that only proves the dual role of T lymphocytes and NK cells in increasing the BAT antitumor activity. Another strain of SCID mice was engrafted with human lymphocytes (human –in-mice model) and then xenografted with human melanoma. In these mice the applied BAT showed again a regression of the tumor cells.

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BP5. TRANSGENIC RABBIT AS AN EXPERIMENTAL MODEL FOR THE STUDY OF LIPID METABOLISM

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There are no ideal models and every model utilized has both advantages and disadvantages. The rabbit is a medium-sized animal that has many cellular and molecular characteristics very much like human. It is an important model for the study of the relationship between plasma cholesterol metabolism, atherosclerosis and heart diseases. To provide new experimental tools, transgenic rabbits have been generated in which individual genes involved in plasma lipoprotein metabolism have been overexpressed.

A construct of the human growth hormone gene directed by the mouse metallothionein promoter was used to investigate transgenic methods in different species by examining construct expression in fetuses and neonates. The transgene was integrated into the rabbit genome. Transgene mRNA was expressed in 25% of the rabbit founders, and one live founder exhibited human growth hormone in serum. This technology is used to the study of lipid metabolism and there are established several transgenic rabbit models for the study of atherosclerosis.

The hepatic lipase transgenic rabbits had reductions in plasma lipid levels compared to normal animals, with total cholesterol decreased up to 40% and total triglycerides decreased by 60% and the lesions in the ascending aorta and the aortic arch of the transgenic rabbits were significantly thicker than in the normal rabbits.

Apolipoprotein E transgenic rabbits that overexpress human apoE had a high level of accumulation of of LDL which might be due to a reduction in LDL receptor activity as a consequence of an increased cholesterol uptake into the liver by VLDL. The predominance of atherogenic IDL might facilitate the initiation of an atherosclerotic process and the increased level of circulating apoE in the rabbit minimizes the deposition of lipid in the artery wall.

Apolipoprotein B mRNA-Editing protein (APOBEC-1) transgenic rabbit is generated by a cDNA encoding rabbit APOBEC-1 in a liver-specific expression vector. The editing factor is found in the intestine but not in the liver of the nontransgenic rabbit, making

this animal species a superb model to investigate the role of apoB editing in lipoprotein metabolism. The transgenic rabbits had significant decreases in VLDL, IDL, and LDL accompanied by an increase in HDL.

An important model for human familial hypercholesterolemia, the Watanabe heritable hyperlipidemic (**WHHL**) rabbit, show hypercholesterolemia due to deficiency of LDL receptors, and very similar lipoprotein metabolism to humans. The incidences of coronary atherosclerosis and myocardial infarction in the original WHHL rabbits were very low. After three rounds of selective breeding, the coronary plaques changed to fibroatheromas with thin fibrous caps and myocardial infarction developed spontaneously. At the opposite end of the spectrum, a partially inbred line of cholesterol-resistant rabbits has been established that does not readily develop atherosclerosis. This may be a result of an enhanced production and secretion of bile salts due to elevated levels of 7α-hydroxylase mRNA.

Transgenic animals will bring new insights into the mechanisms that contribute to the development of various diseases.

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ВР6. ИНУЛИН

Мария Рогова, Красимира Карталова, Тоня Гевара

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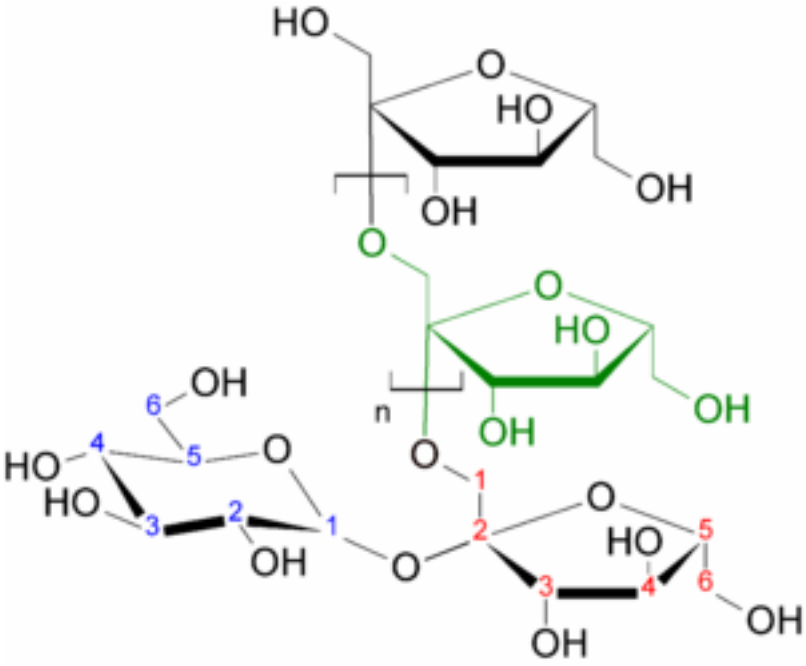
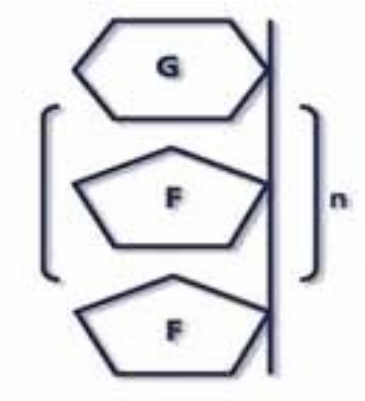


Инулин са 100% разтворими растителни фибри, които се откриват само в растенията и вече хиляди години са част от нашето хранене.
Този продукт е на растителна основа и се среща в корените на растенията от семейство *Asteraceae*, като за промишлени цели се екстрахира от пастението *Cichorium intybus*, където се среща в най-голяма концентрация.

Инулинът е съставен от фрукто олигозахаридни и полизахарид-ни вериги, Химичния състав на инулин - екстрахиран от цикория е G(F)n ,където n варира от 2 до 60 (G=глюкоза, F=фруктоза).



Хранителни качества:
- източник на разтворими фибри;
- пребиотични и здравословни свойства;

- заместител на захарта и мазнините;
- ниска калорична стойност (1.5 kcal/g);
- подходящ за диабетици.
- Напълно натурален продукт
- Няма Е-номер.

Технологични качества:
- структуро-определящ компонент;
- идеална разтворимост;
- подобрява вкуса;
- замества захарта и мазнините;

Приложение:
- при проблеми с храносмилането и за укерпване на имунната система;
- подобрява адсорбцията на минералите и така спомага за заздра-вяването костите;
- контролира телесната маса при Диабет 2.

Препарати съдържащи инулин:

Frutafit инулин, заместители на кафето като ‘Инка’, Инулин Форте® и много други.

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Session C.

Chairpersons:

Assoc. Prof. Anna Tolekova, MD, PhD
Faculty of Medicine, Trakya Universit, Stara Zagora

Assist. Prof. Dimitar Ivanov, PhD
Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences

CO1. SIALYLTRANSFERASE ACTIVITIES OF ZAJDELA HEPATOMA CELLS

D. Ivanov, R. Gavazova

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Sialyltransferases (ST) catalyse the transfer of N-acetylneuraminic acid from CMP-β-Neu5Ac onto carbohydrate groups of glycolipids and glycoproteins. More than 10-12 ST are required to synthetize all the sialooligosaccharide sequences known.

In eucaryotic cells, Neu5Ac (sialic acid) occurs essentially as terminal sugar in α 2,3 and α 2,6 linkages onto terminal Gal residues of N-glycans of the N-acetyllactosamine tipe. Sialic acid is a general term for a family of unique 9-carbon monosaccharides. The most common form of sialic acid form in humans is *N*-acetylneuraminic acid Neu5Ac.

Subcellular localization of the ST has been extensively studied, with preferential localizations in the Golgi apparatus. However, these localizations are not exclusive as ST have been described in the mouse liver mitochondria outer membrane, rat liver nuclei and synaptosomes. Ectosialyltransferases have been described in platelets, lymphoblastoids cells and neuronal cells.

In the Golgi apparatus, ST such as the Gal α 2,6 ST appear generally localized into the trans Golgi cisternae and the trans tubular network in a luminal orientation.

