STRUCTURAL DYNAMICS OF MOLECULAR INTERACTIONS IN LIVING SYSTEMS, THE EFFECT OF ENDOGENOUS AND EXOGENOUS FACTORS

CONTENT

In the field of scientific research, employees achieve results that are published in renowned peer-reviewed journals about the quality of which is also testified by the citation response to them (overview of the publishing activities of employees). Many activities have been awarded and many events are held not only for the staff and doctoral students of the department.

The implementation of doctoral studies (PhD) in the study program *clinical biochemistry* significantly influence the improvement of scientific research work and contribute to the professional growth of employees.

The projects solved at the institute are implemented within the framework of partial orientations, and therefore the following text is divided into according to the scientific-research focus subchapters (complete overview of projects solved at the institute).

A. The usage of molecular-biochemical methods for the diagnoses of selected diseases
B. The spectral fluorescent characterization of complex mixtures
C. The study of selected natural/synthetic compounds and their influence on the biochemical functions of organism (also on the subcellular level)

1. THE CURRENT STATUS OF RESEARCH

A. The usage of molecular-biochemical methods for the diagnoses of selected diseases

In recent years, the significant progress was made in understanding of the molecular mechanisms in the formation of many diseases.

Determination of individual molecular markers for different types of diseases, focused research not only to detection of changes of expression of the selected control genes, but also to identification of the miRNA molecules that are characteristic for the concrete (specific) disorder. Subsequently, more detailed examination of mechanisms of transcriptional regulation allows better understanding of formation, progression of the disease, and monitoring of successful treatment. One area of using molecular-biochemical methods is the detection of early stages of various types of gynecological malignancies (breast cancer, ovarian cancer, uterine cancer and cervical cancer). Besides the basic markers as BRCA1/2 and CA125/Muc16 (relatively low specificity), there are other biomarkers, usually associated with tumor neoangiogenesis, whose expression levels significantly varies during physiological angiogenesis compared to carcinogenesis. To the considered markers belong also Adlican,

COL11A1, GMP6B a DR6, which show 10 -350 times higher expression in the cancer of corpus luteum, endometrium, and placenta. Besides the tumor endothelial cells, these markers are also produced by vascular leukocytes and perivascular cells population. The most recent studies such as the detection of endometriosis as precancerosis of uterine cancer, but also the uterus body cancer focus on the monitoring of the transcriptional activity of genes involved in the process of cell adhesion and the regulation of apoptosis. One of the appropriate marker is the ß -catenin, which affects the regulation of transcription of genes involved in the Wnt signaling pathway and cell adhesion.

So far, exist no suitable marker (from the serum of patients) for screening of development and progression of endometriosis with sufficient sensitivity and specificity, and therefore the use of molecular methods is one of the possible and the prospective ways of detection of endometriosis as a pre-cancerous state, or during the diagnostics of cancer of the uterus.

The second area of using molecular-biochemical methods is monitoring of the development and progression of malignant melanoma (MM). Key role in the metabolism of melanoma has a Microphthalmia Associated Transcription Factor (MITF), which stands in the center of transcriptional regulation of embryonic development of normal melanocytes, through their differentiation, maintaining identity or survival of normal but also malignant melanocytes. Expression of the mentioned transcription factor activates in melanocytes several signaling pathways, and blocking of its expression, or function may lead to the failure in the regulation of anti-apoptotic mechanisms. MITF also influences the activity of matrix metalloproteinase 14, which is responsible for the shape change of the melanocytes and for increase of their mobility and invasiveness. Among the other factors that allow the detection of formation and progression of MM belong detection of polymorphisms of the vitamin D3 receptor (VDR). Its promotor allows synthesis of multiple specific isoforms that are able to affect the formation of MM. The most described polymorphism with different lengths are rs10735810, rs1544410 and rs731236. Analysis of gene expression of VDR and its polymorphisms can provide useful information for the treatment of melanoma using vitamin D and its analogues.

Last but not least way of usage of the molecular-biochemical methods is non-invasive detection of urothelial carcinoma (UC). Upon the occurrence of an aggressive form of UC it has been demonstrated the presence of specific miRNA (miRNA-96, 940 and 135b) in urine and whole blood of patients. Detection and analysis of these miRNAs is possible to determine different stages of bladder cancer, what can contribute to the improvement of diagnosis of early stages of mentioned disease before the appearance of symptoms alone.

