Ectopic fat and metabolic risk

Jeanina Albu

The New York Obesity Research Center, Saint Luke's - Roosevelt Hospital Center, University Hospital of Columbia University, Columbia University College of Physicians and Surgeons, 1111 Amsterdam Ave, New York, NY 10025, USA, jba1@columbia.edu

We are in the middle of a type 2 diabetes mellitus pandemic. Excess body fat but also fat localization contributes to the pathogenesis of type 2 diabetes mellitus and related cardiovascular diseases. An upper (central) body fat distribution is associated with risk factors, i.e. the "metabolic syndrome" components and with a higher probability of developing diabetes mellitus and cardiovascular disease, independent of body weight. An emerging concept is that ectopic fat accumulation is a major determinant of health risk. Fat accumulation in the subcutaneous adipose tissue depot is "safe" while accumulation of fat in the intra-abdominal (visceral) and inter-muscular (inside muscle fascia) adipose tissue depots as well as inside the insulin sensitive tissue (liver, muscle, pancreas and heart) is "deleterious" and represents increased "risk". Imaging techniques have attempted to assess the ectopic fat areas and relate them to specific metabolic, inflammatory and coagulation risks. The fat "overflow" hypothesis proposes that fat in muscle causes insulin resistance and decreased glucose uptake, fat in the liver causes insulin resistance and increased hepatic glucose output and fat in the pancreas causes beta cell destruction and decreased insulin secretion; all these result in type 2 diabetes mellitus in predisposed individuals. Assessment tools of the ectopic fat depots are being refined so better data can be obtained and mechanistic hypotheses can be proposed and studied. Better understanding of these processes will lead to more effective treatments.

Reduced adipose tissue oxygenation in human obesity

Magdalena Păsărică, Olga Sereda, Leanne Redman, Diana Albarado, David Hymel, Laura Roan, Jennifer Rood, David Burk, Steven R. Smith

Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA, pasarim@pbrc.edu

Introduction: Based on preliminary studies in rodents, we proposed the hypothesis that increased adipose tissue mass in obesity without an adequate support of vascularization might lead to hypoxia leading to macrophage infiltration and inflammation.

Methods: Oxygen partial pressure [AT pO_2] in abdominal subcutaneous adipose tissue [9 lean, 12 overweight/obese (obese) men and women] was measured by direct insertion [~1 cm under the skin] of a polarographic [Clark type] electrode. Prior studies in our lab validated the direct method against the method previously described by Hunt [R=0.64, $p<0.01$]. Body composition was measured by DEXA, insulin sensitivity by the hyperinsulinemic euglycemic clamp. Abdominal subcutaneous tissue was used for staining, qRT-PCR and secretion assays.

Results: By design, the obese group had greater BMI compared with the lean group [31.7$\pm$1.9 vs. 22.1$\pm$1.0; kg/m^2] and greater % fat [34.2$\pm$8.2 vs. 20.9$\pm$7.6; %]. As expected, obese subjects had lower insulin sensitivity as shown by the glucose disposal rates [6.0 $\pm$ 2.2] compared to lean subjects [GDR, 11.2 $\pm$ 3.4 mg/min*kg fat free mass]. AT pO_2 was lower in obese [47 $\pm$ 10.6 mm Hg] as compared to lean [55 $\pm$ 9.1 mm Hg]. AT pO_2 was negatively correlated with % fat [R=-0.50, $p<0.05$] and fat mass [R=-0.48, $p<0.05$]. Obese had lower capillary density [172$\pm$60 vs. 308$\pm$135 capillaries/mm^2] and lower expression of Vascular Endothelial Growth Factor [VEGF] [1.04$\pm$0.34 vs. 2.46$\pm$1.11] compared to lean subjects. AT pO_2 positively correlated with VEGF mRNA [R=0.54, $p<0.05$]. CD68 mRNA, secretion of MIP1α negatively correlated with AT pO_2 [R=-0.58, R=-0.79, $p<0.05$].

Conclusions: We provide evidence for the first time of reduced abdominal adipose oxygenation in obese subjects with or without type 2 diabetes mellitus [DM]. The strong correlations with adipose tissue inflammatory markers are consistent with a model where hypoxia drives inflammation. Reduced capillary density, in the face of adipose tissue hypoxia, suggests that defects in neo-vascularization lie upstream of both hypoxia and inflammation in obesity and DM. These results provide new insights into adipose tissue dysfunction in obesity and DM and suggest novel approaches to treat the dysfunctional adipose tissue found in obesity and DM.