Neoplastic transformation of cells has been known to be accompanied by changes in the activity of glycosyltransferases, which transfer sugar residues from "activated" nucleotide-sugar or lipid sugar donors onto growing proteins, glycoproteins or glycolipids. The level of several glycosyltransferases and especially of the ST is abnormal in various malignant cells. Difference in the ST activity in normal and transformed cells is the subject of controversy. Levels of the ST have been reported to be elevated or depressed in cells transformed by viruses. The reasons for most of these differing results are not clear, but they probably reflect differences in function of the enzyme, clonal differences in the established cell lines utilized, method of the assay used, including suitability of acceptors.

Sialyltransferase activity could be studied against different acceptors-desialylated glycoproteins in which the terminal monosaccharide residue is galactose or lactosamine.

The use of different acceptors contributes to obtain information about the predominant isometric enzyme form, catalyzing the sialylation process by forming specific type of linkage - α 2,3 or α 2,6.

Hudgin and Chachter have detected two forms of this enzyme catalyzing formation of sialyl-α (2,3) and sialyl-α (2,6) lactose in liver from rats, pigs, beef and humans. Tsuiki and

Miyagi have described two forms of Gal β1-4GlcNAc α2-6 ST sialylated (transferase I) in rat liver with asialofetuin as an acceptor and the second which is unsialylated (transferase II). In rat hepatomas only sialylated Gal β1-4GlcNAc α2-6 ST identical with transferase I was established. Miyagi et al. using asialoorosomucoid as acceptor, have observed increased Gal β1-4GlcNAc 2-6 activity in different rat hepatomas when compared with host liver, while the activity of Gal β1-3GlcNAc α2-3 ST and ST acting on sialo-bovine submaxillary mucin were decreased in hepatomas. The activity of ST with asialofetuin as an acceptor in normal and host liver and in Zajdela hepatoma cells was decreased in the tumor cells in comparison with normal liver.

We tried to study the individual ST in Zajdela hepatoma cells using the lactose as an acceptor and the results obtained were compared with those control liver. The results of these experiments, show that in liver and in hepatoma homogenates, the ^{14}C-labelled α (2,3) and α (2,6) sialyllactose isomers were identified. It is worth nothing that in the liver, as well as in Zajdela hepatoma cells, the sialyl-α (2,6) lactose was the predominant isomer synthesized and the values of the ratio between the liver and hepatoma enzyme activities attaching Sialic acids in α (2,3) linkages to those attaching the same residue in α (2,6) linkages, were at the same order of magnitude. A suggestion could be made that neoplastic Zajdela hepatoma cells there is an expression of the two CMP-N-acetylneuraminic acid: lactose ST, as it is observed in the parental liver cells, but it tumor the levels of the individual and total enzyme activities are considerably lover than in liver.

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CP1. INFLUENCE OF ELECTRICAL PULSES ON ADHESION BEHAVIOR OF TUMOR AND SOMATIC CELLS

V. Pehlivanova, V. Krasteva, R. Tzoneva, I. Tsoneva

Electroinduced effects in biomembranes, Institute of biophysics, Acad. G. Bonchev str., bl. 21, Sofia 1113

Induced electric fields, both direct and altered are increasingly used in medicine and biothechnology. One of the applications of high-voltage electrical pulses is so-called electrohemotherapy: high voltage pulses and the process electroporation which creates tension on the membrane, leading to electric break and accelerate the penetration of the drugs, DNA or proteins into the tumor cells [1-3]. The influence of electrical pulses with high field intensity on the effectiveness of anti-tumor drugs introduction into the cell is well studied. But the effect of electric filed on adhesive behaviour of cancer and somatic cells is not so well studied.

Cells: Two cell lines from breast cancer were used: MDA-MB-231 - invasive and metastatic cancer cells and MCF-7 - fast-growing noninvasive tumor cells. 3T3 mouse fibroblasts are used as a control.

Electroporation: with "Chemopuls-best" apparatus produced in the Central laboratory for biomedical engineering "Prof. Ivan Daskalov" (CLBME) – BAS, Sofia.

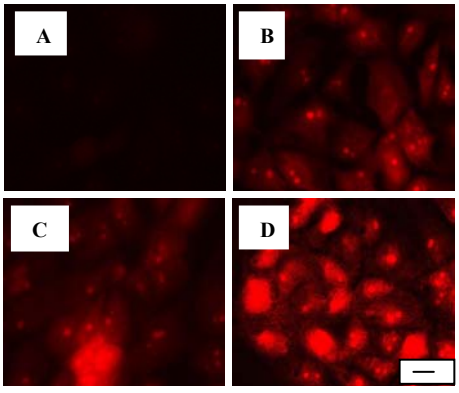
Parameters of electrical pulses: 8 biphasic pulses; 50+50μs, 1ms interval between the phases, frequency of the pulses 1 кHz, 250-1000 V. Induction of 200 – 1000 voltage in the two parallel steel electrodes (length – 22 mm and 10 mm space/distance between them) produces electrical field with intensity of 200, 500 and 1000 V/cm.

Actin staining *(BODIPY 558/568 Phaloidin):* 3T3, MDA-MB-231 и MCF-7 with cell density of 1.5 x10^5 are cultivated on cover glasses (18/18mm) placed in 6 well plates. After 24 hours incubation cells are electroporated in basal cell medium and cultivated additionally for a period of 2, 24 and 48 hours. After the incubation period non-adhesive cells are removed by triple rinsing with PBS, pH 7.3. Adherent cells are fixed by 1ml 3% solution of paraformaldehyde for 15 minutes at room temperature. Fixed cells are permeabilised by 1ml 0.5% solution of Triton X -100 for 5 minutes and then incubated with 1ml 1% solution of BSA (bovine serum albumin) for 15 minutes. Samples are washed with PBS (pH 7.3) three times, and then incubated for 30 minutes at room temperature with BODIPY 558/568 phalloidin. Again washed three times with PBS, pH 7.3 and washed once with distilled water, then installed on object glasses using Mowiol. Preparations are analyzed on fluorescent microscope (Leica, Germany).

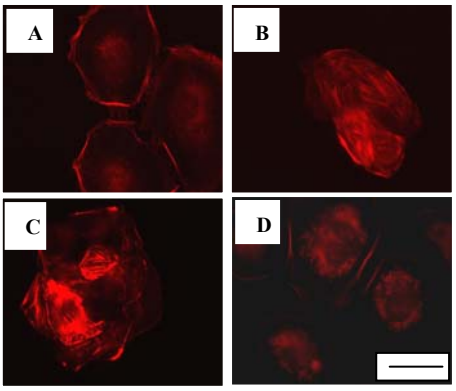
Conclusions: The influence of electropration on the cell adhesion is cell specific. While the invasive cell type responds with increased adhesion, the non - invasive type and somatic cells respond with lowering the cell adhesion. Under the influence of the electric field, the invasive cell type formed well both cell - substrate and cell - cell contacts, while non-invasive cell type increased the formation of intracellular contacts. We could hypothesize that applied electric fields lead to a change in cellular phenotype - a reduced mobility leading to reduced invasiveness.

Acknowledgments: We gratefully acknowledge for the support from The Bulg. Nat. Fund (Grant D0-02/178)

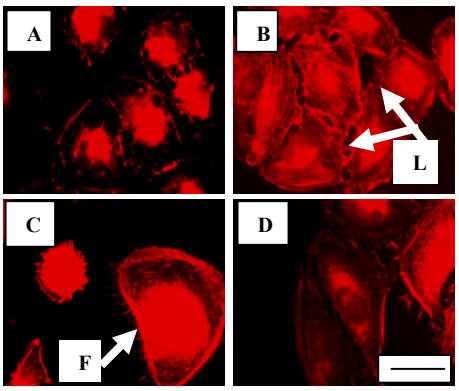
Fig. 1 A. Chemopuls - best apparatus. B. Characteristics of the used electrical pulses



3T3 cells electroporated in the presence of propidium iodide and incubated for 15 minutes. (A) control sample (non-porated cells); (B) cells electroporated at 200 V/cm; (C) at 500 V/cm; (D) at 1000 V/cm. The bar is 40μm.



MDA-MB-231 cells incubated 2 h after electroporation (A) control; (B) electroporated at 200V/cm; (C) at 500V/cm; (D) at 1000V/cm. and stained for actin. The bar is 50µm.



