PROTEOMICS DEPARTMENT

***Felicia Antohe**, PhD*
HEAD OF DEPARTMENT

STAFF

***Luminița (Rădulescu) Ivan**, PhD*
***Elena (Puchianu)Uyy**, PhD*
***Raluca (Haraba) Boteanu**, PhD*
***Viorel Suica**, PhD*
***Aurel Cerveanu-Hogas**, Research assistant*

FORMER RESEARCH STAFF:

Constantina Heltianu, *PhD*
/Head of Cellular Receptors Department: 1979-2004/
Eliza Vasile, Ludy Dobrilă, Leni Georgescu,
Geo Șerban, Viorica Mădălina Cojocaru,
Anca Baciu.

CORE LABORATORY UNIT: ***Mass Spectrometry***

PROTEOMICS DEPARTMENT

Felicia Antohe, *PhD*

Head of Department
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Major position/appointments and professional training

- Member of the Scientific Council
- Ph.D. Coordinator; Advisor for Graduate and Master Programs
- Research fellow: *McGill University, Montreal, Canada*.
- Visiting scientist: *University of Alberta, Edmonton Canada; Max Planck Institute, Bad Nauheim, Germany; University of Texas, Dallas, USA; Molecular Biology and Genetics, Dresden, Germany; Université René Descartes, Paris V, France; L'Université des Sciences et Technologies de Lille I, France.*
- Expert Evaluator/Reporter: *EC Framework; Ministry of Education and Research (MER); National Council of Research and High Education: National Framework for Research, Development and Innovation; Invited peer reviewer for international journals.*
- President of the CNACTCU Commission of MER for high academic degrees (2006-2011).
- Invited Romanian Representative of the Central and Eastern European Proteomic Conference (CEEPC). www.ceepc.eu.
- Member of the Management Comity of the EU FPH 2020 COST Action "CliniMARK" CA 16113 (2016-2020). www.cost.eu/COST Actions/ca/CA16113.

MAJOR RESEARCH INTERESTS

- **Mechanisms of early atherosclerosis and/or plaque instability in Coronary Artery Disease;**
- **Mass spectrometry-based proteomics applied for biomarker research in chronic non-communicable diseases (atherosclerosis, diabetes, cancer, immune disorders);**
- **Membrane microdomains (lipid rafts, plasmalemmal vesicles, caveolae): structural and functional characterization;**
- **Transport of macromolecules in vascular cells: cell receptors, transcytosis, endocytosis.**

PUBLICATIONS

Over 98 original articles (>1950 ISI citations) and data sets were published in collaboration in Web of Sciences journals and 16 monographs in book chapters or reviews during 1979-2019.

- Original data included in Proteomic Database repository: **PRIDE** and **RaftProt**
- One patent in collaboration RO132002-AO
- 3 articles with over 200 citations, 2 articles with over 100 citations

SELECTED NEW FINDINGS OF THE DEPARTMENT

• Alarmins (or DAMPs: danger associated molecular patterns molecules) involved in atherosclerosis include but are not limited to: **HSPs 27, 60, 70, 90, Galectin-3, Annexin A1, Serpin H1, Histone H4 and H1.4**. Their abundance and the complex inflammatory environment generated by hyperlipidaemia are major risk factor for atherosclerosis.

• Mass Spectrometry based proteomics data support the hypothesis that caveolae interact with **cytoskeleton and other structural proteins** (actin, annexin II, filamin and dynamin) and **regulate the transport of macromolecules and the caveolae budding** under high fat stress.

• **HMGB1 protein is an active regulator** of the **vascular barrier** that modulates the expression of specific adhesion molecules for monocytes and macrophages on the endothelial cells surface.

• Mass spectrometry proteomic analysis designated a specific pattern of **S100 family proteins** in the pancreatic cancer.

• The hyperlipidaemic stress induced significant changes in the membrane-cytoskeleton proteome. At least 29 new identified proteins take part in: Regulation of the actin cytoskeleton, Focal adhesion and Adherens junction signalling pathways, **proving membrane-cytoskeleton tight coupling**.

• **Folic acid** is avidly taken up by **activated macrophages** in experimental hyperlipidaemia.

• Altered expression of endoplasmic reticulum **molecular chaperone** (HSPs: Heat Shock Proteins) are potential **active factors in thyroid tumorigenesis**; among them BiP and GRP94 act as biomarkers that discriminate benign follicular thyroid adenoma (FTA) over follicular variant of papillary thyroid carcinoma (FVPTC).

• Increased expression of **HMGB1 protein modulate** the inflammation both in experimental atherosclerosis and diabetes through **RAGE/pAKT1/beta-catenin pathway.**

SELECTED PREVIOUS RESEARCH PROJECTS/ PUBLICATIONS

(In collaboration with former research staff)

• **Endothelial cell receptors; Transport of macromolecules in vascular cells.**
Publications: Antohe et al., Microcirculation Endothelium and Lymphatics, 1986, 1988; Vasile et al., Atherosclerosis, 1989; J Submicrosc. Cytol. Pathol., 1991; Antohe et al., Eur. J Cell Biol.,1993,1999; Antohe and Poznansky, Pharmaceutical Enzymes, 1997; Antohe et al., Hum. Immunol., 2001; Borvac et al., Int Immunol.,1998; Firan et al., Int Immunol., 2001; Antohe et al., Endothelium., 1997; Dobrila et al., Int Immunol., 1992; Antohe et al., Cell Tissue Res., 2005.

• **Albumin binding proteins function in receptor mediated binding and transcytosis of albumin across endothelial cells**.
Publications: Antohe et al. Eur J Cell Biol., 1991, 1993; Georgescu et al. Physiologie, 1986; Heltianu et al., Eur. J Cell Biol. 1994 and Microvasc Res., 1989; Radulescu et al.,Rev.Roum.Biochim.,1997; Antohe et al., Eur J Cell Biol., 1998.

• **Endothelial heart-type fatty acid binding proteins (FABP) are the main carriers for fatty acids.**
Publications: Antohe et al., Eur J Cell Biol.,1991;1998; Antohe et al., J Liposome Res., 2004.

• **Human placental endothelial cells express neonatal receptors (FcRn) which discriminate and monitor the intracellular pathway of IgG.**
Publications: Jinga et al., Placenta, 2000; Radulescu et al., Hum Immunol., 2004; Antohe et al., Hum Immunol., 2001, 2004.

• **Low density lipoprotein (LDL) binding induces asymmetric redistribution of LDL receptors in endothelial cells**.
Publications: Antohe et al., Endothelium: J of Endothelial Cell Res., 1997; Antohe et al., Eur J Cell Biol., 1999.

• **Monoclonal antibodies as therapeutic tools.**
Publications: Radulescu et al., Current Problems in Cell and Molecular Biology, 2001; Antohe et al., J Liposome Res., 2004; Radulescu et al.,Med Sci Monit., 2004 and Hum Immunol., 2004.

• **Role of the folic acid receptors in the pathobiology of cardiovascular diseases.**
Publications: Antohe et al., Cell and Tissue Research, 2005; Antohe, Archives of Physiology and Biochemistry, 2006.

• **Hyperlipidemia induces endothelial cell dysfunction. Cellular and molecular mechanisms involved in the atherosclerotic plaques development**. Project PN II-IDEI-159/2007-2010.
Publications: Radulescu et al., Electrophoresis-Tech. Note, 2003; Antohe et al., Atherosclerosis Suppl., 2006, 2008; Ivan et al., J of Receptors and Signal Transduction, 2010; Uyy et al., Microvasc. Res., 2010; Haraba et al., Int. J of Cardiology, 2011; Haraba et al., Cell and Tissue Research, 2011; Haraba and Antohe, Digest J of Nanomaterials and Biostructures, 2011.

