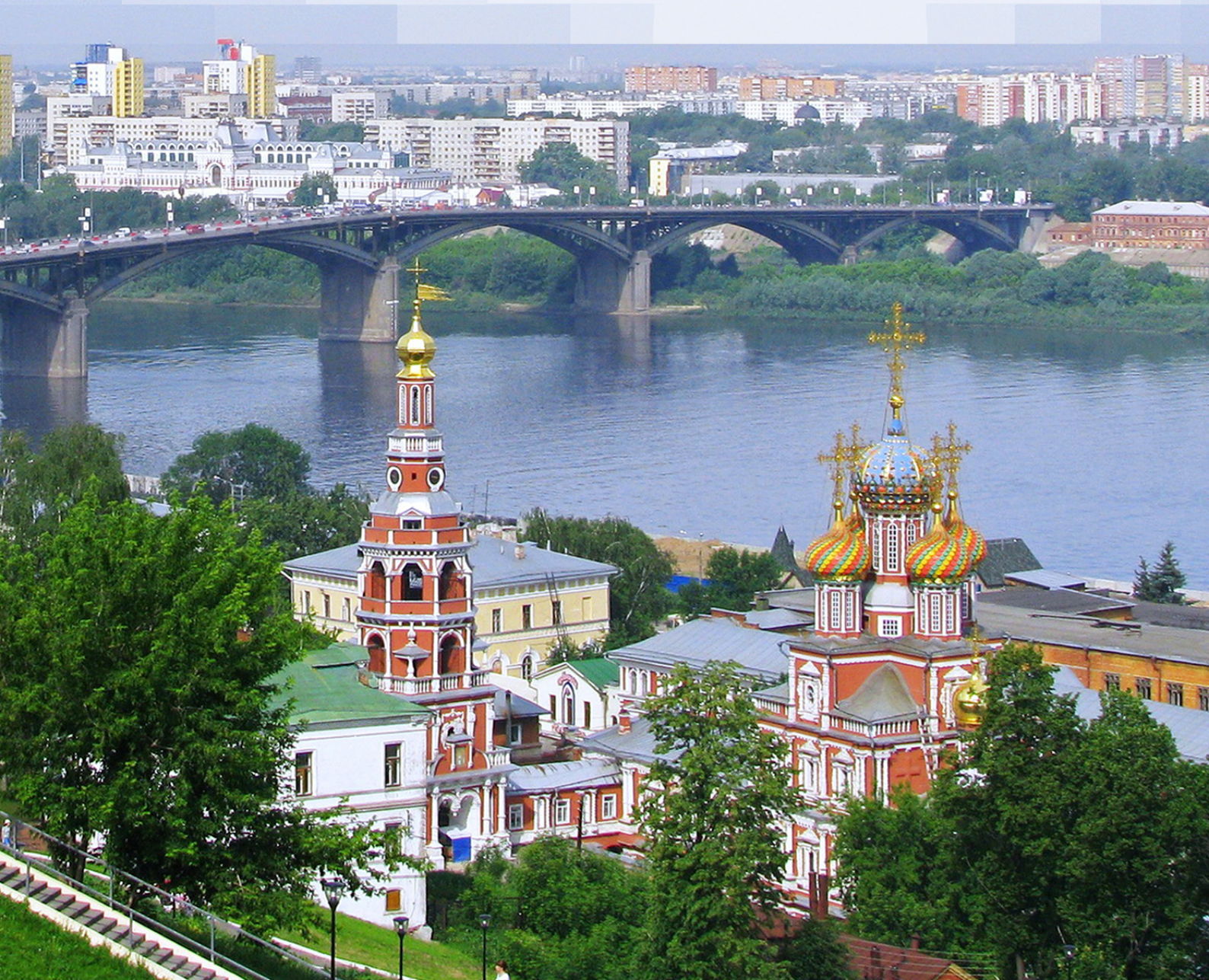