MCF-7 cells incubated 2 h after electroporation (A) control; (B) electroporated at 200V/cm; (C) at 500V/cm; (D) at 1000V/cm. and stained for actin. and stained for actin.. L- lamellopodi; F- fillopodi. The bar is 50µm.

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CP2. INFLUENCE OF ELECTRICAL FIELD ON THE ADSORPTION OF ADHESIVE PROTEINS ON BIOMATERIALS

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Cardiovascular disease is the leading cause of death in the western countries [1] as well as in Bulgaria [2]. Currently available synthetic vascular grafts are limited to large internal diameter (>5 mm) grafts because of frequent thrombosis and occlusion. To overcome these limitations, the scientists and engineers explored a tissue engineering approach to construct small-diameter vascular grafts using new materials and endothelial cells in combination with adhesive proteins [3]. The approach of tissue engineering consists of creating a scaffold which combines the new nano-fibrous polymer materials futures and those of extracellular matrix (ECM) proteins. And so called ECM-mimicking tissue-engineered scaffold will contribute to regeneration of the vascular tissue.

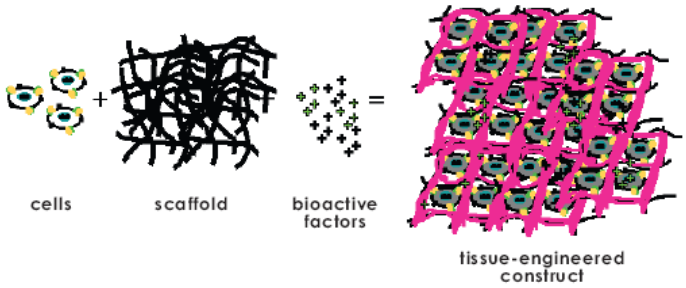

Fig.1: Tissue engineering approach for tissue regeneration. (www.bioeng.nus.edu.sg/research/keyrsch1.htm).

Many biological systems react to applied electric field. For instance, applied alternating electric field influences the quantity and the spatial orientation/conformation of the adsorbed proteins to the solid surface [4]. Subsequently that changes in protein organization leads to shifts in their biological function [5]. Our goal is to create artificial (biomimetic) extracellular matrix using biodegradable polymer nanofibers (synthetic component) and adhesive proteins (biological component) by applying external electrical field. In this respect we investigate the influence of applied electrical field on protein adsorption.

Materials: We used different polymer materials from polyetherimide (PEI) namely: PEI-membrane, PEI-foil, and two nano-fibrous materials with different size of fibres (PEI-1, PEI-2) – all materials are produced at GKSS Research Centre, Teltow, Germany. As a control is used glass surface.

Device for electrotreatment: The device which is new developed in Institute of Biophysics – BAS Bulgaria, is supplied with two graphite electrodes at distance of 1 cm which are connected via stainless plugs to the programmable stimulator (Protek 9205 C, Steinberger, Germany). The outer part of the device is made by Teflon and glass for easy sterilization.

Fig.2. Device for electrotreatment

Method for estimation of adsorbed FNG For electrical stimulation experiments material lists were cut into 1.5cm x 6cm rectangles and placed along the electrodes. Protein adsorption was carried out as FNG in concentration 0,2 mg/ml was added to polymer material at RT for 1 h. under sinusoidal electrical field, frequency - 1Hz, 10Hz or 100 Hz, at 7 V/cm electrical field intensity. Protein concentration is defined spectrophotometrically by Bradford standard assay at 595 nm, 1cm path length cuvette.

Results: The results obtained for FNG show that:

- More FNG is adsorbed in the absence of EF.

- High frequency of applied electrical filed strongly decreases protein adsorption.

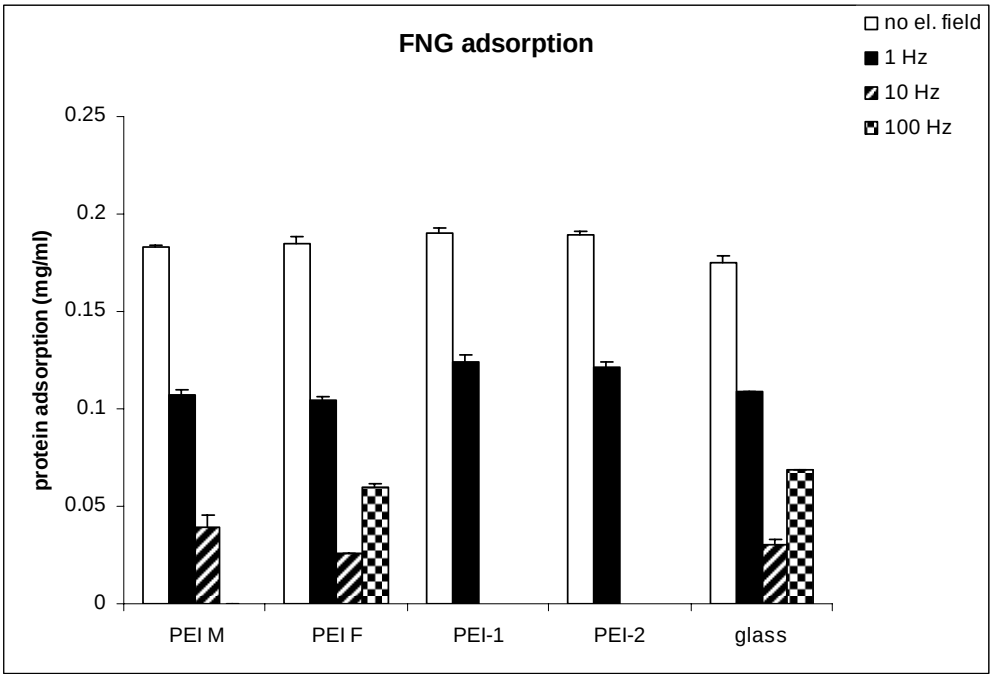

Fig. 2 FNG adsorption to polymer materials under electrical field with different frequency (sinusoidal electrical field, 7 V/cm, 1h, RT).

Conclusion: FNG is the major protein involved in thrombus formation to biomaterials. Applying an alternative low intensity electrical filed to the adsorbed protein layer results in decreasing the adsorbed protein with increasing the frequency of the filed. We could hypothesize that on this way could be formed an antithrombotic surface. Further investigations are needed to confirm that findings.

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CO2. APPLICATION OF THE METHOD OF HEMODIALYSIS FOR EXPERIMENTAL PURPOSES

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The apparatus "Artificial Kidney" is one of life-saving innovations in medicine. Thanks to it, patients with acute or chronic renal insufficiency and those, who for one or another reason need a blood purification from harmful toxins get a chance to continue living. This process is named “hemodialysis” and its principles could be developed to the needs of scientific experiments. On this basis, several methods have been established, one of which is the model of isolated kidney.

This system enables investigation of kidney functions and their change under the influence of various pharmacological and physiological stimuli. It is mainly developed through experiments with mice and rats, but the system is universal and can be used for larger experimental animals. The difference between the hemodialysis and the experimental model is in the object of the dialysis: in the hemodialysis we worked with whole body and blood; in the experiment there is an isolated organ which is not only dialysed but also perfused with a blood like substances.

CP3. IN VITRO RADICAL SCAVENGING ACTIVITY OF MELANOIDINS MEASURED BY DPPH METHOD

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Reactive oxygen species (ROS) are formed during the intermediate steps of oxygen reduction (superoxide anion radical, hydrogen peroxide, hydroxyl radical) or lipid oxidation (alkoxyl or peroxyl radicals). Endogenous sources of ROS are environmental pollutants, drugs and other xenobiotic compounds. Increased production of ROS which cannot be counteracted by the naturally occurring antioxidants (antioxidant enzymes and some vitamins) is termed as oxidative stress. The involvement of free radicals, ROS, and the oxidative stress in the development of degenerative diseases associated with aging (cancer, cardio-vascular diseases, immune system decline, Parkinson disease, Alzheimer disease, cataract) has been repeatedly documented by researchers across the world (Calabrese et al., 2010).

On the other hand ROS are well known as stimulators of intracellular signal transduction (so called redox signaling). Various biological processes (including cell growth, apoptosis, cell adhesion, and HIV activation) are presumably stimulated by ROS. Oxidant-induced Ca^{2+}-signaling and protein phosphorylation are particularly important because Ca^{2+} is a widely used second messenger that regulate muscle contraction, neurotransmission, gene transcription, and cell growth (Suzuki et al., 1997).