• **Eye dysfunction associated with inflammatory systemic disease.**
Publications: Cojocaru et al., Annals of the Rheumatic Diseases, 2004 and 2006; Oftalmologia, 2006; Cojocaru et al., Digest J of Nanomaterials and Biostructures, 2011.

• **Improvement of the diagnostic and follow-up protocols of differentiated thyroid cancer with new markers for a better treatment outcome, prognosis and quality of life**. Project No 135/2012 **GENITIR**, PN-II-PT-PCCA-2011-3, in collaboration with National Institute of Endocrinology "C. I. Parhon".
Publications: Uyy el al., J of Proteome Research, 2016; Baciu et al Roumanian reports in Physiscs, 2017; Popa et al., J of Molecular Endocrinology, 2018.

• **Mass spectrometry analysis of human pancreatic adenocarcinoma cell line (BxPc3) and tissue**. Project No 90/2012 **S100MAP**, PN-II-PT-PCCA-201-3, in collaboration with Fundeni Clinical Institute.
Publications: Antohe, Acta Endocrinologica 2015; Carmen C. Diaconu CC, Antohe F. et al., Patent No RO132002-A0, 2017; Nastase et al., J Transl. Med. Res., 2016; Ilie et al., FEBS J, 2014; Ivan et al., Cajal Symposium, 2011, 2012, 2014.

• **The proteome of new regenerated tissue induced by implants with bio-functional surface which are able to trigger certain healing phases typical for injured bone tissues**. Project No 90/2012 **FABIO3D**, PN-II-PT-PCCA-2011-3, in collaboration with National Institute for Research and Development of Materials Physics.
Publications: Boteanu et al., 2019 in preparation; Grumezescu et al., International J of Pharmaceutics, 2017; Socol et al., Digest J Nanomaterials and Biostructures, 2013; Antohe et al., Int. Winter School on Bioelectronics, 2014; Gadher SJ et al., Expert Rev Proteomics, 2018.

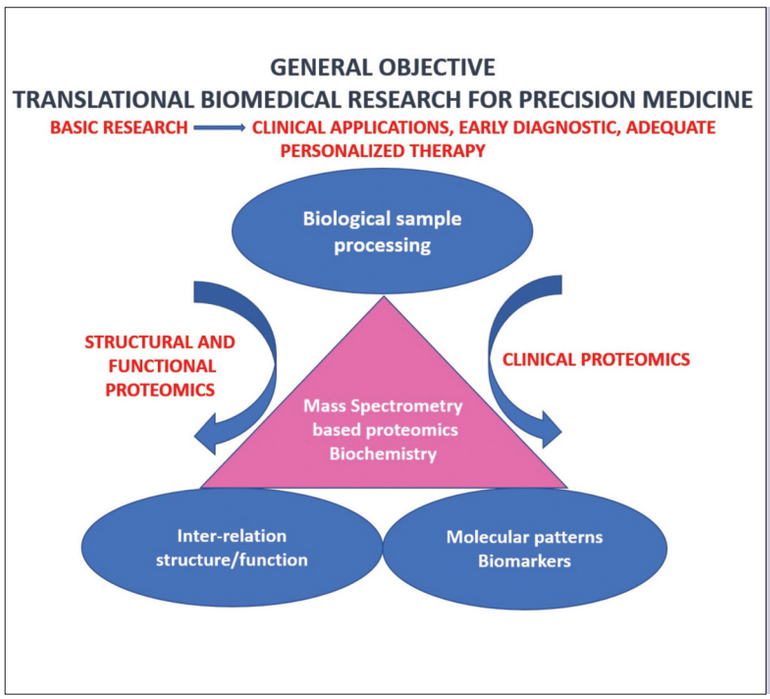
• **Cellular and molecular mechanisms that govern the molecular alteration in response to pathological stimuli in vascular cells by applying mass spectrometry based proteomics**:
analytical and functional proteomics to identify alarmins as biomarkers to be used in clinical practice.
Publications: Suica et al., Proteome science, 2015, BBA-Proteins and Proteomics, 2016; Uyy et al., Romanian reports in physics, 2017, Cell and Tissue Research, 2013; Boteanu et al., J of Proteomics 2017, Archives of Biochemistry and Biophysics, 2015, Cell and Tissue Research 2011, Int. J of Cardiology 2011; Gadher SJ et al., Expert Rev Proteomics, 2015-2019.

THE RESEARCH STRATEGY OF THE DEPARTMENT

To integrate our research activity into the modern medicine, recently called precision medicine, we need both smart approaches and powerful biotechnological methodologies. Thus it will be possible to rapidly transfer the basic research results toward clinical applications for early diagnosis and personalized therapy of each patient. The applied strategy has two steps:

First: the multidisciplinary research approach of the department extended the studies beyond the classical methods (biochemical, immunological, microscopy imaging, et al.) to top level structural and functional proteomic approach using high performance qualitative and quantitative mass spectrometry analysis, and

Second: we focused on alarmins, also known as damage-associated molecular patterns molecules (DAMPs) that are critical molecular biomarkers of the immune response to tissue suffering (Matzinger 1994).



Relevant clinical and surgical laboratory sampling will be biochemically and mass spectrometry analysed and statistically significant changes induced by insults or chronique diseases will be corroborated to generate maps of structure/function correlations that allow identification of molecular patters or biomarkers for clinical applications, early diagnosis and personalised therapy.

GENERAL OBJECTIVES OF THE DEPARTMENT

Using various bio-informatic tools to sort, select and characterize the specific signs of every disease we aim:

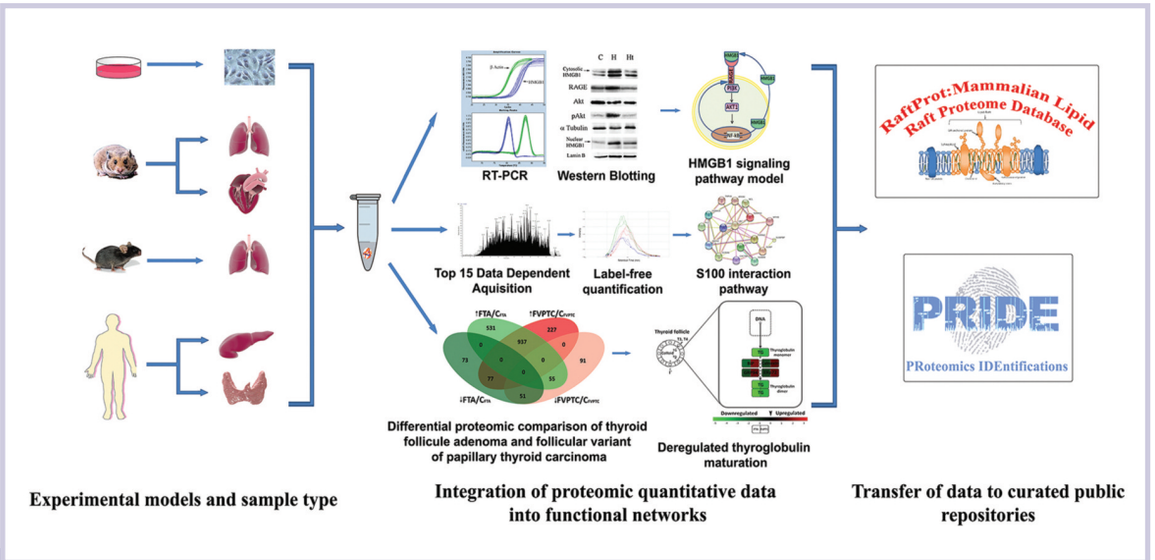
• To achieve the early recognition of the warning signs of a particular disease, cellular dysfunction and systemic inflammation;

• To identify early biomarkers for prevention, diagnosis and progress of the disease;

• To develop adequate strategies for treatment that target the cellular and molecular mediators.