The determination of specific miRNA begins also use in detecting and monitoring of the progression of thoracic aorta aneurysms (TAA). An important role in the pathophysiology of TAA can play a miRNA-19b, miRNA-302D and miRNA-340, whose expression levels were significantly elevated in patients with thoracic aneurysm. Previous studies have shown that miRNA-19a and 19b are members of the cluster miRNA17-92 which regulates the expression of extracellular matrix proteins, for example connective tissue growth factor and thrombospondin 1. During the development and progression of TAA occurs a pathological remodeling of the extracellular matrix (ECM), smooth muscle cell apoptosis and decreased elasticity of the blood vessels. One of the major pathways involved in the formation of the TAA is a signaling mediated by angiotensin 2 induced by transforming growth factor β (TGF-β). Reduced levels of this growth factor on the contrary, reduce the expression of MMPs and thereby prevents dilatation of the aorta and aneurysm progression. In patients undergoing surgical aneurysm repair, in aortic tissue was found increased expression of IL-6 and interferon a (IFN-c). Increased expression of IL-6 lead to the accumulation and induction of monocyte/macrophage activation, what results into activation of chemotactic mechanism through monocyte chemotactic protein 1 (MCP-1). An activated macrophages produce pro-inflammatory cytokines, chemokines, and ROS in the vasculature, what lead to the formation of local inflammation and the ECM changes. Infiltrating leukocytes accelerate the pathogenesis by the production of MMP, which are capable of degrading important components of the aorta wall, including elastin and collagen.

As the level of expression of specific miRNAs in healthy tissue is relatively stable, its changes during pathogenesis are also useful in detection and monitoring of the progression of neurodegenerative diseases, such as multiple sclerosis (MS). MS is a chronic inflammatory disease of the central nervous system, whose mechanisms of forming and progression at the molecular level are not fully understood. Recent research clarified the role of T cells expressing CD40 molecules (TH40 cells). These cells play

a central role in various autoimmune diseases. They are the major sites of IL-17 and INF-γ synthesis. Simultaneously occurs dysregulation of the synthesis of miRNA (e.g. miR-223, miR-15 b) in samples of blood and cerebrospinal fluid (CSF). Proposed comprehensive experimental approach can contributes to the expansion and improvement of knowledge about the progression or recurrence of the disease, what could clarify the prognostic information for doctors and patients, and consequently contribute to simplifying diagnosis, and monitoring during the treatment of MS.

B. The spectral fluorescent characterization of the complex mixtures

The fluorescence belongs to the most universal and the most reliable method for study of living matter and the processes located in the living system. It is non-invasive method between interaction of the light and the matter. Most of the living systems contain molecules which are after interaction with the light energy able to produce the light itself (fluorescence, phosphoresce). The analysis of this light bring us useful information about the structure, changes, dynamics, and chemical interactions of the key molecules present in cells of living systems, what is important and essential of the most modern diagnostic methods. Development of fluorescent labels, probes, and more recently also development of biosensors, form preconditions for more intensive application of fluorescence techniques in diagnosis. Example of the application of chemiluminescent methods is proof of DNA adducts, which can play important role as biomarkers or indicators of genotoxic chemicals exposure, which significantly increase the risk of cancer. In this way, the presence of PAH-DNA (PAH: PolyAromatic Hydrocarbons) adducts was monitored in the blood of the members of the US military during the conflict in Kuwait in 1991. Detection of pathological processes by using of the fluorescent spectras is becoming increasingly widespread. Central role in this area has a diagnostic of precancerous lesions and tumors, e.g. in urology, gynecology, gastroenterology, but also in other fields of medicine.

Fluorescence of protoporphyrine IX after induction of aminolevulinic acid (ALA) is successfully used in combination with endoscopy for localization and visualization of the bladder cancer.