Currently, the use of antioxidant molecules, especially naturally occurring ones, in foods as preventive and therapeutic medicines is gaining popularity. Melanoidins, the brown pigments originated from amino acids or proteins and carbohydrates, and formed during food processing and storage, also possess antioxidant properties (Wagner, K.-H. et al.; Morales F.J. et al.).

In previous studies (Stefanova et al.; Argirova et al.) we have shown that model melanoidins obtained from different amino acid/carbohydrate systems influence contractile activity of rat gastric smooth muscles. The results obtained demonstrated that the most active towards the mechanical activity of gastric smooth muscles were the model melanoidin obtained from glycine and ascorbic acid (Gly-AsA) that evoked muscle relaxation and the model melanoidin obtained from arginine and glucose (Arg-Glc) that caused contraction. Melanoidins isolated from coffee brew also evoked muscle contraction. We tried to clarify the mechanisms underling this activity.

The present study investigates the antioxidant properties of these three melanoidins in order to better characterize their biological properties.

Antiradical activity assay is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Brand-Williams W. et al.). Due to the presence of an odd electron this compound gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured.

When melanoidins are added to the solution containing DPPH, the discoloration of the DPPH radical has to be based either: a) on a combination with melanoidin radicals or b) on a charge transfer induced in melanoidins which may result in hydrogen transfer: Upon reduction, the solution color fades and typically reaches steady state within 30 min.

Because of different kinetic behavior, multiple mechanisms involved in reagent bleaching as well as DPPH instability, the results for radical scavenging capacity are arbitrary compared to the antioxidant power of Trolox, a vitamin E water-soluble analog, and presented as nmol Trolox having the same radical scavenging ability as 1 mg of product of interest. The higher Trolox equivalents, the higher radical scavenging capacity.

Dose-response curve was built for the standard antioxidant Trolox within the concentration range 5 – 25 nmol. Melanoidin samples were tested as 20-ul aliquots taken from concentrated stock solutions. Each melanoidin assay was run in triplicates. Regression analysis was used to calculate the antiradical activity of each sample presented as Trolox equivalents.

The results obtained for the melanoidin Arg-Glc and coffee melanoidin were in the same of magnitude as those obtained for other model melanoidins (Rufian-Henares J.A., Morales F.J.). However, the antiradical capacity of the melanoidin Gly-AsA was one order of magnitude higher than the capacity of other melanoidins. Paramagnetic studies (Wu et al., 2006) have shown that several model melanoidins possess extremely stable free radicals. The positive relationship between the intensity of signals in paramagnetic spectra and the antioxidative effects of melanoidins allows speculating that the major mechanism for the antioxidative effects is the combination between two free radicals. It is quite possible however, the good reducing capacity previously found for the tested melanoidins, to contribute to overall antioxidant capacity, especially for the melanoidin obtained from ascorbic acid.

These findings give a new perspective in studying the mechanism of contractile and relaxation mechanical reaction of the melanoidins on smooth gastric muscles.

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CP4. MANIFESTATIONS OF TACRINE-INDUCED CHOLINERGIC RECEPTORS SENSITIZATION IN RAT'S STOMACH SMOOTH MUSCLES

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The alteration of receptors sensitivity in terms of their natural ligands or relevant drugs is an important experimental and therapeutical problem. Receptor-sensitization/desensitization is defined as a primary independent pharmacological reaction, connected with an increased/decreased sensitivity of receptors towards respective agents.

The aim of the present study is to find out a similar affect of cholinesterase inhibitor tacrine on the cholinergic receptors.

Other members of this drug class possess similar influence (1). Tacrine is a centrally acting drug used to treat dementia associated with Alzheimer's disease (2). Acetylcholine is one of the several neurotransmitters in the brain, used by neurons to communicate one another. Increased level of acetylcholine in the brain is considered responsible for improvement in disturbed cognitive processes.

The drug has some side effects especially in the gastrointestinal tract of patients and experimental animals: abdominal pain, nausea, vomiting, diarrhea, delayed evacuatory function (3, 4). Because of that the contractile activity of smooth muscle (SM) preparations from rat's stomach were used as a model for investigation of sensitization/desensitization processes. A part of the tacrine side effects cannot be explained by the inhibition of acetyl- or butyril cholinesterase activity. Probably they these effects are a result of non-anticholinesterase tacrin-induced reactions.

The strength and character of drug-induced effects were determined by registration of isometric contractions.

Concentration of 1.10^{-8} mol/l tacrine (1.10^{-8} mol/l) was used. This concentration does not diminish cholinesterase activity (5) and it has not significant effect on the SM contractile activity. Thereby the problem of determining the contribution of anticholinesterase- and sensitizing action of tacrine in acetylcholine-induced contractile effects was overcomed.

After a previous 30minutes tacrine incubation a tendency for increase of contractile effectiveness of exogenous acetylcholine (1.10^{-6} mol/l) was observed. It proceeds to a

significant alteration after either 60 or 100 minutes of incubation. The consecutive treatment of SM preparations with acetylcholine only does not induce one-way significant alterations in the acetylcholine – evoked contractions strength.

In presence of tacrine (60 or 100 min) acetylcholine-induced contractions are significantly higher than caused by equimolar concentration acetylcholine got after a change of Krebs solution and recovery of the SM tonus and spontaneous contractile activity.

These results manifest a particularity, typical for the sensitization of cholinergic receptors by others cholinesterase inhibitors (6) – the augmentation of acetylcholine-induced reaction, caused by the sensitization, significantly diminishes after drugs removal from tissue bath.

In conclusion 1.10^{-8} mol/l tacrine increases the contractile effectiveness of exogenous acetylcholine (1.10^{-6} mol/l). The observed effect is not connected with basic, anticholinesterase drug action. It is revealed only after a previous incubation with tacrine (60 or more minutes). The effect may be interpreted as a manifestation of cholinergic receptors sensitization, caused from tacrine.

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CO3. TRICHINELLOSIS – A UNIQUE CHALENGE TO THE ADAPTIVE PROPERTIES OF STRIATED MUSCLE CELL

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With regard to their morphological, physiological and biochemical properties, skeletal muscles represent a unique tissue with widely documented adaptive capability, which reflects to its diverse pathology. Any physiological or pathological change of the usual environment of the myofiber could lead to one of three distinct conditions: 1) hypertrophy, 2) necrosis/degeneration with subsequent inflammation, repair and scar tissue formation, and 3) atrophy, often associated with apoptosis (as reviewed by Bourne, 1960; Huard et al., 2002; Adhihetty and Hood, 2003).

Among all known myopathies, the establishment of nurse cell-parasite complex in result of infestation by the parasitic nematode *Trichinella* is a unique event. This structure derives

from a portion of the striated skeletal muscle cell and develops within 15 to 20 days after larva of *Trichinella* invades the cell (Bruschi and Murrel, 1999). After penetrating the myofiber, the larva induces morphological, functional and enzymatic changes. In result, the occupied portion of the muscle cell transforms toward unknown so far structure called a nurse cell, which is capable of supporting the parasite for years (Despommier, 1998; Bruschi and Murrel, 1999).

Our work is focused on the mechanisms that serve the capacity of striated myofiber to respond the needs of the invasive parasite without any effort to defend. We aim to characterize this dramatic phenomenon in two parallel aspects:

1. The role of the process of apoptosis in the time course of the nurse cell formation with focus particularly on the mitochondrial Apoptosis inducing factor (AIF) and its relationship with other apoptosis related factors some of which are Bax, Bcl-2 and caspase-3;

2. The changes of glycosylation within the occupied myofiber, their ultrastructural localization, molecular identification and biological significance.

For the needs of our work, we induce asynchronous experimental infestation on BALB/C mice with the parasitic nematode *T. spiralis* (ISS03). The experimental procedures are designed to span the very early period of muscle infestation (10 days post infestation, d.p.i.) over the time course of striated myofibers de-differentiation (14-16 d.p.i.) up to the end of nurse cell formation (45 d.p.i.).