EXPERIMENTAL PROCEDURES

To reach the proposed objectives a variety of experimental procedures was applied to evaluate the inflammatory response associated with chronic non-communicable diseases: atherosclerosis, diabetes and cancer.

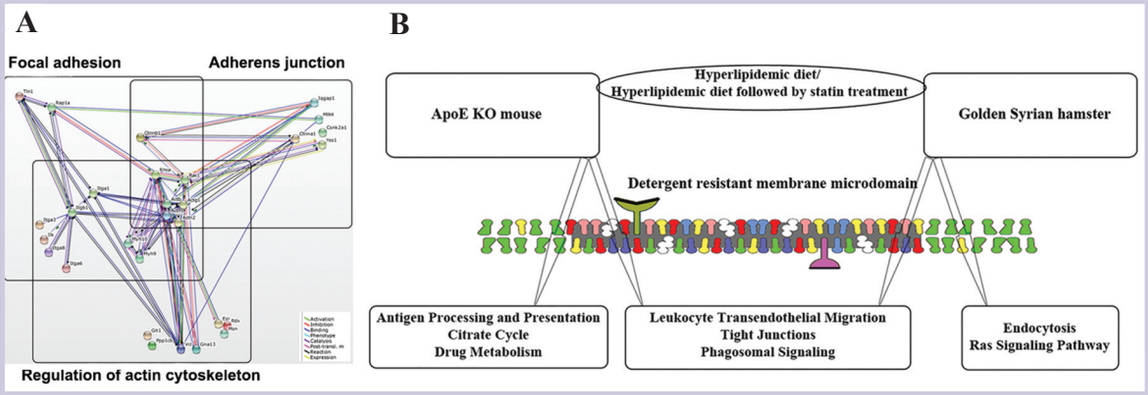


Graphical work flow of the powerful proteomic analysis using high performance Easy nano-LC II LTQ Orbitrap Velos Pro equipment (ThermoScientific) for the investigation of the inflammatory response induced by the expression of main alarmins (HMGB1, HSPs, S100 family) released under various stress factors as hyperlipidaemia, hyperglycaemia, or insults generating cancer, in different experimental biological system (cell culture, small animal models and human tissue).

RESULTS

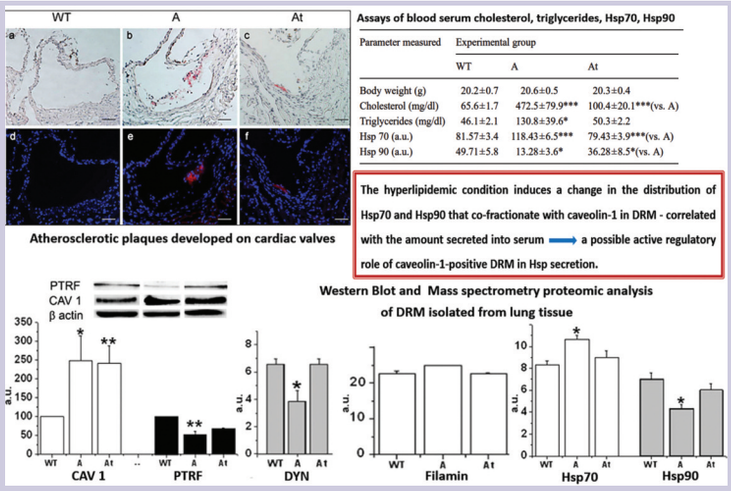
The designed experimental procedure, based on biochemical and mass spectrometry proteomic data uncovered the role of several heat shock proteins (HSPs), high-mobility group box 1 (HMGB1) protein and S100 proteins as the main alarmins involved in maintaining and amplifying inflammation in atherosclerosis, diabetes and cancer (RadulescuL., 2013; Antohe F., 2015; Boteanu R., et al., 2017).

The hyperlipidemic stress induced significant changes in the membrane-cytoskeleton proteome: 654 (out of 1925) proteins have statistically significant altered abundance and are involved in 13 KEGG signaling pathways while 29 of them take part in: Regulation of the actin cytoskeleton, Focal adhesion, Adherence junction **proving membrane-cytoskeleton coupling** and induced alteration by hyperlipidemia and/or statin treatment (A). (Suica et al., Proteome Sci., 2015).



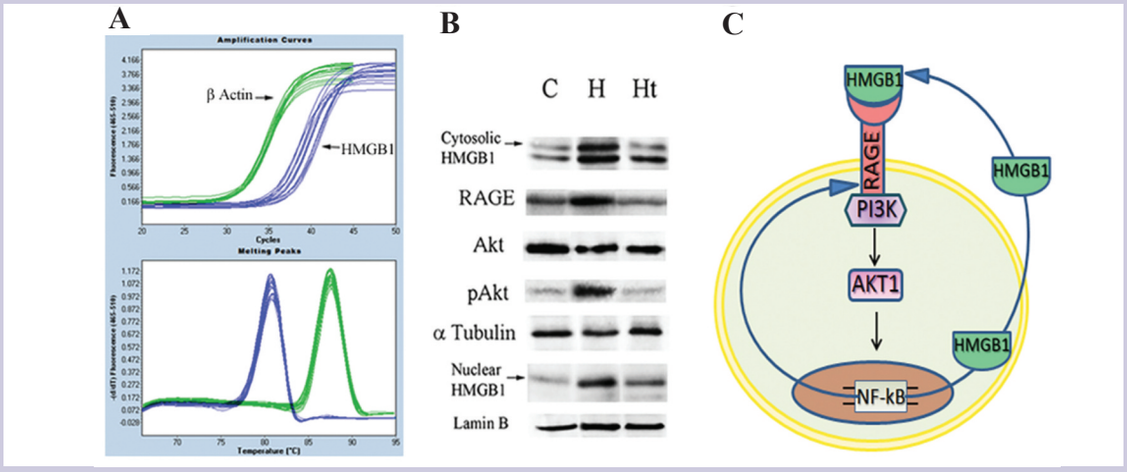
Employing latest generation liquid chromatographic and mass spectrometric approaches followed by specialized software analysis allowed us to discover with high degree of confidence protein molecules' inter-relation maps affected by hyperlipidemia and statin treatment such as leukocyte trans-endothelial migration, tight junctions, phagosome signaling, common to both types of organisms. However, the different methods to induce the high fat stress revealed uniquely altered signaling pathways in antigen processing and presentation, citrate cycle, extracellular matrix-receptor interaction, adherence junction and focal adhesion in one or the other organism (B). *(Suica et al., BBA, 2016).*

Mass spectrometry analysis of the detergent resistant membrane microdomains isolated from hyperlipidemic animals exposed or not to fluvastatin versus control demonstrated severe alterations of membrane protein composition. Alarmins involved in the atherosclerotic process include but are not limited to: HSPs 27, 60, 70, 90, Galectin-3, Annexin A1, Serpin H1, Histone H4 and H1.4. Their abundance correlated with the complex inflammatory environment generated by hyperlipidemia, a major risk factor for atherosclerosis (Boteanu et al., 2017).