Lights and shadows in exploration of the arterial smooth muscle cells dysfunction in hyperglycaemia

Doina Popov

Institute of Cellular Biology and Pathology "Nicolae Simionescu",
8 B.P. Hasdeu Street, Bucharest 050568, Romania, doina.popov@icbp.ro

Given the important role of smooth muscle cells (SMCs) in arterial wall dysfunction in diabetes, as well as in diabetes associated with accelerated atherosclerosis, I'll present a brief review of the recent achievements in identification of signaling molecules underlying SMCs altered responses in hyperglycaemia (HG). Basically, activation of glycoxidation and non-enzymatic glycation of proteins, of polyol and diacylglycerol pathways, of various protein kinase C isoenzymes, and of transcriptional activities leading to insulin resistance and local overproduction of free radicals takes place in SMCs exposed to HG. The overall result is the excessive growth, migration, and proliferation of arterial SMCs, and the disturbance of the delicate balance that maintains their normal contractile phenotype, as the dysfunctional cells turn into secretory and/or osteoblast-like cells. Several lines of evidence identified the dysmetabolic insults that promote HG-induced calcium deposition in the aortic wall, such as inflammation, shear and oxidative stress, hyperphosphatemia, and elastinolysis. However, biology of medial SMCs calcification is still poorly understood, and the current endeavors are directed towards limitation of calcium deposition by targeting the metabolism of vascular matrix vesicles, and introducing strategies to enhance their clearance. The original results emerging from the Golden Syrian hamster (rendered diabetic by streptozotocin injection) and from human aortic SMCs cultured in 5 and 25 mM glucose (to mimic physiological and diabetic condition, respectively) are presented in the context. I'll conclude the lecture with several open issues disclosed by the most recent literature that deserve essential attention for targeting the translational medicine.

This work was supported by grants from the Romanian Academy.

O foaie de parcurs pentru cercetarea diabetologică românească

Constantin Ionescu-Tîrgovişte

National Institute of Diabetes, Nutrition and Metabolic Diseases "N.C. Paulescu",
5-7 Ion Movilă Street, Bucuresti, cit@paulescu.ro

Diabetul zaharat de tip 2 şi celelalte două tulburări ale metabolismului energetic înrudite (obezitatea şi sindromul metabolic) domină patologia metabolică, care explică mortalitatea crescută prin boli cardiovasculare. Comunitatea ştiinţifică internaţională este profund implicată în prevenirea acestor tulburări sau, în cel mai rău caz, în diagnosticarea precoce, înaintea instalării complicaţiilor cronice ireversibile.

În cadrul Asociaţiei Medicale Române, funcţionează de 2 ani „Alianţa Ştiinţifică Românească", care are ca scop identificarea cercetătorilor români care lucrează permanent sau temporar în diferite centre de cercetare din lume, în vederea stabilirii unei comunicări directe pe temele de cercetare de interes comun. În cazul de faţă, în patologia metabolică şi cardiovasculară. Prin intermediul lor, dorim realizarea unor parteneriate stabile de colaborare între centrele în care ei lucrează şi centre omoloage din România. Această colaborare poate facilita găsirea unor parteneri solizi

Din punctul de vedere al cercetării diabetologice, principalele obiective înscrise ca priorităţi în foaia de parcurs a acesteia, menţionăm: (a) modificarea criteriilor de definire şi de diagnostic a diabetului, care în prezent nu mai corespund cu realitatea clinică; (b) stabilirea factorilor patogenetici operanţi în diferitele fenotipuri ale sindromului diabetic, îmbinând analiza factorilor de mediu cu cea a factorilor genetici şi epigenetici; (c) dezvoltarea unei metode de evaluare neinvazivă a masei β celulare, condiţie esenţială pentru realizarea primelor două puncte; (d) identificarea unor molecule „naturale" (existente în alimente, în alimentele-medicament sau în plantele-medicament) cu potenţial de modulare a funcţiei β celulare şi de regenerare a sa. Este bine de ştiut că cea mai utilizată clasă medicamentoasă în tratamentul diabetului zaharat este cea a biguanidelor, descoperită iniţial în planta *Galega officinalis* şi transpus apoi în industria farmaceutică. Întrucât numărul plantelor utilizate în diferite ţări în tratamentului diabetului zaharat este mare, se poate presupune că şi numărul moleculelor cu efect antidiabetic trebuie să fie şi el foarte mare.