As shown by immunohistochemistry, in the context of low Bax and caspase-3 expression and strong AIF release in the sarcoplasm and translocation to the nucleus at the very early stage of infection, we suppose that AIF-mediated and caspase and Bax independent signaling is involved in the apoptosis activation in the area of *Trichinella* occupation. In the time course of nurse cell formation Bax, Bcl-2 and caspase-3 migrate into the enlarged nuclei but in the end of encapsulation of *Trichinella*, caspase-3 and AIF disappear. It seems that up-regulation of certain factors of apoptosis may be implicated in the mechanisms of de-differentiation of the occupied muscle cell rather than in the processes leading to its death. Our future work aims to establish the kinetics and the time sequence of expression of these and other apoptosis related factors during the process of transformation of the myofiber.

Currently, we demonstrate very early changes in glycosylation of striated muscle cell within the area of occupation. On one hand, an increased expression of glycoconjugates was found, which are reactive to three different lectins, specific for sialic acid (SiA). This phenomenon seems to be a characteristic of the nurse cell since it remains stable even after the transformation is completed. In attempt to identify these SiA-modified glycoconjugates, protein samples from mouse striated muscle tissue, previously invaded by *T. spiralis*, were analyzed using a proteomic approach. Based on the most significant score, three different proteins were identified so far, which are involved in the glucose uptake and regulation of the calcium transport. However, none of them is likely to bear SiA-modification. Further studies are necessary to identify the nature of this sialylated glycoconjugate.

Additionally, a coarse pattern of staining with *Helix pomatia* agglutinin (HPA), which binds terminal O-GalNAc residues, was demonstrated. It seems to be a characteristic only of the early stages of transformation of the occupied striated muscle cell and identification of its ultrastructural localization within the myofiber is on focus of our further work.

Taken together our data and the results of many other research teams propose the nurse cell-*Trichinella spiralis* complex as an excellent model to explore the broad and still unidentified adaptive properties of the striated muscle tissue.

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CP5. PLASTICITY IN BEHAVIORAL DEVELOPMENT AND FLIGHT MUSCLE CONTRACTILE PROTEINS OF HONEY BEES

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Honey bee workers typically switch from jobs inside the colony to collecting nectar and pollen at three weeks of age. However, bee behavioral development is plastic, if colony conditions require, bees may initiate foraging as early as one week of age. Endocrine and neural developments of precocious foragers are indistinguishable from typical foragers. However, there is accumulating evidence that precocious foragers are not as successful as typical foragers. We tested the hypothesis that flight muscle development differs between precocious and typical foragers, explaining performance differences. We measured nectar load, wing beat frequency, and contractile protein profile of typical and precocious foragers. Our results showed that older typical foragers collected significantly more than younger typical foragers. Typical foragers collected significantly more nectar than precocious foragers. Age effects wing beat frequency. In comparison of wing-beat frequency, directly related to muscle contraction rate, there was a significant increase with age. Youngest foragers of 7 days of age had a wing beat frequency between 100 and 150 per second; where as the oldest foragers of 27 days of age had wing-beat frequency between 200 and 250 per second. When we examined the flight muscle contractile protein profile in a SDS-polyacrilamide (12%) gel, we found 7 areas that are different among 1 day old bees, nurses, new foragers and experienced foragers.

Session D.

Chairpersons:

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DO1. ASSOCIATION OF FOKI POLYMORPHISM OF THE VITAMIN D RECEPTOR (VDR) GENE WITH BONE MINERAL DENSITY (BMD) IN A BULGARIAN POPULATION SAMPLE

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Objective: The aim of this study was to search for possible association of low bone mineral density (BMD) with the FokI polymorphism of the vitamin D receptor (VDR) gene in Bulgarian population.
Patients and Methods: 400 Bulgarian women and 74 Bulgarian men participated in this study. BMD was measured at the lumbar spine. Four subgroups were identified: women with low BMD (n=220, cases), women with normal BMD (n=180, controls), men with low BMD (n=44, cases) and men with normal BMD (n=30, controls). The genotype frequencies FF, Ff, ff were investigated by PCR and electrophoresed through an agarose gel after enzymatic digestion of the PCR products by FokI.
Results: The genotype frequencies were 54 % for the FF, 40 % for the Ff and 6 % for the ff genotype in women controls, and 25 %, 48 % and 28 % in women cases, respectively. The genotype frequencies were 43 % for the FF, 57 % for the Ff and 0 % for the ff genotype in men controls, and 20 %, 41 % and 39 % in men cases. The different genotypes were significantly associated with BMD. The relative risk for low BMD was high for the FokI marker (RR=3,67). The association between low BMD and the polymorphism under study was described by an etiological factor (EF) of 0,55.
Conclusions: The specific FokI polymorphism of the VDR gene is associated with low BMD in Bulgarian population. It might therefore be useful genetic marker in osteoporosis risk assessment in our population.

DO2. MALDI-TOF MASS-SPECTROMETRY IN HIGH-THROUGHPUT SNP GENOTYPING

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Introduction of high-throughput methods became cruicial in genetic variation studies in postgenome sequencing era. About 4 000 000 SNPs, considered to be the hallmark of genetic diversity in human population, were identified (1). Genetic variations are linked to simple and complex diseases etiology, as well as to the predicted individualization of therapy or diversity in drugs response. Therefore, application of high-troughput analysis is of major importance for genetic studies and pharmacogenetic research in predisposition to diseases.

MALDI-TOF MS (Matrix Assisted Laser Desorbtion/Ionisation Time-Of-Flight Mass Spectrometry) SNPs analysis is considered as the most powerful and reliable (1). In general the mass spectrometry is based on the production, separation and detection of gas phase ions. The sample is embedded in the crystline structure of small organic compounds (matrix) and further irradiated with a laser beam resulting in desintegration of the crystal. The laser energies cause structural decomposition and generates a cloud of particles from which ions are extracted by an electrical field. After acceleration, the ions drift through a field-free path (1-2 meters) and finally reach the detector. Ion masses (mass-to-charge ratios) are typically calculated by measuring the time-of-flight (TOF) reaching several microseconds, which is longer for larger compared to the smaller molecules (2).

The method requires multiple steps prior to the direct messurement. Products covering the SNPs regions are amplified by multiplex PCR, followed by inactivation of the remaining dNTPs and primer extention reaction (PEX-reaction). The specifically designed extention primers are annealed next to the SNP site and allele specific terminated extension fragments are generated in the presence of ddNTPs (Fig. 1). Due to the nucleotide status of SNP, a shorter or longer extension product is generated. In case of heterozygosity both products are generated. The two SNP alleles appear as two distinct signals, due to the precise separation by their respective different small molecular masses (2, 3).