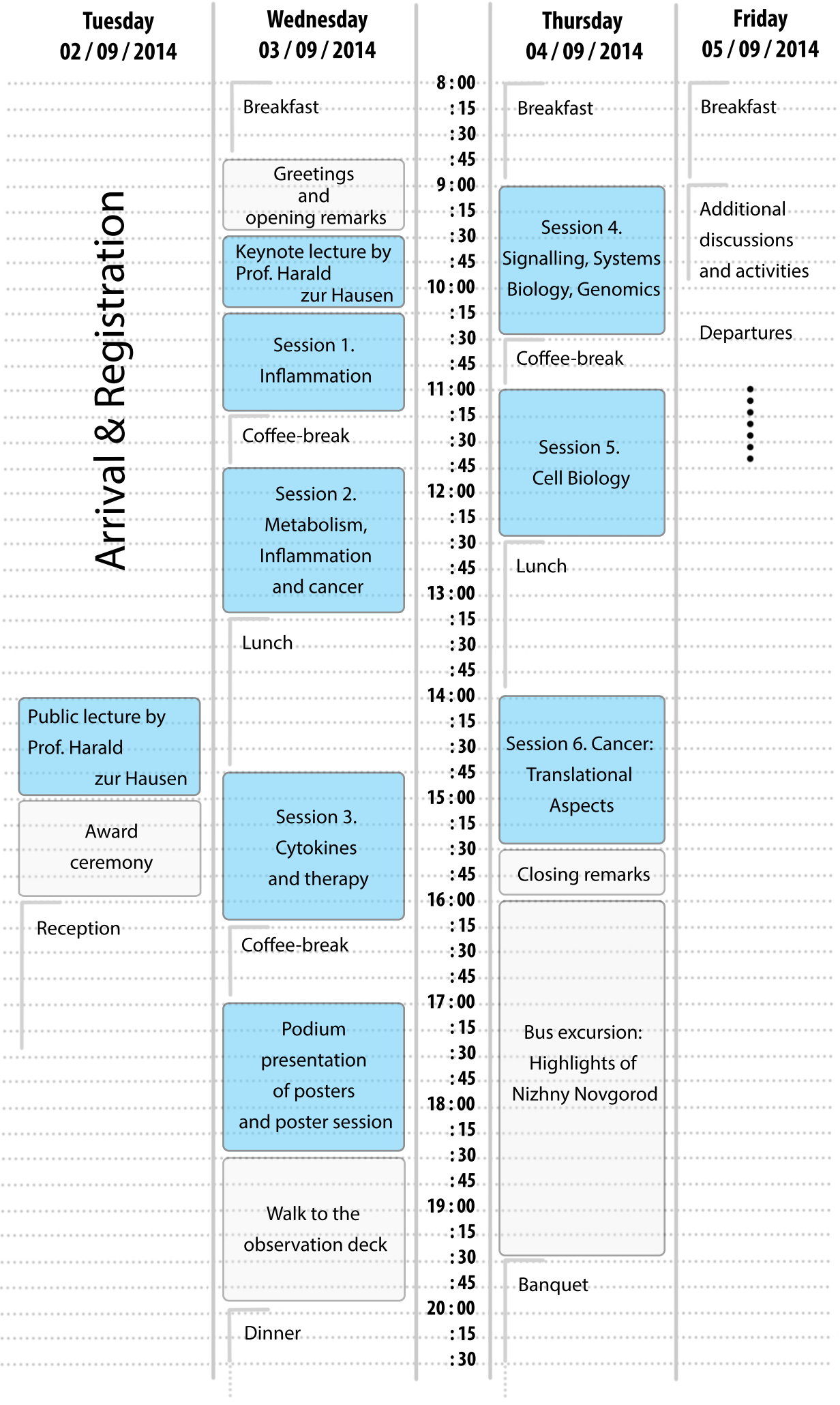
Immunology & Cancer '2014