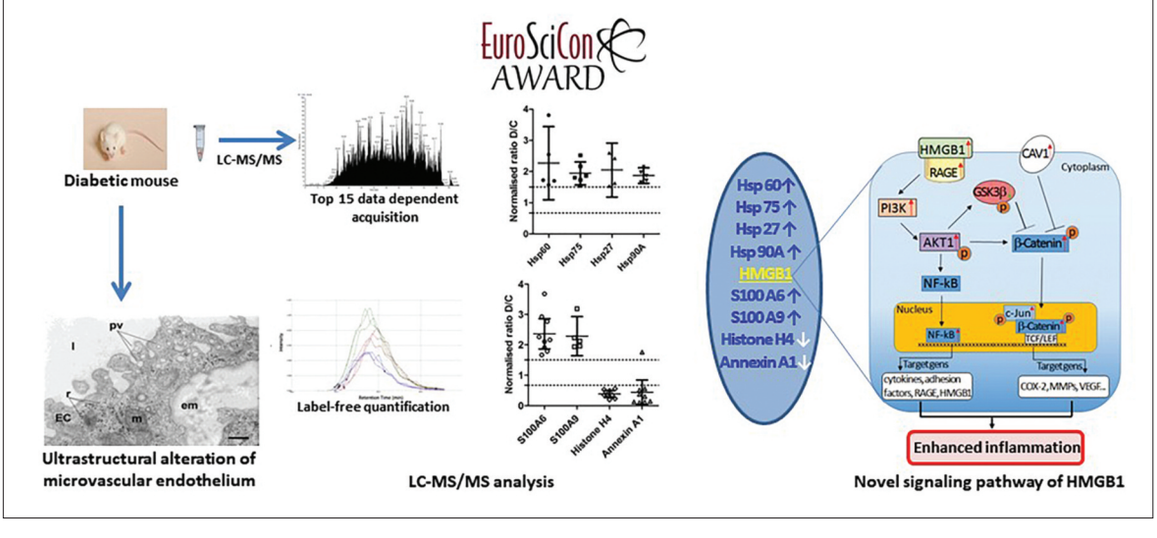
Mass spectrometry and biochemical data supports the hypothesis that caveolae interact closely with the cytoskeleton elements and other structural proteins including: ***actin, annexin II, filamin and dynamin*** *to regulate the transport of macromolecules and the budding dynamics of caveolae under high fat stress* *(Uyy et al., 2013).*

In experimental hyperlipidemia increased expression of HMGB1 was evidenced in serum, lung and cardiac tissue, closely related to the up-regulation of RAGE and AKT1 phosphorylation. HMGB1 is an active regulator of the vascular barrier that modulates the expression of specific adhesion molecules on the endothelial cell surface causing a particular behavior of the monocytes and macrophages (Boteanu et al.,2015; Haraba et al 2011).



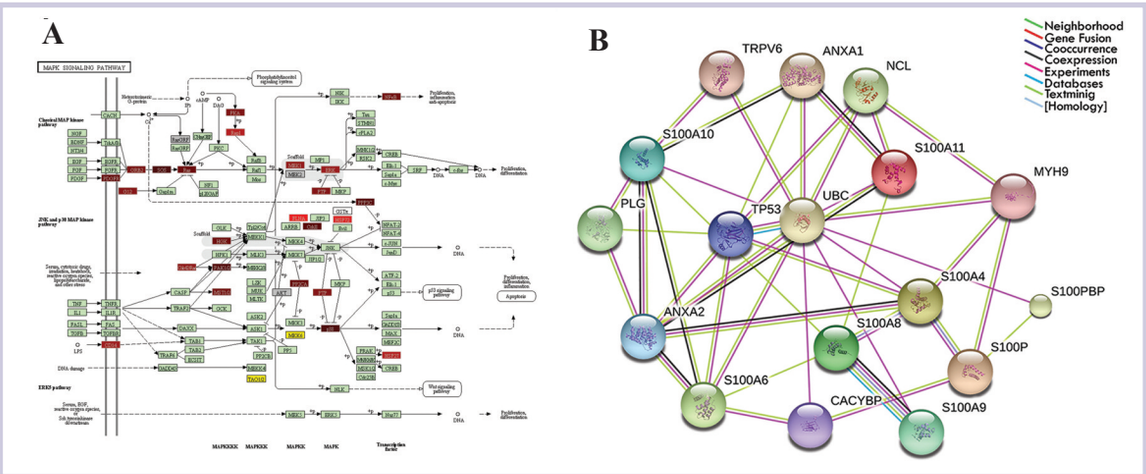
Representation of HMGB1 signaling events in the heart tissue of animals fed a lipid rich diet. (A) mRNA level of HMGB1 normalized to β-actin (real time PCR); (B) immunoblotting using HMGB1, RAGE, AKT1, phospho-AKT1 (Thr-308), a-Tubulin and Lamin B antibodies followed by the appropriate HRP coupled secondary antibodies; control group: C, hyperlipidemic group: H, fluvastatin treated hyperlipidemic group: Ht; (C) Original representation of HMGB1 signaling events in the heart tissue of animals fed a lipid rich diet (Boteanu et al., 2012).

Diabetes induced vascular ultrastructural changes related with differential expression of potential biomarkers, such as alarmins or damage associated molecular patterns (DAMPs) which stimulate and amplify the inflammatory process at molecular level.



In diabetes systemic inflammation leads toward metabolic and chronic complications sustained by activated endothelial cells that display a higher number of caveolae and up-regulation of alarmins, such as AnnexinA1, Histone H4, HSPs and S100 proteins. Damaged pancreatic beta-cells release HMGB1 that initiate an inflammatory autoimmune response through RAGE/AKT1/beta-catenin new demonstrated signalling pathway (Boteanu et al., 2015).

The research performed in the PROTEOMICS department added new lines of evidence based on mass spectrometry research and powerful bioinformatics that S100 protein family is often associated with ductal pancreatic adenocarcinoma (DPAC) and the significant alteration of proteins involved in the MAP4K4 signaling pathway.



(A). MAP4K4 signaling pathway in human DPAC showing the 27 out of 38 identified proteins that were significantly altered in malign tissue. (B). Multiple interactions of identified S100 proteins in DPAC revealed by STRING 9.1 analysis, predicting the functional patterns in pancreatic cancer (coloured lines show the types of association between proteins).

Inflammation and cancer are closely connected since tumour development requires a special microenvironment that seems to be secured by an intrinsic inflammatory mechanism. The applied shotgun nano-flow liquid chromatography coupled to high-performance tandem mass spectrometry identified and quantified proteins that are significantly altered in the thyroid hormone synthesis pathway in benign follicular thyroid adenoma versus follicular variant of papillary carcinoma.

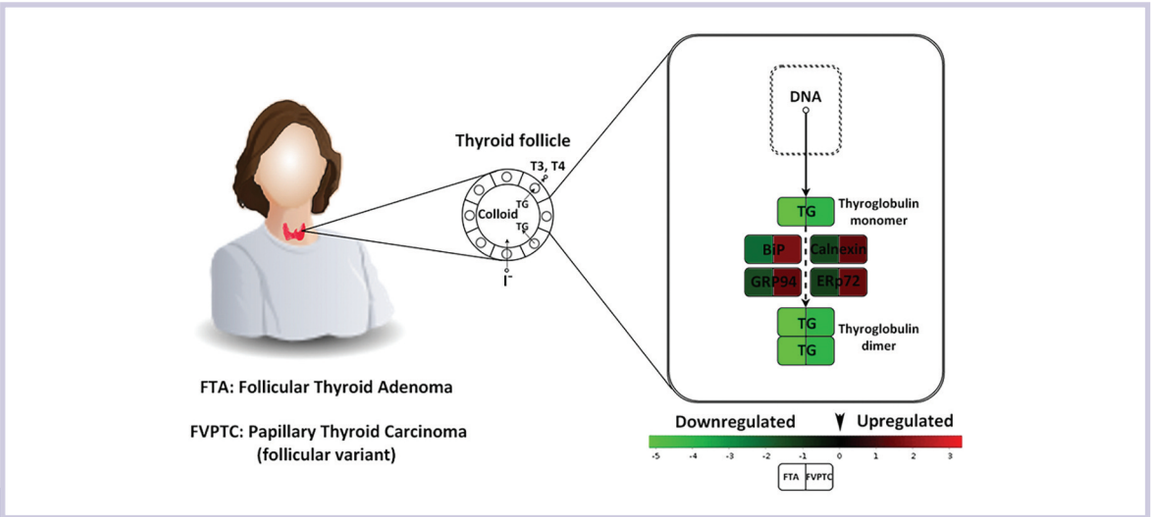


Diagram showing the deregulated expression of four endoplasmic reticulum chaperones BiP, GRP94, CANX and ERp72 (78kDa glucose-regulated protein, endoplasmin, calnexin, protein disulfide isomerase A4 respectively) that highly discriminate between benign and malignant thyroid lesions and may represent good candidates as molecular biomarkers to be used in clinical practice. Data are available via ***ProteomeXchange PRIDE with identifier PXD004322.*** *(Uyy et al., J Proteome Res. 2016).*

The results of the department were published in peer reviewed papers and selected data sets were included in centralized, standards compliant, public domain repositories: *The* ***RaftProt*** *(Mammalian Lipid Raft Proteome)* and ***PRIDE*** *(PRoteomics IDEntifications)* databases as reliable mass spectra validated evidence, handled by expert bio-curators (PRIDE EMBL-EBI, http://www.ebi.ac.uk/pride).