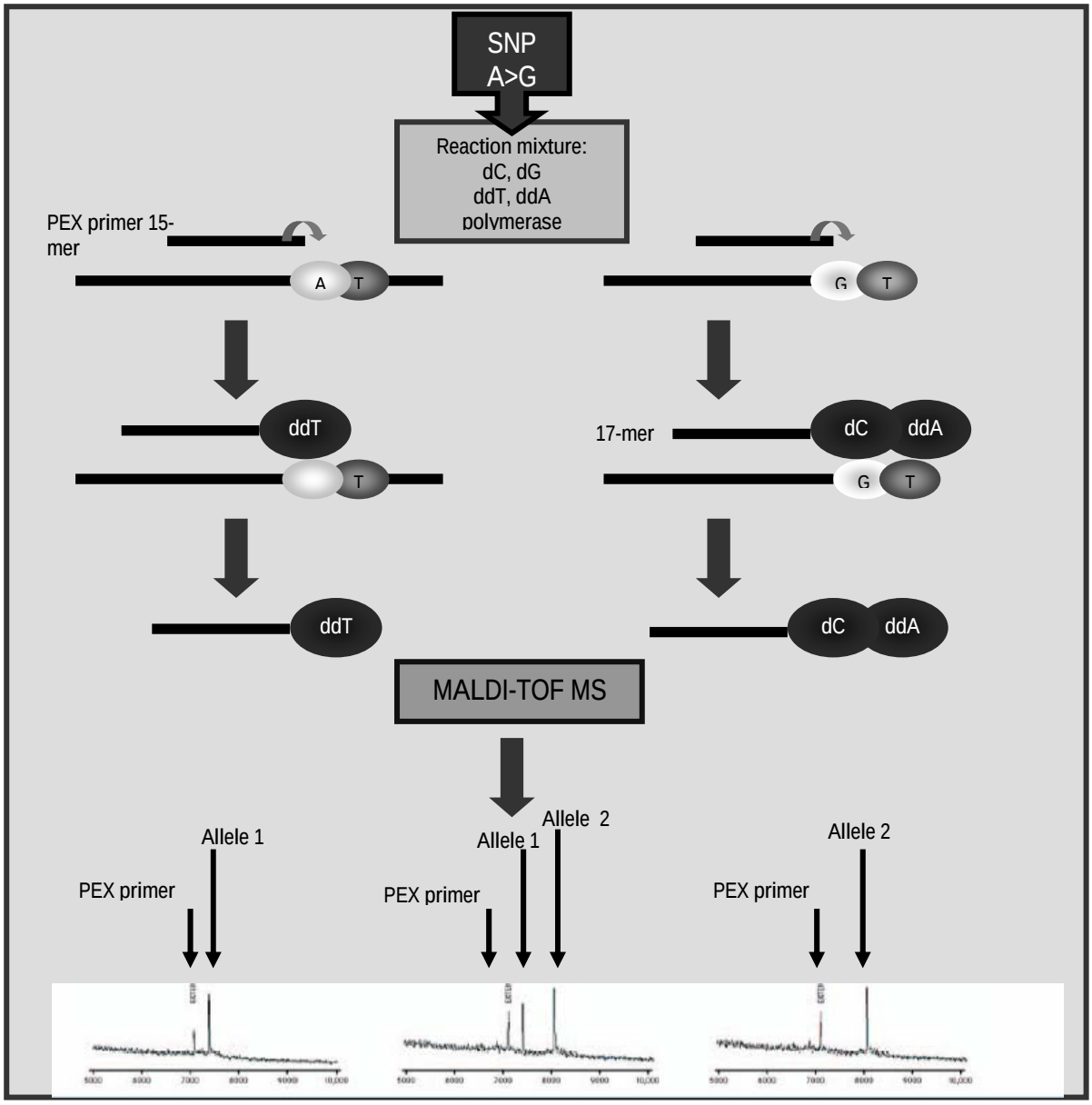
Among the advantages of MALDI TOF thechnology is its analytical accuracy (about 0.1 to 0.01% of the determined mass), resulting in the fact that the mass spectrometric based methods have been reffered to as a gold standart for SNPs analysis. The analytical accuracy of the method allows a 15mer DNA (4500 Da) to be unambiguosly distinguished from a 16mer with approximately 300 Da or 6.6% higher mass. The method is based on the direct measurement of masses and the incertainities caused by indirect detection are avoided, conferring 100% specificity.

Current generation MALDI-TOF instruments are cappable of recording a single spectum within less than 1 second (1). Automation and minituarization, on the other hand, allow 1ng or less DNA to be used per genotype. Moreover, multiplexing allows several different SNPs to be combined and analized in a single tube (well). In combination with the possibility of parallel detection of many samples in 384-well format makes possible to analize 30 000 SNP genotypes per day, achieving high quality at a low price per sample (2).

MALDI-TOF technology is also appicable in other genetic approaches, such as quantitative analysis of SNPs in pools of DNA, highly informative molecular haplotyping, resequencing and mutation detection. Furthermore, MALDI-TOF measurment is used for epigenotyping in studies on gene regulation and function, and RNA expression profiling (1, 4).

Recently, the MALDI-TOF MS high throughput technology has strengthen its possition as a reliable and precise method in diferent areas of genomic research.

Fig. 1 The principle of the PEX reaction for A>G substitution.



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DO3. MASS-SPECTROMETRY IN CANCER PROTEOMICS AND DRUG DISCOVERY

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Completing the Human Genome Project drew the focus on the gene expression and the encoded proteins function. Hence, demands for high-throughput proteomic approaches for identifing and characterising proteins in the underlying biological proceses. The high-throughput mass spectrometry techniques provide powerfull tools for fast progressing cancer proteomics in different asspects, as identification of early detection markers, better understanding the carcinogenic processes, studing drug resistance patherns, improving individualized responses to therapies and novel therapeutical agents discovery.

In this respect two dimencional gel electrophoresis (2DE) followed by mass spectrometry, namely peptide mass fingerprinting (PMF), provides rapid and efficient detection and bioinformatically aided identification of the proteins expressed under different conditions and processes. Denaturating 2DE-gel has the power to separate thousand polypeptides based on their charge (pI) and molecular mass (Mr). Further steps include scanning the gel image, followed by excision of the spots of interest, obtaining peptide fragments after enzyme digestion, which masses are further measured by MS and identification of the PMF derived structural protein information in the public protein databases (1).

MALDI-TOF MS (Matrix Assisted Laser Desorbtion/Ionisation Time-Of-Flight Mass Spectrometry) measurement is based on the ability to transfere peptides in gass phase under soft ionisation conditions minimizing spontaneous fragmentation effects. Briefly, the samples of peptide mixtures are co-crystalized with small organic compounds (matrix) and further put under laser–triggered short ion-pulses. The generated singly charged ions are accelerated in vacuum tube and are separated according to their mass-to-charge ratio, calculated on the basis of their time-of-flight (TOF). Lighter ions arrive first at the detector and the resulting mass–spectra profile is visualised and further identified in the protein databases (1).

Recent technical advances resulting in growing published approaches, identifying new biomarkers for cancer diagnostics, lead to new information in the field of cancer diagnosis and prognosis. Results of MS analyses of blood proteome from healthy controls were found to differ significantly from the paterns shown in early stage breast cancer patients (2). Another recently published study on the proteomic characteristics of endometrical cancer cells identified differences in proteins expression compared with normal cells. Namely Cyclophilin A was found to be overexpressed and associated with poor survival and thus considered as a promissing novel prognostic factor and possibly an attractive terapeutic target (3).

Furthermore, mass spectrometry innovation techniques are applied to drug discovery and development approaches to identification of new drug targets and drug mechanisms of action. Proteins as functional molecules in cells are the major targets for drugs. Proteomic analysis using 2DE and MALDI-TOF of neuroblastoma patients before and after treatment with Xanthoangelol, revealed that DJ-1 protein is involved in the drug-induced apoptosis and showed its cytotoxic effect on drug resistant neuroblastoma cells. The revealed mechanism was found promissing in a therapeutic approach to advanced neuroblastoma (4). Identification of potential drug targets enables search for targeted therapy to specific pathways concerning breast, colorectal, prostate, cervical and hepatocellular cancers (5).

Furthermore, the possibility of certain metabolites and specifically modified proteins identification resulted in studies on toxicity and side effects of known drugs and therapies (6).

Mass-spectrometry provides powerful and rapid means in proteins analysis separated on a 2D gel and already gives promising results in the identification of cancer related proteins, as well as in their control and function.

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DP1. ДНК И КРИМИНАЛИСТИКАТА

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Молекулата на дезокси-рибо-нуклеиновата киселина /ДНК/ е организирана като две свързани, комплементарни една на друга вериги образуващи спирала. Този модел на ДНК е създаден от Уотсън и Крик през 1953, за което бяха удостоени с Нобелова награда.

В хода на разследването, криминалистите могат да използват ДНК от открит на местопрестъплението биологичен материал (кръв, семенна течност, кожа, слюнка, косми). За да са годни за извличане и изследване на ДНК, в пробите, които се събират, трябва да има съдържащи ядра клетки. Вече е възможно да се изследват и безядрени клетки /в това число и косми без космената луковица/, като това става посредством анализ на митохондриална ДНК по метода на секвенирането.

Относително къси блокове от тандемно повторени нуклеотидни последователности, означени като "минисателити" или "микросателити", са открити през 1980г. Те са с висока генетична информативност. С това откритие става възможно получаването на уникалния генетичен профил на всеки отделен индивид. По аналогия с уникалните пръстови отпечатъци те са наречени "ДНК пръстови отпечатъци" или "генетични отпечатъци". Процесът на идентифициране чрез проба от ДНК се нарича "генетичен отпечатък" (от англ. fingerprinting) или "ДНК профил". При него се съпоставят относително големи участъци от ДНК веригата. Методът е разработен през 1984 г. от английския генетик Алек Джефрис и е използван за пръв път през 1986 г. в разследване по дело за случай на убийство.