The native fluorescence of collagen and of the end product of glycation during 3D scanning of the fluorescent matrices of the skin allows further identification of the skin lesions and tumors. Very effective is also diagnostic application of the sophisticated fluorescence technique in gynecology. The native fluorescence of cervical tissue is studied in relation to the presence of intraepithelial neoplasia and dysplasia. Similarly, the endogenous fluorescence of normal and malignant esophageal tissue scanned during endoscopic examination showed conformity with conventional biopsy. Better visualization can significantly improve the information about the size and extent of lesion essential for subsequent treatment. Other applications of fluorescent and luminescent techniques in general biochemistry are so extensive, e.g. study of biological membrane, apoptosis, oxidative stress, mitochondria, free radicals, environmental biomarkers, neurodegenerative diseases, qualitative and quantitative identification of medicines and drugs.

C. The study of selected natural/synthetic compounds and their influence on the biochemical functions of organism (also on the subcellular level)

Biochemistry of reactive oxygen species (ROS) and nitrogen (RNS) and the mechanism of their formation are important in the assessment of physiological processes in the body, but also in the etiopathogenesis of many disease conditions. The physiological processes regulated by redox balance are sensitive to excessive production of ROS from any source, either endogenous or exogenous. Their importance is underlined by the disruption in various diseases associated with oxidative stress. The redox regulation changes are foundation of pathophysiology processes associated with the aging process (e.g. telomere shortening) and a number of diseases (e.g. cancer, type 1 diabetes mellitus, atherosclerosis, neurodegenerative disease, rheumatoid arthritis, HIV infection, ischemic reperfusion injury, obstructive sleep apnea).

Low levels of ROS are tolerated by cells, but high levels are induced oxidative stress. The reactive species of oxygen and nitrogen (RNOS) act as redox messengers in intracellular signaling pathways and regulation on the physiological levels. Activation of the internal signaling pathways leading to activation of transcription factors that regulate the expression of specific genes necessary for ensuring the various

functions. Overproduction may affect cell function by damage of nucleic acids, protein oxidation, lipid peroxidation and lead to cell death promoting internal apoptotic pathways. It depends on the balance between production and uptake of reactive oxygen species in the right place and at the right time, whether becomes damaged structures, activates the defense or will act as signaling molecules.

Antioxidant defense system which includes enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and low-molecular metabolites such as glutathione, tocopherol, carotenoids and flavonoids it provides an effective inactivation. Antioxidant system is affected by a number of other factors and circumstances (e.g. receiving exogenous anti - or pro-oxidants, intake, transport and binding of metals to organic compounds in the body).

The system is inducible and is thus a suitable subject for study. The monitoring of RNOS production of selected materials and the adaptive response of cells by endogenous antioxidants it is currently one tool leading to the knowledge of mechanisms of selected substances action that can be specifically used in the prevention or treatment.

2. OWN CONTRIBUTION TO THE STATE OF RESEARCH

A. The usage of molecular-biochemical methods for the diagnoses of selected diseases

The determination of expression of tumor vascular markers (TVM) in the peripheral blood is one of the less burdensome method for the patient which could help in differential diagnosis of different types of cancer (e.g. of the female reproductive system). Increased level of gene expression of these markers in the affected tissue, peripheral blood or umbilical blood has been confirmed till now just on a small number of patients. Therefore, it is important to confirm or deny the correlation of changes in mRNA levels of selected genes involved in neoangiogenesis at the molecular level with the amount of the corresponding proteins in the peripheral blood of patients. There is a close relationship between the level of angiogenesis, and metastasis. Highly sensitive methods of molecular diagnosis are suitable for detection of TVM. We are currently studying the expression of genes (DR6, GPM6B) in peripheral blood of gynecological patients, while our results confirm their increased expression.

Our research on malignant melanoma development and progression is focused on the microphtalmia-associated transcription factor (MITF), specifically to detect changes in the expression of MITF itself, as well as the effector proteins of its signaling pathway (e.g. MMP14, GLI2). At the same time, we monitor the presence of different polymorphisms of vitamin D receptor, which are associated with a worsened prognosis of the disease. Study and detailed understanding of the regulation of MITF pathway may contribute to the development of new diagnostic procedures, subsequently applicable in treatment course monitoring of malignant melanoma patients.