Inflammatory up-regulation of fractalkine and MCP-1 in human smooth muscle cells under high glucose conditions

Elena Dragomir

Institute of Cellular Biology and Pathology "Nicolae Simionescu",
8 B.P. Hasdeu Street, Bucharest 050568, Romania, elena.dragomir@icbp.ro

The major complication of diabetes mellitus is accelerated atherosclerosis that entails an inflammatory process, in which fractalkine and MCP-1 have key roles. We investigated the effect of diabetes-associated high glucose (HG) on these chemokines and signalling mechanisms involved in human aortic smooth muscle cells (SMC).

Exposure of SMC to HG resulted in an increase of fractalkine and MCP-1 expression and the activated MAPK signalling pathway, a process associated with elevated oxidative stress. Transfection with decoy oligodeoxynucleotides identified the involvement of transcription factors AP-1 and NF-κB in the observed up-regulation of chemokines. The MAPK inhibitors blocked the phosphorylation of IkBα and c-jun, indicating the role of MAPK in NF-κB and AP-1 activation in SMC under HG conditions. The up-regulation of MCP-1 and fractalkine was associated with increased adhesive interactions between HG-exposed SMC and monocytes. Treatment of HG-exposed SMC with PPARα activators (fenofibrate and clofibrate) resulted in a reduction of mRNA and protein expression of MCP-1 and fractalkine.

In conclusion, HG up-regulates the expression of fractalkine and MCP-1 in SMC leading to increased monocyte-SMC adhesive interactions by a mechanism involving activation of MAPK, AP-1 and NF-κB. The increased expression of these two pro-inflammatory chemokines and the ensuing increased adhesion between SMC and monocytes may trigger the inflammatory process associated with further vascular complications of diabetes.

New insights into the regulation of apoptosis in hematopoietic tumor cells

Alexandru Almăşan

Department of Cancer Biology, Bridge appointment with Department of Radiation Oncology, Taussig Cancer Center, Lerner Research Institute/ NB40, 9500 Euclid Avenue, Cleveland, OH 44195, USA, *almasaa@ccf.org*

The BH3-only members of the Bcl-2 family regulate apoptosis by either neutralizing the pro-survival family members or by activating their pro-apoptotic relatives. We found that levels of PUMA are upregulated in human tumor cells following genotoxic stress such as irradiation. Overexpression of Bcl-2 and knockdown of PUMA protected against radiation-induced cell death, while its overexpression sensitized Bcl-2-expresing cells to radiation at a degree comparable to parental cells, suggesting that a physiological balance between the pro- survival proteins and their BH3-only antagonists is required for an effective apoptotic response. Treatment of the isolated mitochondria with a PUMA-based peptide triggered cytochrome C release, inhibited by pretreatment with a Bim-BH3 masking antibody. Moreover, the interaction of Bim with Bcl-2, Bcl-xL, and Mcl-1 was greatly diminished following irradiation. Concomitantly, the association of PUMA with these anti apoptotic molecules was significantly increased in irradiated cells, indicating that PUMA acts as a sensitizer with Bim serving an activator function. Irradiation and PUMA overexpression lead to Bax activation and apoptosis. Bax translocation to mitochondria and its oligomerization were late events. PUMA expression and binding to Bcl-2 family proteins as well as Bim displacement were also late events, providing further support for an amplification stage required for apoptosis. PUMA levels and association with Bcl-2 increased in response to irradiation in primary cells obtained from patients but not in normal B cells derived from peripheral blood. Use of BH3 mimetics, such as ABT-737, is being currently examined in the clinic. ABT-737 acts as an antagonist of the anti-apoptotic family members Bcl-2 and Bcl-xL. Since acquired resistance is likely to emerge for even the most effective cancer chemotherapies, we examined the mechanism of resistance to ABT-737. The resistant cell lines we have developed from sensitive hematopoietic B-cell derived tumor lines have increased levels of Mcl-1. Increased Mcl-1 levels were primarily controlled at the transcriptional level, with the stability of the protein a contributing factor. The protective effect of increased Mcl-1 expression is based on its ability to associate with the BH3- only protein Bim that is diplaced from bcl-2 by ABT-737. ABT-737 is unique as a drug because it is very specific and its mechanism of cell killing has been well characterized. To overcome ABT-737 resistance we are using clinical-grade therapeutics that are known to block Mcl-1 transcription.