NEW ORIGINAL FINDINGS (2009-2019)

• **Alarmins** can serve as valuable biomarkers of chronic non-communicable diseases (atherosclerosis, diabetes, cancer).

• **High lipid stress** induces increased expression of specific endoplasmic reticulum **HSPs** in endothelial cells.

• **HMGB1** modulate the **inflammation** in **atherosclerosis** and **diabetes through RAGE/ pAKT1/ β-catenin pathway**.

• Mass spectrometry reveals specific **patterns of S100** proteins in pancreatic cancer.

• Endoplasmic reticulum chaperones **(HSPs)** are potential **active factors** in thyroid **tumorigenesis**.

• Selected pattern of **HSPs** forms a potential biomarker panel that discriminates benign **follicular thyroid adenoma (FTA) over follicular variant of papillary thyroid carcinoma (FVPTC)**.

CURRENT PROJECTS

1. Mechanisms of early atherosclerosis and/or plaques instability in Coronary Artery Diseases. European Joint Transnational Collaborative Project on Cardiovascular Diseases (EU JTC 2017, ERA-CVD, **XploreCAD** No.41/2107, Project Coordinator: Rune HANSEN, PhD; Project Romanian Coordinator: Felicia ANTOHE, PhD);

2. Targeting innate immune mechanisms to improve risk stratification and to identify future therapeutic options in myocardial infarction (PN-III-P4-ID-PCCF-2016-0172, **INNATE-MI**, No. 5/2018, Project Coordinator: Acad. Maya Simionescu; Colaborator Proteomic Department);

3. Dynamics of the endothelial junctional proteins in HMGB1-induced angiogenesis; Clinical implications (PN-III-P1-1.1-PD-2016-1369, **Ep-Angio**, No. 138/2018, Project Coordinator: Raluca Boteanu, PhD);

4. Multifunctional microbubbles for improved image-based diagnosis and drug delivery (FRI Grant awarded by FRIPRO-program from The Research Council of Norway. **MULTIBUBBLE** No: 240410/F20, 2015-2018. Project Romanian Coordinator Felicia ANTOHE, PhD).

1. XploreCAD PROJECT

State of the art:

Atherosclerosis is considered a multifactorial disease with risk factors ranging from high-fat diet, hypertension, smoking, diabetes, to genetic susceptibility and other factors. Classic statin treatment has led to significant reduction of clinical events, but considerable residual risk of cardiovascular related mortality still remains.

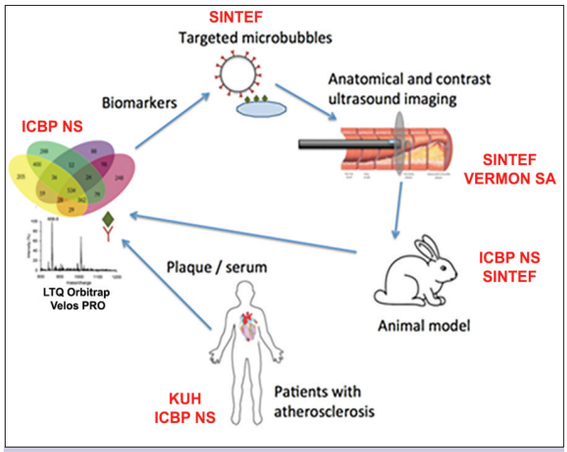
These days the scientific community face a significant change in the pathology of atherosclerotic lesions that is gradually shifting from the lipid rich plaque towards more stable, fibrous and less inflammatory lesion. Further studies are much needed to understand these complex mechanisms. Many hopes were raised by the development of OMICS projects with the promise to achieve personalized healthcare improvements.

Objectives:

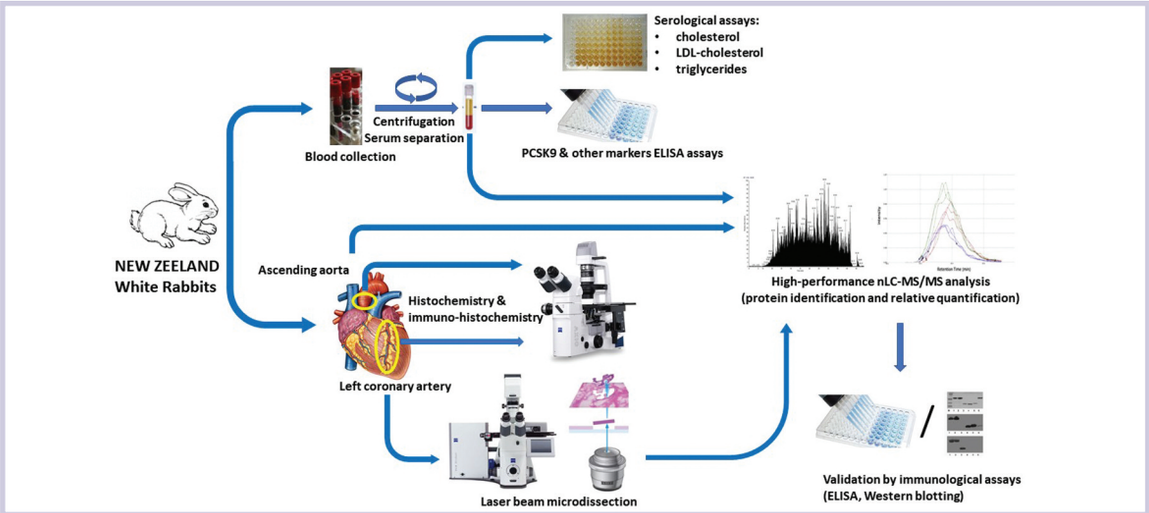
The strategy of this European collaboration project is to extend the studies beyond the classical methods, to top level proteOMIC approach using high performance qualitative and quantitative mass spectrometry equipment, namely LTQ Orbitrap VelosPro with the highest power of resolution and sensitivity of targeted proteomics and the extremely selective triple quadrupole mass spectrometer TSQ Vantage. These equipments were recently acquired in the frame of a grant from the European Community Structural Funds, CARDIOPRO Project (2009-2012).

The transnational EU ERA-CVD JTC project put together the efforts of four European partners (Stiftelsen SINTEF, Norway; ICBP NS, Romania; Vermon SA, France and Karolinska University Hospital (KUH), Sweden) aiming:

- **to identify potential biomarkers** of coronary artery disease
- to use the best biomarkers with **novel ultrasound technology** for improved **imaging of coronary plaque**



Collaborative activities within consortium in the work flow during XploreCAD project implementation.