Our research on urothelial tumors is focused on the detection and analysis of specific miRNA (miRNA-96, 135B and 940). Based on the interaction of DNA / protein with chromatin complexes analyzed by specific chip-qRT-PCR method (chromatin immunoprecipitation capture quantitative real-time PCR) we can verify the links between the transcriptional activity of the relevant genes with the formation/presence of transcriptionally active euchromatin or transcriptionally inactive heterochromatin in urinary bladder cancer. The results achieved at project should increase our knowledge on progression or recurrence of the disease, and should contribute to the improvement of the prognostic information for doctors and patients, and consequently can be used for early, non-invasive diagnosis of urinary bladder cancer.

Our research on aneurism of thoracic aorta is focused on the detection of specific miRNA expression (e.g. miRNA-19b, miRNA-302D, miRNA-340). Previous studies have pointed out, that miRNA-302D regulates the transcription of the TGF-β. In addition, we also monitor the changes in the expression of selected genes (e.g. TGF-β, inflammatory interleukins IL-6 and 10, and matrix metalloproteinases 2 and 9). Till now, there was just a small part of miRNA molecules, expressed in the cardiovascular system, described. Identification of additional miRNA and the subsequent analysis of their functions should provide an innovative view to the mechanisms controlling cardiovascular development as well as to its function / dysfunction.

In sclerosis multiplex (SM) research, we detect the expression of specific blood or cerebrospinal fluid miRNA (e.g. MiR-223, miR-15b) and subsequently correlate it with the stage of the disease.

We also monitor the changes in the expression of inflammatory mediators (IL-1β, IL-6, IL-17, TNF-α, IFN-γ) in mRNA level by qRT-PCR as well as in protein level by biochip immunoassay. Our results should help to confirm and determine the stages of SM.

B. The spectral fluorescent characterization of complex mixtures

Many years of experience with luminescence techniques in the workplace led to the reprocessing and own development of methods for needs of clinical, biochemical, biological, biophysical, pharmacological, but also food research. Biological fluids are complex mixtures containing a number of substances. Endogenous fluorescence spectral analysis reflects not only the presence and quantity of fluorescent substances, but also many other factors such as the polarity, pH or presence of the quencher.

The tumor histological-morphological manifestations changes are before changes in the tumor cells metabolism. The biochemical changes detect even before the onset of symptoms of the disease that may significantly affect the treatment and prognosis of the disease. High-sensitivity fluorescence can in many cases and distinguished the presence or absence of changes and differentiate biological material of healthy and sick. The biological fluid of first choice for fluorescence spectral analysis in the workplace is urine (non-invasive, almost indefinitely repeatable collection, reflects the metabolic pathways and regulation of homeostasis). For the urinalysis was developed a unique process, which significantly contributes to the detection and identification of changes caused by exogenous and endogenous components. The specific synchronous fluorescence measurements allow create three-dimensional fluorescent matrix, which graphically define the composition of the urine under normal and pathological conditions. We have experiences with fluorescence sensing of other biological fluids such as plasma, cerebrospinal fluid, saliva, amniotic fluid, or synovial fluid, each with its own characteristics in our department.

Thanks to a long-term active cooperation with several clinical departments (e.g. Clinic for Children and Adolescents, Department of Gynecology and Obstetrics, East Cancer Institute, Department of Plastic and Reconstructive Surgery, Department of Internal Medicine) is available quite extensive database of measured spectra. Processing and evaluation of measured data is realized through custom software applications developed in cooperation with the Faculty of Electrical Engineering and Informatics of Technical University of Košice. The fluorescent spectrum analysis of our department includes early detection of cancers of various organs (e.g. breast, cervical, and uterine body cancer, ovarian cancer, malignant melanoma), as well as renal diseases or degenerative diseases of the joint apparatus.

We have rich experience with monitoring changes in native fluorescence and polarization of mitochondrial membranes caused by the influence of various endogenous and exogenous factors (e.g. toxic substances, medicaments). The mechanism of this response is selectively specific to individual substances or physical effects.

C. The study of selected natural/synthetic compounds and their influence on the biochemical functions of organism (also on the subcellular level)

Within the *in vitro*, *in vivo* and human studies that have been conducted in our Department several experiments were aimed to detect organ specific responses, relations between site of production and site of action of isoenzymes.

In vitro studies were focused on the ability of various plant extracts or humic acids to induce or scavenge RNOS. Furthermore, we studied the ability of cyclic chalcone derivatives to induce oxidative stress. Our objective was to elucidate the mechanisms of their action in the human body, and to define the formula with substantially cytotoxic or cytoprotective effect, which would be suitable as a pharmacologically active agent.