Role of Sphingolipids in Cancer Therapy

Claudia Alexandra Dumitru

University of Duisburg-Essen, Medical School Essen, Dept. of Molecular Biology, Hufelandstrasse 55, D-45122 Essen, Germany, claudia.dumitru@uni-due.de

Although recent advances in cancer research led to a greatly improved prognosis for cancer patients, many malignancies remain without a cure to this day.

A growing body of evidence suggests that alterations in the apoptotic machinery of cancer cells are important modulators of both tumorigenesis and response to therapy. Apoptosis can occur via two major pathways, namely the extrinsic (death receptor-initiated) or the intrinsic (mitochondria-initiated) pathway. The two pathways converge on activation of caspase-3 and subsequently on other proteases and nucleases that drive the terminal events of apoptosis. In turn, every step in the apoptotic cascade is monitored and controlled by certain pro-survival signals provided by families of anti-apoptotic molecules such as NF-κB, Akt/PKB, Bcl-2 and IAPs.

Over the last years, many groups demonstrated the involvement of sphingolipids, especially ceramide, in apoptosis, induced by a wide variety of stimuli. Ceramide has been shown to be a crucial mediator of death signals triggered by members of the TNF family of ligands, irradiation, chemotherapy, UV light or heat shock. Ceramide can be generated either by *de novo* synthesis via activation of ceramide synthase or by sphingomyelin hydrolysis, following activation of the acid, neutral or alkaline sphingomyelinases. The main degradation pathways of ceramide are mediated by ceramidases and glucosyltransferase, which convert ceramide to sphingosine and glucosylceramide, respectively. While ceramide accumulation leads to cell death, sphingosine and/ or glucosylceramide are mainly anti-apoptotic. Therefore, the balance between ceramide production and conversion pathways is critical for the cell's fate.

Our studies focus on elucidating the exact role of ceramide in signalling induced by several stimuli currently used in cancer treatment. This will permit a better modulation of ceramide metabolism in the targeted cells and may lead to development of new and improved therapeutic strategies.

What can we learn from computer simulations of biomolecular systems?

Vlad Cojocaru

EML Research gGmbH, Schloss Wolfsbrunnenweg 33, 69118 Heidelberg, Germany
Vlad.Cojocaru@eml-r.villa-bosch.de

Computer simulations (also called in sillico experiments) have become very powerful tools for studying biological processes, largely due to the rapid increase in computer power and improved accuracy. In molecular dynamics simulations, trajectories of biomolecular assemblies are build by numerically solving the Newton's law of motion for each atom of the system. The trajectories reveal the dynamics of the studied system, thus giving imporant information about the interplay between its structure and function. However, the time scales of real biological processes span a range from picoseconds to years. In computer simulations, only those processes that occur in picoseconds to microseconds are available. The time scales available to the simulations is restricted by the choice of the time step, which is limited to 1 to 2 femptoseconds (fs) because the fastest motions in such systems (vibrations of the bonds involving hydrogen atoms) occur in approximately 10 fs. However, due to rapid increase in the availability of supercomputers it is now possible to simulate systems as big as one million atoms and time scales approaching 10 microseconds. Thus, processes such as the folding of small proteins may be simulated in real time. If such resources are not available or the investigated process occurs on a time scale beyond microseconds, a wide range of methods (some of which I will describe) are being developed to accelerate biological processes in sillico.

To emphasize the power of computer simulations in the investigation of the dynamics of complex biomolecular structures, I will present simulations that reveal a large conformational transition in an RNA motif, named the kink-turn motif and different approaches to study substrate access and product release pathways from the buried active site of the drug-metabolizing cytochrome P450 enzymes.