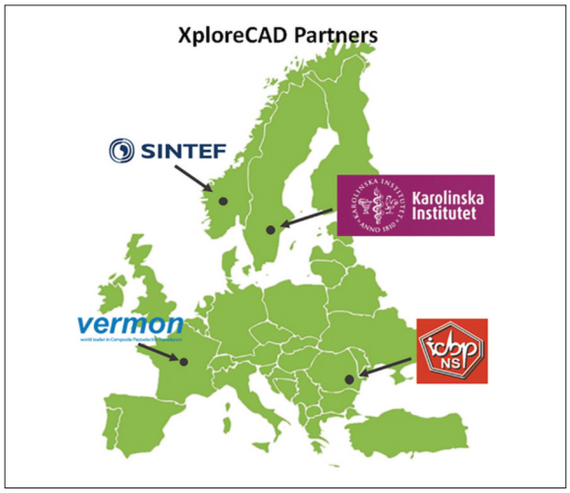
The novel design of the experimental model for atherosclerosis and the smart selection of clinical, biochemical and mass spectrometry technologies were carefully selected to unveil the potenial biomarkers responsible for the residual risks in cardiovascular disease under treatment with low lipid drugs.

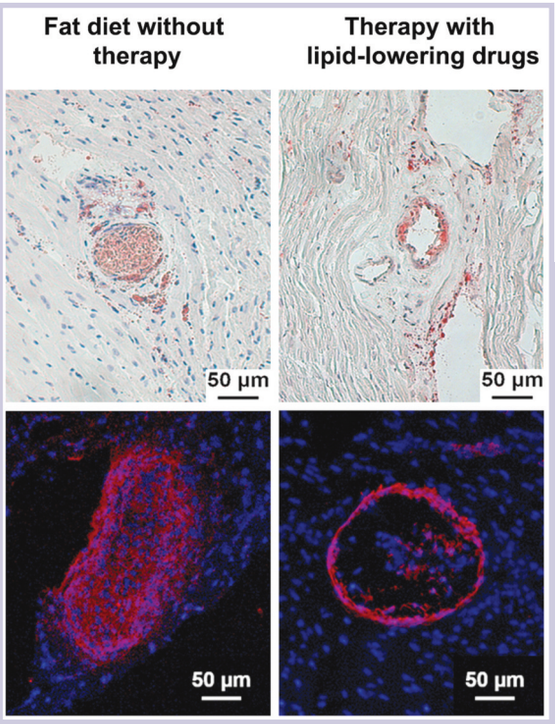
Methodology:

Alarmins, the host biomolecules that can initiate and perpetuate a noninfectious inflammatory response are critical players of coronary artery disease (CAD) plaques progression. By analyzing blood and plaque samples from both patients and animal models, the XploreCAD project will identify potential biomarkers of coronary artery disease. Using a new atherosclerotic animal model, the New Zeeland White Rabbits exposed to hyperlipidemia in the presence or absence of statins in association with PCSK9 inhibitors, we can safely assume that the performant technologies proposed will evidence modified profiles of pro- inflammatory cytokines, adhesion factors and regulatory proteins that can reduce atheroma plaques for the benefit of human health.

Progress / Intermediary results

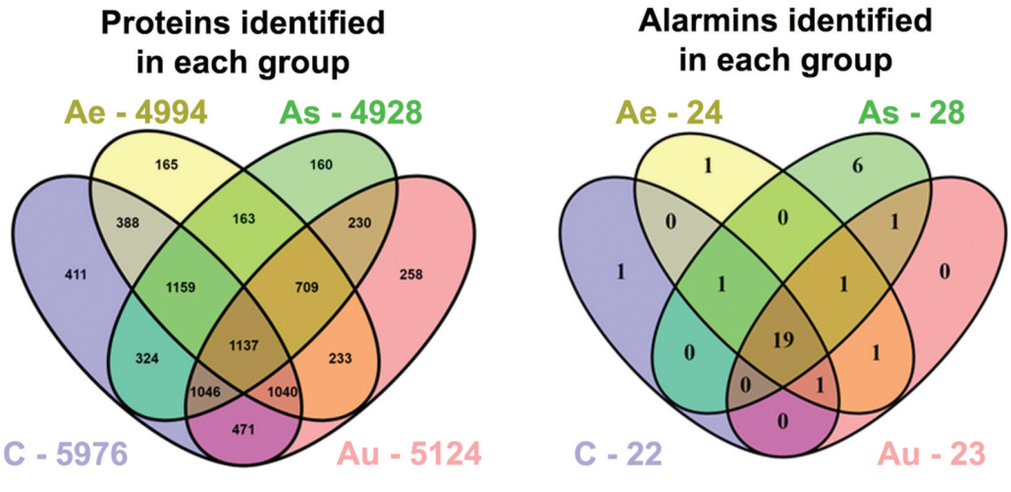
• After 12 weeks the tissue fragments harvested from all animal groups demonstrated that atherosclerotic advanced unstable plaques were developed in the lesion pron-areas of all animals fed high fat diet and the applied treatment reduced significantly the plaques dimension and also changed the histologycal aspects of the lesion.





Fat diet without terapy
Therapy with Lipid-lowering drugs

Atherosclerotic plaques developed in coronary arteries in New Zeeland white rabbits. Semi-thin frozen sections stained with Hematoxilin and Oil Red O (upper panel) and indirect immunofluorescence of semi thin frozen sections stained with Anti-alfaSMA (Alexa 594) and Höechst (lower panel).



Mass specrometry based proteomics analysis revealed a high number of identified proteins in all axperimental groups. After appropriate validation, the normalised alarmins quantified may be used as biomarker candidates. C: control standard diet; Ae: athero diet followed by standard diet; As: athero diet followed by standard diet and treatment 1 month;

Au: athero diet 3 months.

2. INNATE-MI PROJECT

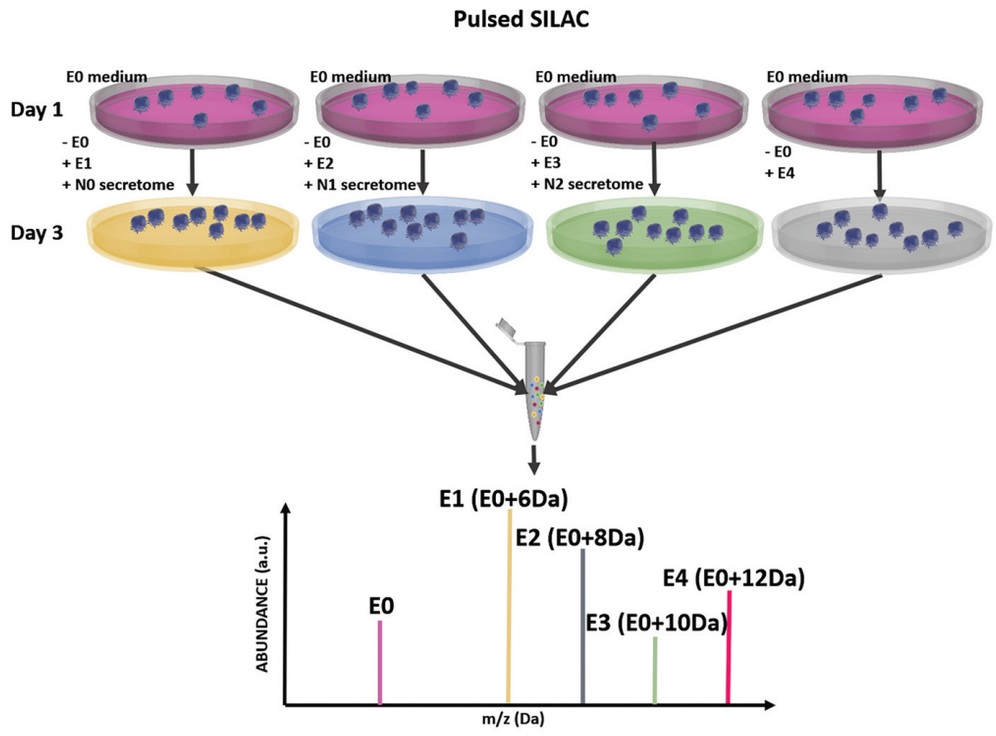
Objectives:

The PROTEOMICS department will add the human and technological resources to the complex and innovative **INNATE-MI** research project coordinated by Acad. Maya Simionescu.