In vivo studies conducted in our Department contributed to clarifying the role of retinoic acid in the development of spinal cord and liver redox state during prenatal period; to clarify the role of melatonin

in nitrosourea-induced mammary carcinogenesis, as well as in understanding the maintenance of PUFA oxidative stability during their long term supplementation fortified with plant extracts.

In human studies Se-AOX (ClinicalTrials.gov Identifier: NCT02026856), there have been studied the effect of sodium selenite pentahydrate administration to patients with systemic inflammatory response, sepsis, severe sepsis and septic shock in order to adapt the conditions of strong oxidative stress and improving their survival.

3. SCIENTIFIC GOALS – REASONING AND ANALYSIS

A. The usage of molecular-biochemical methods for the diagnoses of selected diseases

The benefit of modern biochemical-molecular methods is the possibility of more accurate detection and classification of the cancer stages by non-invasive examination methods of body fluids with the possibility of practical outcomes in routine examinations during preventive examinations.
For the verification of results obtained within fluorescence analysis will contribute the correlation with clinical and biochemical measurements (e.g. cytokines, CA), with the analysis of nucleic acids (e.g. miRNAs), with the identification of DNA/protein interactions as well as with the concentrations of specific proteins. In the solving of this issue is possible:

- to isolate the specific miRNA from biological material and to analyze them with the possibility of their use in clinical biochemical diagnosis of selected diseases (e.g. bladder cancer, multiple sclerosis);
- to verify the importance of establishing of newly selected vascular tumor specific markers for different cancers (e.g. gynecological, urological);
- to monitor the expression of specific genes (e.g. those associated with inflammation, involved in angiogenesis) for a more accurate determination of progression of the disease, as well as for monitoring of the treatment success;
- to determine the activity of selected metalloproteases that correlate with increased invasiveness of tumor cells, malignant melanoma, or those that are involved in the remodeling of the aorta wall in the progression of aneurysm thoracic aorta;
- to track the transcription mechanisms either by analysis of the presence of transcriptionally active euchromatin or by the identification of DNA/protein interactions.

B. The spectral fluorescent characterization of complex mixtures

The aim of working group is to continue in established methods of urine fluorescence fingerprints analysis and to improve the processes leading to the identification of pathological characters of fingerprints. It will be continued in expansion of applications of fluorimetric techniques for *in vitro* diagnostics.

It will be continued in monitoring of less common body fluids (e.g. the tears, sweat, exudates, cervical mucus) in relation to particular diagnoses to determine the molecular mechanism of the diagnostic benefits for ophthalmology, dermatology, oncology, as well as for internal medicine or surgery (e.g. identification of 5- hydroxyindolacetic acid, which is a marker of acute appendicitis).

We plan to apply fluorescence in vivo in clinical material, e.g. to monitor transparency quality of the lens, the identification of skin precancerosis. In the solving of this issue is possible:

- to develop (in close cooperation with clinical departments) the graphic definitions (fluorescent matrices, fingerprints) of various biological materials based on special fluorescent measurements;
- to develop exact algorithms of evaluation of fluorescent matrices of biological material at specific physiological and pathological conditions;
- to apply the fluorescent techniques in clinical and biochemical diagnostic of selected diseases (e.g. cancer in early stages of the disease and precancerous conditions) gynecological, urological, neurological and others;

- to refine fluorescent monitoring of subcellular processes with a focus on biological membranes and their dynamics under normal and pathological conditions.

C. The study of selected natural/synthetic compounds and their influence on the biochemical functions of organism (also on the subcellular level)

The study of oxidative stress we plan to focus on the research of the impact of selected substances on the function of mitochondria (e.g. from heart, liver). Study will be carried out by dynamic measurement of respiration, of the activity of selected enzymes (e.g. ATPase, SOD, GR, GPX), by detection of fluorescent cofactors (e.g. NADP/NADPH, NAD/NADH) and by determination of the DNA damage degree (e.g. Comet Assay). The goals of the study of natural substances present in the diet and their effects on the body are linked to previous research results and are based on the needs of medical practice. The assessment of the ability to eliminate ROS by compounds with antioxidative properties represents available potential not only in the prophylaxis or direct treatment of conditions associated with oxidative stress, but also in reducing the severity of adverse events.
In the solving of this issue is possible:

- to study the antioxidative effect of selected natural and synthetic compounds,
- to focus on a targeted selection of substances with the greatest antioxidative effects,
- the substances with the most prominent antioxidative properties can be further deeply analyzed to characterize their effect in complexity,
- to study both the LDL and HDL fractions in relation to selected diseases (e.g., cardiovascular disease, obesity, metabolic syndrome),
- to study the effects of various natural substances (of plant or synthetic origin) on antioxidative and respiratory activity of mitochondria from different tissues.

4. PROPOSAL OF ACHIEVEMENT OF THE SCIENTIFIC OBJECTIVES

A. The usage of molecular-biochemical methods for the diagnoses of selected diseases

Biochemical-molecular methods are currently very accurate and very sensitive for monitoring the early pathological changes:

- to follow the changes of gene expression in various types of diseases by the use of mRNA qRT-PCR in combination with gel electrophoresis;
- for monitoring protein levels with corresponding gene of interest use vertical electrophoresis, followed by transferring the separated proteins from polyacrylamide gels to nitrocellulose membrane using a semi-dry Western blot technique;
- to detect changes in the expression of specific miRNAs are using real-time PCR cycler with a highly sensitive analyzer melting oligonucleotide fragments;
- to determine the presence of transcriptionally active euchromatin and monitor DNA / protein interactions uses a method Chip qRT-PCR.
- monitor changes in expression at the protein level we use ELISA immunoassays Biochip Array.

B. The fluorescence spectral characterization of complex mixtures

For rapid diagnosis is essential instrumentation and methodological background of department. The device LS 55 allows to make superior luminescent, fluorescent, phosphorescent, chemiluminescent, and bioluminescent measuring of biological fluids, cells, tissues and body surfaces (mucous membranes, skin).

The fluorescence measurements are highly reproducible and sensitive. Luminescent spectrophotometer LS 55 allows the determination of a wide spectral range (200-900 nm). In addition to conventional excitation and emission spectra allows extra scan spectra with synchronous shift monochromator, which further increases the resolving power and selectivity of measurement data,

measurement further polarization anisotropy and biological systems. Complex characterization of fluorescent samples that provides a better overview of the dynamics of physical and chemical processes during different stadium in biomacromolecules, biological membranes of cell organelles and whole cells of various tissues. Fluorescent characterization in combination with the simulation micro perfusion extends the *in vitro* studies of physiological and pathological processes during ischemia and hypoxia processes. Used methods:

- three-dimensional excitation and emission spectra with high resolution for detection of native fluorophores in biological materials;
- three-dimensional synchronous fluorescence spectra in the form of contour maps, so called synchronous fluorescence fingerprint (3D SFF), (priority of workplace) – used for high resolution potential particularly suitable of the analysis of complex mixtures and differentiation (of body fluids, pharmacologic and cosmetic products, the determination of the originality and quality control of product stability study of biological materials);
- anisotropy - measuring of fluorescence polarization - study of interactions between biomacromolecules and other molecules (interaction of receptors and signal molecules, the mechanism of antigen - antibody "expand and shrink" molecules of proteins and nucleic acids, molecular dynamics of biological membranes, transport across the membrane interactions on membranes, characterization of ambient fluorophores in cells and cell organelles – determination of the polarity, pH, type of interaction;
- chemiluminescent measurement - study detailed mechanisms and energy of some biochemical reactions;
- application of fluorescent labels and their interactions e.g. with cells, cell organelles, intracellular structures;
- in fluorescence scanning (synchronous spectra) hard surface skin and mucous membranes is a unique method of early diagnosis of precancerous conditions;
- analysis by high performance liquid chromatography (HPLC) with UV / VIS and fluorescence detection for fractionation of biological material required for exact identification of molecules;
- practical possibility of combining the designated spectral fluorescence techniques with other methods (e.g. microscopy), separation (e.g. HPLC, electrophoresis), an immunology method (e.g. ELISA), isolation and molecular-genetic methods (e.g. PCR, Western blot, electrophoresis).