Процесът по изготвяне на генетичен отпечатък започва с извличане на ДНК. Следващата стъпка в теста е извършването на анализ на ДНК въз основа на полиморфизмите по дължината на рестрикционните фрагменти в човешкия геном. Това се извършва, като чрез специфичен ензим се фрагментира ДНК и получените фрагменти се разделят според молекулната си маса чрез процес на електрофореза. Съвременните протоколи включват

амплифициране на ДНК чрез полимеразно верлижна реакция (ПВР) и белязане на полиморфните микросателитни участъци със специфични флуоресциращи багрила. ПВР позволява използването на изключително малко количество от материала на пробата, като резултатът е много дискриминативен, с шансове за случайно съвпадение сведени до едно към милиарди. При електрофорезата, с помощта на специален лазер се регистрира излъчената флуоресценция и ДНК профилите се анализират с помощта на специален софтуер.

Методът за изследване на генетичния отпечатък използва много-вариантните, повтарящи се последователности от бази /"микросателитите"/. Двама души, между които няма връзка, биха имали различен брой и вид микросателити в дадено местоположение на веригата на ДНК. Определяйки броя на повторенията и техния вид в дадено място, става възможно да се установи съвпадение, което е почти невъзможно да възникне случайно. Все пак, колкото по-голям е броят на анализираните полиморфни маркери толкова по-малък е шансът за случайно съвпадение на ДНК профилите при несвързани индивиди.

Методът се използва в криминалистиката, за да се свържат конкретни заподозрени с проби от кръв, косми, слюнка или семенна течност, открити на местопрестъплението. Чрез ДНК анализ може да се достигне и до изключване на конкретен заподозрян.

Употребата на ДНК отпечатъците не се ограничава само с приложението й в криминалистиката – този способ дава възможност за изследване на популации на диви животни, провеждане на тест за бащинство, идентификация на трупове, откриване на епидемични щамове бактерии и вируси и много други. Този метод може да подпомогре и изграждането на хипотези относно моделът на човека и човешкото общество в праисторическите времена.

DP2. ПОЛИМЕРАЗНА ВЕРИЖНА РЕАКЦИЯ

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DO4. CELL CYCLE

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DP3. MALIGNANT CELL TRANSFORMATION

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DP4. APOPTOSIS

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DP5. HEAT SHOCK PROTEINS

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DP6. ANGIOGENESIS

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Angiogenesis is a process by which new blood vessels are formed from preexisting ones. New vessels are needed for the distribution of oxygen and nutrients to the growing organs of the embryo or for the reparation of wounded tissue in the adult. A lot of factors participate in the process- growth factors and growth factor receptors, signaling molecules, adhesion molecules, extracellular matrix proteins, proteases, maturations, morphogenic and guidance molecules, transcription factors. Angiogenesis play a crucial role in cancer, eye diseases, diabetes, arthritis, atherosclerosis and etc. Tissue activity of angiogenesis depends on the balance of many stimulating or inhibiting factors.

The process of angionegesis begins with stimulating signal (growth factor or cytokine) and its binding to the receptor, activation and proliferation of the endothelial cells, digestion of the basal membrane, remodeling of the extracellular matrix, tube and loop formation and vascular stabilization (accumulating of mural cells over the new vessel).

The best studied factor and its receptor is VEGF/VEGFR (Vascular Endothelial Growth Factor and its Receptor). There are also co-receptors. One of the ways of angiogrnesis is the VEGF pathway. There are five ligands (VEGF/VEGF-A, PLGF, VEGF-B, VEGF-C and VEGF-D) and five receptors (VEGFR1, VEGFR2, VEGFR3, NRP1, and NRP2). When the VEGF binds to VEGFR activation of Tyrosine Kinase Domain occurs through ligand-induced dimerization and receptor autophosphorylation at multiple tyrosine residues in the intracellular domain. Another way is DLL/Notch pathway. Interaction of Notch receptors with Notch ligands, such as Delta-like or Jagged, between two bordering cells leads to a cascade of proteolytic cleavages and this is the triggering of the angiogenesis. Both pathways lead to further activation of other molecules and finally leading to activation of endothelial cells.

Angiogenesis is used by tumor cells for oxygen and nutrient delivery. Blocking angiogenesis is one of the main possible ways to cope with tumor cells. It can be achieved by targeting VEGF-A with monoclonal antibodies such as bevacizumab and VEGF-trap, to inhibit VEGFR2 with specific antibodies and a variety of small-molecule VEGF Receptor Tyrosine Kinase inhibitors that inhibit ligand-dependent autophosphorylation of VEGFR2 ,to disrupt VEGFR1 with anti-VEGFR1 antibodies, and to block the interaction between VEGF-A and VEGFR2 with soluble

VEGFR1 protein. Blockade of Notch signalling can be accomplished by using different strategies, including anti-DLL4 monoclonal antibodies, gamma-secretase inhibitors, soluble DLL4-Fc, anti-Notch1 neutralising antibodies, and Notch1-trap.
In struggling with cancer widely used drug is anti-VEGF antibody Avastin (bevacizumab), often in combination with 5-Fluorouracil or carboplatin and paclitaxel. Another anti-VEGF antibody is Lucentis(ranibizumab).
Angiogenesis plays an important role in many diseases and deeper understanding of the process would give us more ways to control the angiogenesis.

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Session E.

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EO1. ПРОУЧВАНЕ НА JC ПОЛИОМАВИРУСНА ИНФЕКЦИЯ СРЕД КРЪВНИ ДОНОРИ

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EO2. REACTIVATION WITH HUMAN HERPES VIRUS TYPE 6 (HHV-6) IN PATIENTS WITH DIFFERENT DISORDERS IN CENTRAL NERVOUS SYSTEM (CNS) AT 2009 IN BULGARIA

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Infections with Human Herpes virus type 6 (HHV-6), a β-herpes virus are very common, approaching 100% in seroprevalence. Primary infection with HHV-6 causes *roseola infantum* or *exanthema subitum*, a common childhood disease that resolves spontaneously. After primary infection, HHV-6 is a major cause of opportunistic viral infections in the immunosuppressed, typically AIDS patients and transplant recipients, in whom HHV-6 infection/reactivation may culminate in rejection of transplanted organs and death. A possible correlation between active HHV-6 infection and multiple sclerosis and other disorders in CNS has been the focus of much attention in the past few years. Our purpose is to identify the putative acute HHV-6 infection/reactivation in patient with different disorders, mainly in CNS. Twenty serum samples were analyzed from patients with hepatitis (5), infectious mononucleosis-like illness (4), encephalitis (3), multiple sclerosis (3), child cerebrals paresis (2) and chronicle tiredness (3). The criterion standard for diagnosis of HHV-6 infection was the presence of high titer of IgG human herpesvirus type 6 antibody without serological evidence of alternative virus from *Herpesviridae* or another virus. Ten cases of positive HHV-6 infection were identified: 3 with diagnosis multiple sclerosis, one aged three with viral encephalitis, in 3 between 2 and 5 years old with child cerebrals paresis, in 2 aged 34 and 28 respectively with diagnosis chronicle tiredness and one with infectious mononucleosis-like illness.
This is the first description of participation of HHV-6 in patients with neurological disorders in Bulgaria.

EP1. THE SEROEPIDEMIOLOGY OF VARICELLA/HERPES ZOSTER VIRUS (VZV/HZV) IN BULGARIA AT 2009

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Varicella is a widespread highly contagious infectious disease in all human populations, caused by the Varicella/Herpes zoster virus (VZV/HZV). Primary VZV infection (Varicella, chickenpox) results in the diffuse vesicular rash in childhood and lifetime immunity. Secondary reactivation of this neurotropic virus leads to herpes zoster (shingles), a painful, unilateral vesicular eruption in a restricted dermatomal distribution. Varicella is seen more commonly in children, whilst herpes zoster is mainly observed in the elderly. Although uncommon, disease

complications secondary VZV/HZV may be severe and life-threatening especially at the extremes of age, during pregnancy and in the immunocompromised. The aim of this investigation was to assess the prevalence of antibody Varicella/Herpes Zoster virus in different age and groups from Bulgarian population at 2009, as well as to see epidemiological and seasonal variations of this disease. The enzyme linked immunosorbent assay (ELISA) method was used to assess the presence of anti-VZV/HZV antibody. A total of 376 serum samples were collected for VZV/HZV at 2009 in our Laboratory. From all of this 259 samples were immunity of VZV/HZV. Age specific prevalence of IgG antibody to VZV/HZV showed a progressive increase with age in both males and females. Prevalence of VZV IgG antibodies was 39.8% in the age group of less than 10 years, 80% in 10 – 14 years, 86% in 15 – 19 years, 88% in 35 – 40 years. Our results correlate with data in Turkey, Australia and Italy, which reported level of immunity by the ages of 10 – 14 years 85%, 83% and 82% respectively. The data show that the best option to reduce the circulation of wild type VZV in the population would be the immunization of young children.