References:

1. **Vlad Cojocaru**, Peter J. Winn, and Rebecca C. Wade. 2007. Exploring the routes out of the active site of cytochrome P450 2C9. *Proceeedings of the 15th international conference on cytochromes P450, Bled, Slovenia, July 2007*.
2. **Vlad Cojocaru**, Peter J. Winn, and Rebecca C. Wade. 2007. The ins and outs of cytochrome P450s. *Biochimica et Biophysica Acta*. **1770**:390-401.
3. **Vlad Cojocaru**, Reinhard Klement, and Thomas M. Jovin. 2005. Loss of G-A base pairs is insufficient for achieving a large opening of U4 snRNA K-turn motif. *Nucleic Acids Research* **33**:3435-3446.
4. **Vlad Cojocaru**, Stephanie Nottrott, Reinhard Klement, and Thomas M. Jovin. 2005. The snRNP 15.5K protein folds its cognate K-turn RNA: A combined theoretical and biochemical study. *RNA* **11**:197-209.
5. **Vlad Cojocaru** 2005. Molecular motions at the 5' stem-loop of U4 snRNA: Implications for U4/U6 snRNP assembly. Doctoral dissertation published online by the Georg-August- University Goettingen.

Analysis of molecular determinants of PRL-3, a protein tyrosine phosphatase involved in cancer metastasis

Ştefan Szedlacek

Enzymology Department, Institute of Biochemistry from the Romanian Academy,
296 Splaiul Independentei, Bucharest 060031, Romania, szedlacs@yahoo.co.uk

PRL-3 (phosphatase of regenerating liver) belongs to the dual specificity protein tyrosine phosphatases. The special interest for these PTPs in the last years is due to its overexpresssion in different cancer forms as well as in metastasis of colorectal cancer. A large number of studies have been performed related to this protein; however, only few data are available at present as concerning its molecular determinants. To evaluate whether a C-terminal polybasic sequence represents a nuclear localization signal (NLS) we obtained several truncated and mutant forms of PRL-3 and analyzed their subcellular localization as compared to the wild type form. Our results invalidate the hypothesis that this is an NLS. We also studied the influence of the C- and N-terminal residues on the phosphatase activity of PRL-3. Our results prove that the C-terminal CAAX motif, besides directing the protein farnesylation, plays an additional regulatory role by inhibiting the catalytic efficiency of PRL-3. Accounting for the results we are reporting here, as well as for those reported in literature, we propose a hypothetical molecular mechanism for the nucleocytoplasmic localization and transfer of PRL-3.

Glyconectins – a primordial class of cell adhesion molecules involved in the emergence of multicellular organisms

Octavian Popescu

Babeş-Bolyai University, Institute for Interdisciplinary Experimental Research, Faculty of Biology and Geology, Molecular Biology Center, 42 August Treboniu-Laurian Street, 400271 Cluj-Napoca, Romania opopescu@biolog.ubbcluj.ro

In order to understand the molecular basis for primordial self-recognition and non-self discrimination during the emergence of multicellular organisms, we focused our attention on the role of cell surface glycoconjugates in Porifera xenogeneic cellular interactions, as the evolutionary most compatible model system for ancestors of Metazoans. The investigations started in the beginning of the 20th century on dissociated marine sponge cells provided important phenomenological evidence for cell sorting. However, these and subsequent experiments from 1960 to 1980 used semi-purified and chemically ill-defined extracts, termed "aggregation factors". After 1980, the first structure-to-function-related studies have been performed on glycoconjugates from *Microciona prolifera*. Using more complex purification and biochemical characterization of these "aggregation factors" we have shown that they contain a new class of large cell surface proteoglycan-like molecules, heavily covered by long glycan chains. These molecules are different from classical mammalian glycoconjugates, suggesting that sponge proteoglycans define a new class of primordial cell adhesion molecules, named by us **glyconectins** (GNs) [1]. We have isolated GNs from three marine sponge species: *M. prolifera* (GN1), *Halichondria panicea* (GN2), and *Cliona celata* (GN3). Using atomic force microscopy we have demonstrated that the strength of GN1-GN1 binding generates essential cell cohesion forces in the sponge *M. prolifera*, as previously implied by functional investigations [2, 3]. The binding strength between homotypic pairs of glycans (400 pN) is higher than those between heterotypic pairs (20 pN). We performed physico-chemical, biochemical and structural analyses of GN glycans isolated from the three sponge species. The sequential and selective chemical degradation of these glycans and subsequent mass spectrometric and NMR analyses revealed that each GN presents novel and highly species-specific sequences. All three GNs include distinct acid-resistant and acid-labile carbohydrate domains, the latter composed of novel repetitive units. Seventeen novel, species-specific carbohydrate sequences were revealed [4, 5]. These differences are sufficient to explain the species-specific separation of glycan-coated beads *in vitro* and the sorting of sponge cells *in vivo*. The molecular mechanism of glycan-mediated homophilic GN interactions in Porifera is based on highly species-specific and Ca^{2+}-dependent associations, and approaches the degree of selectivity of the evolutionarily advanced heterophilic immunoglobulin superfamily recognition system. Our studies establish a new paradigm for the pivotal role of primordial GN glycans in the self-assembly and non-self discrimination pathway of cellular adhesion prior to the evolution of multicellularity.