September 2-5, 2014

Nizhny Novgorod, Russia

SCIENTIFIC PROGRAM




Tuesday
02 / 09 / 2014
Wednesday
03 / 09 / 2014
Thursday
04 / 09 / 2014
Friday
05 / 09 / 2014
8 : 00
: 15
: 30
: 45
9 : 00
: 15
: 30
: 45
10 : 00
: 15
: 30
: 45
11 : 00
: 15
: 30
: 45
12 : 00
: 15
: 30
: 45
13 : 00
: 15
: 30
: 45
14 : 00
: 15
: 30
: 45
15 : 00
: 15
: 30
: 45
16 : 00
: 15
: 30
: 45
17 : 00
: 15
: 30
: 45
18 : 00
: 15
: 30
: 45
19 : 00
: 15
: 30
: 45
20 : 00
: 15
: 30
Arrival & Registration
Public lecture by Prof. Harald zur Hausen
Award ceremony
Reception
Breakfast
Greetings and opening remarks
Keynote lecture by Prof. Harald zur Hausen
Session 1. Inflammation
Coffee-break
Session 2. Metabolism, Inflammation and cancer
Lunch
Session 3. Cytokines and therapy
Coffee-break
Podium presentation of posters and poster session
Walk to the observation deck
Dinner
Breakfast
Session 4. Signalling, Systems Biology, Genomics
Coffee-break
Session 5. Cell Biology
Lunch
Session 6. Cancer: Translational Aspects
Closing remarks
Bus excursion: Highlights of Nizhny Novgorod
Banquet
Breakfast
Additional discussions and activities
Departures

Tuesday, September 2, 2014

	Morning arrivals, registration
13:30	Bus transfer to Lobachevsky University
from 14:00	**1.** Address by Rector of Lobachevsky University Prof. E. V. Chuprunov **2.** Introduction for Prof. H. zur Hausen by Prof. S.A. Nedospasov
	Public lecture by Professor Harald zur Hausen *(Heidelberg)* «Cancer prevention by vaccination»
	• Award ceremony for Doctor Honoris Causa title to Prof. Harald zur Hausen; • Short presentations by representatives of DWIH, DFG and Helmholtz Association in Russia.
	Reception at Rectorat, Lobachevsky University

Wednesday, September 3, 2014

8:00	*Breakfast*
8:45	**Greetings and opening remarks** V.V. Novikov, S. Rose-John, S.A. Nedospasov, M. Krispin, J. Achterberg, E. Eremenko
9:30	**Keynote lecture** **Harald zur Hausen** *(Heidelberg)* «Are some cancers and chronic diseases zoonoses?» *(speaker introduction by F.L. Kisselev)* Abstract on page 11

SESSION 1: «Inflammation»

10:15 – 11:15

Chairman: S. Rose-John

10:15	**Eicke Latz** *(Bonn)* «The role of inflammasome activation in chronic inflammatory diseases» Abstract on page 12
10:45	**Alexander Poltorak** *(Boston, Petrozavodsk)* «Experimental models of sepsis» Abstract on page 13
11:15	*Coffee-break*

SESSION 2: «Metabolism, Inflammation and Cancer»

11:45 – 13:15

Chairman: S.A. Nedospasov

11:45	**Matthias Heikenwalder** *(Munich)* «Metabolic activation of intrahepatic CD8+ and hepatocellular carcinoma via a Light-driven cross-talk with hepatocytes and NKT-cells causes nonalcoholic steatohepatitis» Abstract on page 14
12:15	**Thomas Wunderlich** *(Cologne)* «Obesity promotes cancer development» Abstract on page 15
12:45	**Elena Zagainova** *(Nizhny Novgorod)* «Detection of metabolic tumor status, using geneticaly encoded sensors and optical imaging» Abstract on page 16
13:30	*Lunch*

SESSION 3: «Cytokines and Therapy»

14:45 – 16:15

Chairman: E. Latz

14:45	**Sergei Nedospasov** *(Moscow, Berlin, Nizhny Novgorod)* «Functions of TNF from distinct cellular sources and new approach to anti-TNF therapy» Abstract on page 18
15:15	**Andrey Kruglov** *(Berlin, Moscow)* «Lymphotoxin-directed mechanisms of intestinal immunity and microbiota control» Abstract on page 19
15:45	**Stefan Rose-John** *(Kiel)* «The pro- and anti-inflammatory activities of Interleukin-6: therapeutic consequences» Abstract on page 20