EP2. HERPES SIMPLEX VIRUS

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Herpes simplex virus types 1 and 2 (HSV-1 and -2) are members of a *Herpesviridae* family. Recognized since ancient Greek times, herpes infections still attracts the attention of scientific and medical community. Some more details: 1) The vast majority of the world population is infected with at least one member of the human Herpesvirus family. HSV infections cause mucocutaneous infections such as herpes labialis (cold sores) and herpes genitalis (genital herpes). HSV persists in a latent form for the life of the host, periodically reactivating and often resulting in significant psychosocial distress for the patient. The virus can also induce sight-impairing or life threatening disease mainly in immunocompromised patients, pregnant women and newborns (Kleymann, 2003a,b); 2) Neonatal herpes still presents diagnostic and management problems in view of continuing high mortality and morbidity rates (Kimberlin, 2004); 3) HSV infections are important causes of morbidity and mortality for patients with neoplastic diseases, especially haematological malignancies (Wade et al., 2006); 4) Several independent studies suggest that HSV-2 infections correlate with a higher than normal incidence of cervical cancer (Jones, 1995; Pisani et al., 2004; Smith et al., 2002); 5) Oncolytic herpes simplex virus type 1 (HSV-1) vectors are emerging as an effective and powerful therapeutic approaches for cancer and other diseases (especially for those affecting the central nervous system, such as Parkinson's disease or maligtnant glioma) (Jacobs et al., 1999; Varghese, Rabkin, 2002); 6) Curently no cure is available. Antiviral therapy is the main treatment modality, used either orally, intravenously or topically to prohibit further replication of the virus and thereby minimize cellular destruction (Kleymann, 2003a,b). Short-term treatment with acyclovir can accelerate the healing of an acute outbreak, and continuous acyclovir therapy is often prescribed for people with frequent recurrences.

While Acyclovir, the gold standard of antiherpes treatment, can reduce the recurrence rate by 60-90%, it can also cause a wide array of side effects, including renal failure, hepatitis, and anaphylaxis. The development of acyclovir-resistant strains has also beer reported especially in

immunocompromised patients. New, well tolerated antiherpes agents with novel mechanisms of action and low resistance rates that significantly reduce time to healing, prevent rebound of disease after cession of treatment, reduce frequency and severity of recurrent disease are therefore needed (Bacon et al., 2003; Gaby, 2006).

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EP3. BOVINE HERPESVIRUS-4 (BHV-4)

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BHV-4 is a gammaherpesvirus which attracts the interest of scientists because of the following main reasons: 1) Gammaherpesviruses are members of an emerging subfamily among the *Herpesviridae*. Two genera are discriminated: i) lymphocryptovirus, including its type species Epstein-Barr virus (EBV), and ii) rhadinovirus, including viruses of interest for medicine, veterinary medicine, and biomedical research, i.e. alcelaphine herpesvirus 1, bovine herpesvirus-4, equine herpesvirus 2, human herpesvirus 8, mouse herpesvirus 68 and ovine herpesvirus 2 (Ackermann, 2006). 2) The BHV-4 infection is distributed worldwide. The virus is not strictly species-specific: infection was also proved in American bison (Bison bison), African buffalo (Syncerus caffer), sheep and cat (Thiry et al., 1990). 3) On one hand BHV-4 is not known to cause any disease which makes it easier for experimental work (Trapp et al., 2003). It has recently been reported that this virus can play a direct or indirect role in the aetiology of bovine mastitis; therefore its importance and its economic impact needs further attention (Wellenberg et al., 2002). On the other hand, BHV-4 genome consists of 5 gene blocks conserved among the gammaherpesviruses and particularly within the Epstain Barr virus (which causes infectious mononucleosis and is associated with Burkitt's lymphoma and nasopharyngeal carcinoma; Pattle, Farrell, 2006) and

herpesvirus saimiri (T-lymphotropic virus which establishes specific replicative and persistent conditions in different primate host species; Fickenscher, Fleckens, 2001) genomes (Lomonte et al., 1996). 4) Finally, BHV-4 shares antigenic and genomic relationships with alcelaphine herpesvirus 1, the causal agent of the African form of malignant catarrhal fever in cattle and some wild rumonants (Thiry et al., 1990).

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EP4. ENZOOTIC LEUCOSIS IN CATTLE AS A MODEL OF SOME HUMAN LEUKAEMIAS

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The enzootic leucosis in cattle (ELC) caused by bovine leukaemia virus (BLV) is widespread throughout the world due to easy transmission of the infection. The economic losses from ELC are significant but the scientific interest to the disease is prompted because of the genetic relatedness and identical replication strategy of BLV to a large group of retroviruses causing leukaemia in humans, such as HTLV-I, HTLV-II, HIV. As for as the genome of BLV and HTLV I and II there is a similarity of over 50% in their nucleotide sequences.

The successful study of the ELC like a model of some human leukaemias is dependent to a great extent on the possibility of modelling the disease in experimental animals, to create a smaller, easily manipulated model with short incubation period. According to the literature data rats and rabbits are immunoreactive to BLV infection. The present investigation aims to prove some morphological changes as well. For the purpose rats and rabbits were inoculated with BLV-producing cells from the FLK-BLV permanent line and with leukocytes from a cow, suffering from ELC. Infection in approximately 1/3 of the experimental animals was established, performed by a primary disease with clinical (weight loss, alopecia, rhinitis, pneumonia), haematological and histopathological findings, which were not detected in the controls. Leukocytosis and immature cells in the peripheral blood, enlarged lymph nodes, lymphoid cells infiltrations in most parenchymal organs and activation of the reticuloendothelial system suggested a carcinogenic action of BLV 16-18 months p.i..- pathology similar to the alterations induced by the field strain BLV in its natural host. It makes rats and rabbits suitable for studying lymphoid leukemias in methodological aspect - detailed establishment of pathogenesis of the disease and in practical aspect - to improve the diagnostic, clinical and therapeutic techniques for these diseases in laboratory conditions.

EP5. APPLICATION OF ELECTRONIC LEARNING COURSE: SPECIFIC PREVENTION AND THERAPY OF VIRAL DISEASES

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The development and the diffusion of ICT and Internet expand opportunities for communication, information exchange, changing work environment and daily lives.
The combination of elements of traditional and e-learning allows quick and easy access to educational content and learning activities, ways of monitoring and evaluation of the achievements of trainees, the opportunity for optimal contact with the teacher, timely feedback, active participation in the learning process. At the same time it is a challenge for teacher preparation in the design of the course, structuring of information and training materials. Adequate assessment is that which helps the teacher to choose what he will include in the electronic part of the course and what will give on hardcopy as manuals, books, journals and others. We analyzed the strengths and weaknesses of the electronic course, given the specificities of the trainees and selected the most appropriate form of education blended learning between classical and electronic learning.

It is defined as "Learning, in that the primary function of the virtual learning environment is the preservation and provision of additional educational resources that fulfill the secondary function of otherwise conventional form of education" (Sharpe et al 2006).

Blended training is characterized by integrating various information and communication technologies in traditional educational context. In organizational and meaningful terms this integration can be very diverse as a result of a significant proportion of traditional and electronic educational technologies. Technology can be used to support teaching, learning and teacher's interaction. The aim of this study is to present the mozaic organized electronic and teaching resources with the structure that meets the needs of the course: Specific Prevention and Therapy of Viral Diseases of postgraduate training of senior and semi-higher medical staff. In this study we offer a description of the steps in the creation of Web-based course with dynamic content, interactive opportunities for its inclusive and legal documentation, which includes the following centers: general theory, basic principles in the production of viral vaccines and their application in practice, therapy of viral infections.

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