1. **Popescu O, Misevic GN**, Self-recognition by proteoglycans, *Nature*, **386**, 231-232, 1997.
2. **Dammer U, Popescu O, Wagner P, Anselmetti D, Guntherodt HJ, Misevic GN**, Binding strength between cell adhesion proteoglycans measured by atomic force microscopy, *Science*, **267**, 1173-1175, 1995.
3. **Popescu O, Checiu I, Gherghel P, Simon Z, Misevic GN**, Quantitative and qualitative approach of glycan-glycan interactions in marine sponges, *Biochimie*, **85**, 181-188, 2003.
4. **Misevic GN, Guerardel Y, Sumanovski L, Slomianny MC, Demarty M, Ripoll C, Karamanos Y, Maes E, Popescu O, Strecker G**, Molecular recognition between glyconectins as an adhesion self-assembly pathway to multicellularity, *J. Biol. Chem*., **279**, 15579-15590, 2004.
5. **Guerardel Y, Czeszak X, Sumanovski L, Karamanos Y, Popescu O, Strecker G, Misevic GN**, Molecular fingerprinting of carbohydrate structures phenotypes of three porifera proteoglycan-like glyconectins, *J. Biol. Chem*., **279**, 15591-15603, 2004.

Endotheliopathy in atherosclerosis

Maya Simionescu

Institute of Cellular Biology and Pathology "Nicolae Simionescu",
8 B.P. Hasdeu Street, Bucharest 050568, Romania, maya.simionescu@icbp.ro

Major human maladies are associated with-, or are due to vascular diseases that entail the direct participation of endothelial cells (EC). Initially considered a gratuitous cellophane-like sheet, the EC have evolved to a cell respected by biologists, pathologists and pharmacologists.

The **cell biologists** discovered that EC have a large array of paracrine, endocrine and autocrine functions: controlled permeability, synthesis of basal lamina and extracellular matrix components, guard vascular tone (by balanced synthesis of PGI2, NO, EDHF, endothelin), administer haemostasis (via vWf and PAI-1). We have found that EC perform transcytosis of macromolecules (LDL, albumin) by a receptor-mediated or independent mechanism, that caveolae are differentiated membrane microdomains and the cargo-carrier for endocytosis and transcytosis.

The **cell pathologists** uncovered that EC dysfunction as a "syndrome" consist of perturbed transcytosis, disturbed synthetic capacity, corrupt cross-talk with neighbouring cells, unbalanced antithrombogenic capacity, impaired regulation of the vascular tone, and disturbed proliferative capacity. Our data obtained on hyperlipemic rabbit and hamster and on human early atherogenesis revealed that in response to hyperlipemia, the vascular endothelium undergo gradual modifications that consist initially in *EC modulation of constitutive function* (increased LDL transcytosis, subendothelial accumulation of modified lipoproteins) and increased synthetic capacity. The dual assault of hyperlipemia and modified lipoproteins on endothelium generate a multipart inflammatory process and *EC dysfunction* expressed by alteration of plasma membrane components, endoplasmic reticulum stress, recruitment of monocytes, anoikis, alterations of EC junctions, deficiency in lysosomal acid lipase, and ultimately the formation of endothelial- derived foam cell, *EC injury and apoptosis.*