C. The study of selected natural/synthetic compounds and their influence on the biochemical functions of organism (also on the subcellular level)

The set scientific goals that we want to implement by the methods used in our department as well as the introduction of new methods and procedures. It is a method:

- clinical biochemistry metabolites (e.g. glucose, urea, creatinine), enzymes (e.g. SOD, ALP), proteins (e.g. Hsp, cytokines);
- isolation of mitochondria from different organs can be extended to other subcellular particles, biological membranes, whole cells, etc.;
- the use of mitochondria as defined living system to test substances interactions of their influence on cell respiration using Clark's oxygen electrode;
- monitoring DNA damage by comet assay method.

5. RESEARCH INFRASTRUCTURE

Overview of the institute's instrumentation:

- ***Luminescence spectrophotometer Perkin Elmer LS55*** – with additional accessories: polarizer, the biokinetics, remote fiber optic
- ***Randox Monza RX*** – semi-automated biochemical analyzer with variety of clinical application available in serum, plasma, urine and CSF of patients

- ***Quantimetrix LipoPrint*** – electrophoretic separation of lipoproteins, which allows to identify and determine the percentage of atherogenic sub fractions in the blood of patients
- ***Becker Coulter Optima MAX-XP*** – ultracentrifuge suitable for the preparation and isolation of subcellular particles, especially mitochondria
- ***Automated device to determine the solubility of solids*** - original equipment to determine the dissociation constants, stability constant of a ligand of metals in living systems (potentiometric device with the solubility measurement)
- ***HPLC (Shimadzu)*** – liquid chromatography system with UV/VIS a fluorescence detection
- ***PCR cycler TECHNE TC/3000 (Barloworld Scientific)*** – conventional PCR system
- ***Real-time BIORAD CFX96*** –apparatus for quantifying gene expression with precise thermal control
- ***Qiagene Rotor-Gene Q*** – ***Real***-time PCR cycler and high resolution melt analyzer - device for quantifying gene expression
- ***ROCHE LightCycler® 480 Instrument II*** – Qualitative and quantitative detection of nucleic acid mutation scanning and analysis of single nucleotide polymorphisms (SNP)
- ***Randox evidence investigator-Biochip Array*** – immunoassay analyzer, single nucleotide polymorphism genotyping, gene expression monitoring, detection of pathogens and mutations
- ***Elisa immunoanalyser Dynex DS2*** – fully automated ELISA processing system for quantification of specific labeled proteins
- ***Syngene G:Box system*** – detection and documentation system for the visualization and evaluation of UV and chemiluminescent signal, cooled camera photo shoot
- ***Biorad Trans-Blot SD*** – semi-dry apparatus for DNA/RNA/protein transfer from separating gel to NC membrane
- ***Spectrophotometer NanoDrop 2000c*** – UV/VIS spectrophotometer, measurements in wide spectral range (190 – 840 nm), require only 0,5-2 µl of sample, suitable for nucleic acids or protein quantification
- ***Fluorescence spectrophotometer NanoDrop 3300*** – measurements in spectral range 300-750 nm, require only 0,5-2 µl of sample
- ***Hansatech Oxygraph Plus (Clark electrode)*** – PC operated oxygen electrode system allows respiration measurements
- ***Additional equipment*** – e.g. analytical balance, electrophoresis apparatus, spectrophotometers, pH meters, thermostats, equipment for deionized water

6. EXPECTED BENEFITS TO SOCIETY

A. The benefit lies in the possibility of detection of different phases of cancer using non-invasive examination of body fluids with possible practical outcome in routine screening during the preventive examinations. Furthermore, research could contribute to earlier relapses onset detection, therapy monitoring, and prognosis evaluation whit an impact on the quality of life of patients.

B. The findings of fluorescent profile comparative analysis of body fluids should bring novel, accurate, fast and reliable algorithms helping in disease diagnostics (e.g. oncology, metabolic). Getting more detailed knowledge of the molecular mechanisms of interactions of substances in living systems, especially in biological fluids could then contribute to the early detection of various pathological conditions.

C. The study of reactive species of oxygen and nitrogen, as well as antioxidants and their role in numerous physiological processes contribute to the extension of knowledge base about oxidative stress, and could be used in the prevention and treatment of selected diseases (e.g. atherosclerosis).