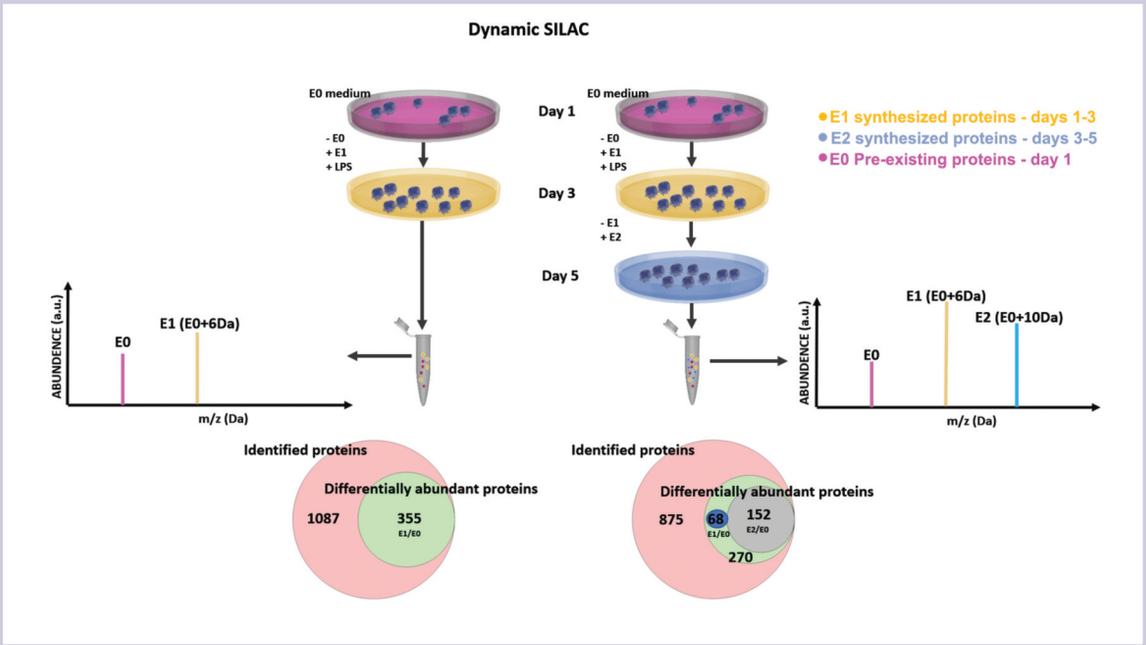
• To unravel key immune mediators that induce macrophage polarization under the influence of neutrophil secretome using high-precision proteomics.

Methodology:

The Stable Isotope Labeling with Amino acids in Cell culture (SILAC) approach will be used. The technique based on mass spectrometry will allow the quantitative detection of differences in protein abundance among samples using non-radioactive isotopic labeling. The choice of SILAC, a very powerful method to study cell signaling, post translation modifications such as phosphorylation, protein–protein interaction and regulation of gene expression will also allow the global study of secreted proteins and secretory pathways during cardiac recovery post myocardial infarction.



Using pulsed SILAC, newly synthesized macrophage proteins under the influence of secretomes of various phenotypes of neutrophils are tagged with stable isotopes. Parity mixture of all cell lysates can then be used to detect in a single experiment the influence of the secretomes on the macrophage protein level.



Dynamic SILAC is used to detect the macrophage protein turnover as a consequence of incubation time and various stimuli.

Progress/ Intermediary results

The complex methodological approach and up-dated bioinformatics will identify some immune mediators and signaling pathways that govern the crosstalk between polymorphonuclear (PMN) and macrophage (Mac) sub-populations that control the inflammation/repair balance post myocardial infarction. Preliminary results evidenced proteins with an altered mass spectra abundance were mostly of cytoplasmic, cytosolic, membrane and nuclear origin implicated in regulation of biological processes and cell organization and biogenesis with molecular function of protein interactions and catalytic activity. In addition statistically relevant cellular pathways, such as Carbon Metabolism, Glycolysis and/or Gluconeogenesis or Pentose phosphate pathways were pointed out. Further studies were in progress to validate these results.

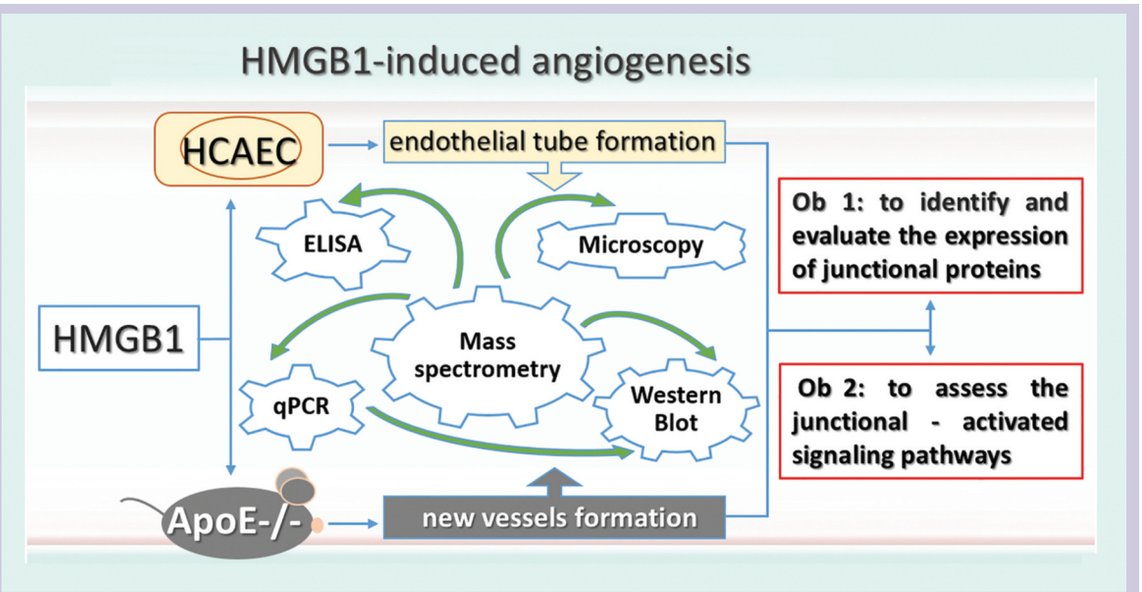
3. EP-ANGIO PROJECT

Objectives:

To further unveil the mechanism of plaque development through active angiogenesis stimulated by the high mobility group box 1 (HMGB1) we designed experiments on culture endothelial cells:

• To identify and evaluate the expression of junctional proteins (integrins, zonula occludens proteins, catenins, etc.) and

• To assess the junctional-activated signaling pathways that mediate various steps of angiogenesis: migration, invasion, tubulogenesis.



The project chart indicates the in vitro and in vivo models of angiogenesis induced by HMGB1 and the methodology that will be used to accomplish the proposed objectives.

Progress / Intermediary results

The preliminary results showed that the HMGB1 increases significantly the migration of cultured human coronary artery endothelial cells (HCAEC) and maintained their capability to generate tubes and to form networks, indicating the pro-angiogenic capacity of this alarmin.

Perspectives

Despite the progress of medical sciences of the past 30 years, many disorders of the cardiovascular system remain unsolved. The need for early recognition of the warning signs of particular diseases, cellular dysfunction and systemic inflammation translated by early biomarkers would be the real support for prevention, diagnosis and adequate strategies for treatment that can selectively target the cellular and molecular mediators.