The **cell pharmacologists** found that, directly or indirectly, drugs such as Ebselen, ACE inhibitors, statins, and others, correct EC dysfunction. We have found that enoxaparin has antioxidant properties on human EC exposed to pro-oxidative conditions and aspirin and PPAR activators reduce MCP-1 expression, in high-glucose exposed EC. Since EC dysfunction is a gradual process, early markers and a combination of agents and drugs tailored for a specific dysfunctionality hold the future for therapeutic interventions to reverse EC dysfunctions and atherosclerosis. The wealth of data on the role of EC in health and disease may constitute a complex discipline that includes *endotheliology, endotheliopathy and endotheliotherapy.*

Funding: work supported by grants from the Romanian Academy, Ministry of Education and Research, Romania, NIH-USA and European Community Programme.

Endocan, a new marker for endothelial activation

Bogdan Dragoş Grigoriu

University of Medicine and Pharmacy, 16 Universitatii Street, Iasi 700115, Romania and INSERM U774, Pasteur Institute, Lille 59019, France, b_grigoriu@hotmail.com

Tumoral development implies interaction between the tumor cell and its environment (extracellular matrix, stromal cells, tumor endothelium and immune cells) which contributes actively to tumor development.

Endocan, a lung- and kidney-selective endothelial cell specific dermatan sulfate proteoglycan, is a molecule present in this tumoral environment and is overexpressed in various tumors as lung, breast, colon, or kidney cancers. *In vitro*, endocan amplifies the growth factor mitogenic effect on cells of epithelial origin. Endocan is also putatively involved in regulation of leucocytes transmigration from blood to tissues by blocking LFA-1/ICAM-1 interactions, an essential step in firm adhesion of leukocytes to the endothelium and an important cofactor involved in cytotoxic leukocyte function.

We show that, *in vivo*, endocan overexpression by non-tumorigenic epithelial cells induces tumor formation, while overexpression by tumorigenic cells sharply increases the growth rate of resulting tumors. However, endocan alone does not influence proliferation of tumor or endothelial cells. Thus, endocan tumor-promoting capabilities result from amplification of growth factor effects on tumor cells. VEGF is a powerful inducer of endocan secretion by endothelial cells and endocan and VEGF expressions are correlated in non-small cell lung cancers. In these tumors, endocan is overexpressed and this concerns mainly the tumorigenic, exon 2 containing endocan isoform. Moreover, serum endocan values are correlated with patient survival, with a shorter survival in patients with high circulating levels of endocan. Interestingly serum endocan levels were correlated with presence of metastasis and nodal involvement but not with the primary tumor size.

In sepsis patients, where endothelial stimulation is an important pathological phenomenon, endocan levels are also elevated and associated with increased sepsis severity and higher mortality.

In conclusion we show that endocan play an important role in tumoral development by amplifying growth factor effects on tumoral cells and by inhibiting immune cell recruitment into the tumor. Serum endocan levels reflect the tumoral angiogenic stimulation and represent a prognostic factor in non-small cell lung cancers. Endocan is also expressed by the endothelium in sepsis and has a prognostic significance.

Transcriptional regulation of NADPH oxidase in human aortic smooth muscle cells: role of renin-angiotensin system

Adrian Manea

Institute of Cellular Biology and Pathology "Nicolae Simionescu"
8 B.P. Hasdeu Street, Bucharest 050568, Romania, adrian.manea@icbp.ro

Objective: Oxidative stress is implicated in the pathophysiology of cardiovascular diseases, such as hypertension and atherosclerosis. Among others, NADPH oxidase is one of the most important sources of superoxide in vascular cells. Here we investigate the role of NF-kB and AP-1 in the regulation of NADPH oxidase expression and function in human aortic smooth muscle cells.

Methods and results: Lucigenin-enhanced chemiluminescence assay established that NF-kB and AP-1 inhibitors reduced the angiotensin II (Ang II)/tumor necrosis factor α (TNFα)-stimulated NADPH oxidase activity. Real time polymerase chain reaction (PCR) analysis showed that NF-kB/AP-1 decoy oligodeoxynucleotides, BAY117085 and SP600125 prevented the Ang II/TNFα-dependent up-regulation of Nox1, Nox4, $p47^{phox}$, and $p67^{phox}$ mRNA expression. Computer analysis revealed the presence of putative NF-kB and AP-1 elements in the promoters of human Nox1, Nox4, $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$. Luciferase gene reporter assay, real time PCR, and Western blotting analysis showed that NF-kB/AP-1 inhibitors significantly diminished the Ang II/TNFα-stimulated $p22^{phox}$ promoter activity, mRNA, and protein expression. Transient overexpression of p65RelA, IKKβ, c-Jun or c-Fos up-regulated $p22^{phox}$ gene promoter activity. Transcription factor pull-down assay and chromatin immunoprecipitation demonstrated the physical interaction of p65 and c-Jun proteins with predicted NF-kB or AP-1 binding sites.

Conclusions: Regulation of NADPH oxidase by NF-kB and AP-1 may represent a possible mechanism whereby pro-inflammatory factors induce oxidative stress in hypertension and atherosclerosis. Because NF-kB and AP-1 itself are redox-sensitive transcription factors, a positive feedback mechanism whereby ROS, possibly generated by the NADPH oxidase, may be important for sustained reactive oxygen species production.

This work was supported by grants from Romanian Academy and Romanian Ministry of Education and Research.

Associations of apolipoprotein A5 with metabolic syndrome

Loredan Ştefan Niculescu

Institute of Cellular Biology and Pathology "Nicolae Simionescu"
8 B.P. Hasdeu Street, Bucharest 050568, Romania, loredan.niculescu@icbp.ro

Introduction: The dyslipidemia, such as hypertriglyceridemia and hypercholesterolemia, is considered an independent risk factor for cardiovascular diseases. The APOA5 was recently identified as a new apolipoprotein gene located 30kb upstream of the well-characterized APOA1/C3/A4 gene cluster on 11q23 and it was shown to be strongly associated with plasma triglycerides levels. We demonstrated that ApoA5 accelerates the triglyceride-rich lipoprotein catabolism through a direct activation of their hydrolysis by lipoprotein lipase and their removal from plasma, and not by affecting VLDL-triglycerides liver secretion. Published data reveal that common APOA5 gene polymorphisms are associated with increased plasma triglycerides levels, a risk factor for the metabolic syndrome. We previously demonstrated that APOA5 -1131C allele is associated with the risk for metabolic syndrome in Romanian subjects. To date, few studies associate plasma apoA5 levels and main lipid parameters in human subjects with dyslipidemia.

Aim: to determine the association of APOA5 gene variants and biochemical parameters with apoA5 levels in plasma and HDL fraction from patients with metabolic syndrome from a Romanian urban population.

Methods: APOA5 gene polymorphisms (-1131T>C and c.56C>G), apoA5 levels in plasma and HDL fraction were assayed in 279 subjects, and correlated with plasma biochemical parameters. The subjects were divided in 2 groups: control and metabolic syndrome (MS), and classified according to their weight status, following the WHO criteria.

Results: We measured a decrease in apoA5 levels in plasma and HDL fraction from MS patients, carriers of APOA5 -1131C allele, as compared with MS non-carriers. In contrast, MS patients having the APOA5 c.56G allele had higher levels of plasma and HDL-associated apoA5 levels, as compared with the MS non-carriers. Higher apoA5 levels in plasma and HDL fraction from MS patients, carriers of c.56G allele, as compared with MS patients having the -1131C allele and with those having the common APOA5 genotype were measured. A redistribution of apoA5 from HDL to VLDL particles was observed in plasma from obese MS patients. We observed a positive correlation between the levels of apoA5 in plasma and HDL fraction with triglycerides concentrations in MS patients, carriers of APOA5 -1131C allele. In addition, plasma and HDL-associated apoA5 levels positively correlated with triglycerides and glucose levels in MS patients with APOA5 c.56G allele. In MS patients, carriers of c.56G allele, HDL-cholesterol negatively correlated with apoA5 concentrations in plasma and HDL fraction.

Conclusion: Our results demonstrate that the c.56C>G polymorphism resulted in higher plasma apoA5 levels, being a functional polymorphism, whereas -1,131T>C gene variant may not be a functional polymorphism.

Funding: Romanian Academy and Romanian Ministry for Education and Research grants.