The general future focus will be directed toward the rapid transfer of proteomics research data generated with the top mass spectrometry technology to clinical practice for the benefit of patients and the development of precision medicine.

COLLABORATION

INTERNATIONAL

- Münster University, Germany (Prof. F. Spener)
- University of Texas at Dallas, USA (Prof. V. Ghetie; Prof S.E. Ward; Dr. J. Borvac; Dr. M. Firan)
- University of Alberta, Edmonton, Canada (Prof. M.J. Poznansky; Prof. T. Allen)
- McGill University, Montreal, Canada (Prof. J.J.M. Bergeron and Prof. B.M. Kopriwa)
- Purdue University, Indiana, USA (Prof. P.S. Low)
- Stiftelsen, SINTEF, Trondheim, Norway (Dr. R. Hansen)
- Vermon, SA, Tours, France (Dr. M. Legros)
- Karolinska University Hospital, Sweden (Dr. K. Caidahl)
- The Luxembourg Clinical Proteomics Center (LCP), (Dr. B Domon)
- University of Groningen, Groningen, Netherlands (Prof. Dr. Rainer Bischoff)
- Clinical and Cancer Proteomics, Erasmus MC, Rotterdam, Nederlands (Prof. T.M. Luider).
- Proteomics and Metabolomics Scientific ThermoFisher research group (Dr. M. Oppermann)

NATIONAL

• National Institute of Endocrinology "C.I. Parhon"
• "Cantacuzino" National Institute of Research and Development Medical-Military
• Fundeni Clinical Institute
• Virusology Institute "Ștefan S. Nicolau"
• Oncology Institute "Alexandru Trestioreanu"
• Institute of Biochemistry of the Romanian Academy
• National Institute for Research and Development of Materials Physics
• National Institute for Research and Development of Physics of Laser, Plasma and Radiation
• ProAnalysis Sistem, research department.
• Agilrom Scientific SRL, research department.
• SC TECHNOMED IMPEX CO SA
• SC RNTECH SRL

PARTICIPATION TO- AND GRANTS AWARDED BY COMPETITION (1979-2020)

Leadership and collaborative participation in 45 competitive research projects and 11 international research projects.

SELECTED INTERNATIONAL GRANTS

• **2001-2004** Grant FP5 ICA1-CT-2000-70020, Centre of Excellence of the European Community, Function and dysfunction of blood vessels: transcytosis in normal and pathological states, alterations in atherosclerosis and diabetes; their therapeutic control.
• **2005-2007** Grant FP6, SSA-EC 16873 Strengthening the European Research Area by Reinforcement of Romanian Research Competency in Genomics and Proteomics of Major Global Risk Diseases: Atherosclerosis, Diabetes and its Complications.
• **2008-2012** POS-CCE 143/SMIS CSNR 2667 Extension and modernization of the research infrastructure in order to increase competitiveness in the field of cardiovascular diseases, diabetes and obesity (CARDIPPRO), European Community Funds.
• **2015-2018** FRI Grant awarded by FRIPRO-program from The Research Council of Norway: MULTIBUBBLE No: 240410/F20.
• **2018-2020** JTC 2017 ERA-CVD project XploreCAD 41/2017, Advanced ex vivo analysis and multi-frequency ultrasound technology for improved evaluation and diagnosis of coronary plaque. European Community Funds.
• **2018-2022** Member of the Management Comity of COST Action CA 16113 CliniMARK: good biomarker practice to increase the number of clinically validated biomarkers. European Community Funds.

AWARDS

• **"Emil Racovita"** Prize of Romanian Academy, 1991 (Antohe F.)
• **EURESCO** Prize of the Euresco Conferences, Membrane Dynamics in Endocytosis-Molecular Regulation, Tomar, Portugal, 2001 (Antohe F.)
• **BIO-RAD Laboratories** Prize for valuable research, 2002, (Rădulescu L., Antohe F.)
• **3rd Award** of the International Congress of Medical Sciences, Bulgaria, 2003 (Cojocaru V.M., Antohe F.)
• **Summer School Fellowship:** Soft Condensed Matter Physics in Molecular and Cell Biology, Edinburgh, Scotland, UK, 2004 (Puchianu E.)
• Best scientific paper of the **National Congress of Internal Medicine** 2008, Romanian Society of Internal Medicine (Cojocaru V.M., Antohe F.)
• **"Ion Moraru" Award for Basic Immunology** 2004 *"Immnunodetection of caveolin-1 in membrane pulmonary fractions in diabetic mouse"*, The 34th National Immunology Conference, Bucharest, Romania (Puchianu E., Radulescu L, Popov D, Antohe F.)**.**
• **Best Poster of the Conference:** "*8th International Conference, Vascular Endothelium: Translating Discoveries into Public Health Practice*", 2005**,** Knossos Royal Village, Greece, (Rădulescu L., Stancu C., Antohe F).

• **COST STSM** 2008, in Proteomic analysis of lipid rafts in normal and hyperlipidemic condition. Host: Juergen Eckel, Institute of Clinical Biochemistry and Pathobiochemistry, Düsseldorf, Germany (V.-I.Suica PhD. student, Antohe F. PhD. coordinator).

•**3rd Award** for poster presentation at the Annual Meeting of the Romanian Society for Cellular Biology. (Suica VI, Ivan L, Uyy E, Haraba RM, Antohe F., Bistrița 2009).

• **3rd Award** for poster presentation at the Annual Meeting of the Romanian Society for Cellular Biology. (Haraba RM, Suică VI, Uyy E, Ivan L, Antohe F., Constanța 2010).

• **1st Award**: Phospho-proteome mass spectrometric investigation of folicular thyroid adenoma, (Suica VI, Uyy E, Boteanu RM, Ivan L, Antohe F, SRBC, 2016).

• **Scientific Achivements Journal Article Award** of Ministry for Education and Research. (Uyy E, Antohe F, Ivan L, Haraba R, Radu D, Simionescu M., 2010).

• **"Excellence Award"**, ASM Congres, Bucharest, 2015. Exression of S100 proteins in pancreatic cancer by nano-LC-MS/MS"- (Boteanu RM, Uyy E, Suica VI, Antohe F).

• **UEFISCDI Award**: Boteanu RM, Uyy E, Suica VI, Antohe F.- High-mobility group box 1 enhances the inflammatory process in diabetic lung. Arch Biochem Biophys. 583, 55-64, 2015.

• **Best presentation Award**: Suica VI, Uyy E, Boteanu RM, Antohe F, Detergent resistant membrane microdomains signaling pathways in two atherosclerotic animal models, 9th Central Eastern European Proteomics Conference (CEEPC), Poznan, Poland, 2015.

• **CNCSIS Award**: Lixandru BE, Cotar AI, Straut M, Usein CR, Cristea D, Ciontea S, Tatu-Chitoiu D, Codita I, Rafila A, Nica M, Buzea M, Baicus A, Ghita MC, Nistor I, Tuchiluş C, Indreas M, Antohe F, Glasner C, Grundmann H, Jasir A, Damian M.-Carbapenemase-Producing Klebsiella pneumoniae in Romania: A Six-Month Survey. PLoS One. 2015.

• **UEFISCDI Award**: Boteanu et al., J. Proteomics, 2017; Uyy et al., Romanian Reports, 2017; Baciu E., et al., Romanian Reports, 2017; Uyy et al., Journal of Proteome Research, 2016; Suica V., et al., Biochimica et Biophysica Acta-Proteins and Proteomics , 2016.

• **COST Action CliniMARL CA 16113 STSM 2019** for SRM training program for method implementation in the Proteomics Department of the Institute of Cellular Biology and Pathology "N. Simionescu", the Home Institute. Host institution: University of Groningen, Groningen, Netherlands, Dr. Rainer Bischoff (Researh fellow: Aurel Cerveanu-Hogas, Antohe F. PhD coordinator).