16:15	*Coffee-break*

Podium presentations of posters and poster session

Chairman: D.V. Kuprash

17:00	**Florian Reisinger** *(Munich)* «Specific and non-hepatotoxic degradation of nuclear hepatitis B virus cccDNA by lymphotoxin-beta receptor activation» Abstract on page 21
17:10	**Caroline Winsauer** *(Berlin)* «The contribution of T cell-derived TNF to colitis» Abstract on page 22
17:20	**Vladimir Ilyuha** *(Petrozavodsk)* «RIP1 kinase-dependent inflammatory response» Abstract on page 23
17:30	**Grigory Efimov** *(Moscow)* «Novel recombinant anti-TNF antibody-based fusion proteins for diagnostics and treatment» Abstract on page 24
17:40	Abstract on page 25 **Marina Shirmanova** *(Nizhny Novgorod)* «Study of immunogenicity of the Killer-Red expressing tumors»
17:50	**Ilgiz Mufazalov** *(Mainz)* «IRF4 but not RORγt is indispensable for IL17A and GM-CSF production *in vivo*» Abstract on page 26
18:00	**Ksenia Shakhova** *(Nizhny Novgorod)* «Soluble forms of membrane differentiation molecules in monitoring of oncological diseases» Abstract on page 27
18:30	Walk to the observation deck near Kremlin (Chkalov stairs, Chkalov monument), group photo.
20:00	*Dinner*

Thursday, September 4, 2014

8:00	*Breakfast*

***SESSION 4*: «Signalling, Systems Biology, Genomics»**

9:00 – 10:30

Chairman: A. Poltorak

9:00	**Athena Chalaris-Rißmann** *(Kiel)* «ADAM17 as proteolytic gatekeeper of inflammation and cancer» Abstract on page 28
9:30	**Inna Lavrik** *(Magdeburg)* «The chains of death: A new view on caspase-8 activation at the DISC» Abstract on page 29
10:00	**Evgeny Rogaev** *(Worcester, Moscow)* «Neurodegenerative versus carcinogenic molecular-genetic pathways» Abstract on page 30
10:30	*Coffee-break*

***SESSION 5*: «Cell Biology»**

11:00 – 12:30

Chairman: M. Heikenwalder

11:00	**Karl Lenhard Rudolph** *(Jena)* «Essential role of telomerase in aneuploidy-induced transformation of human cells» Abstract on page 32

11:30	**Alexey Tomilin** *(*Saint Petersburg) «Human Artificial Chromosomes for Regenerative Medicine and Gene Therapy» Abstract on page 33
12:00	**Maria Lagarkova** *(Moscow)* «Comparison of isogenic human ES and iPS cell lines reveals no specific traces of the reprogramming process» Abstract on page 34
13:00	*Lunch*

***SESSION 6*: «Cancer: Translational Aspects»**

14:00– 15:30

Chairman: L. Rudolph

14:00	**Cécile Gouttefangeas** *(Tübingen)* Abstract on page 35 «Therapeutic vaccination against tumors: the Tübingen experience»
14:30	**Nadezhda Cherdyntseva** *(Tomsk)* «Immune system contributes to the efficacy of cancer chemotherapy and outcome» Abstract on page 36
15:00	**Dmitry Kuprash** *(Moscow)* Abstract on page 38 «Exploring the autoantigenic repertoire of thyroid tumors for differential diagnostics»
15:30	**Closing remarks**
16:00	**Bus excursion:** Highlights of Nizhny Novgorod
19:30	**Banquet**

ABSTRACTS

ARE SOME CANCERS AND CHRONIC DISEASES ZOONOSES?

H. zur Hausen[1] and E.-M. de Villiers[1]

[1]*Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, Heidelberg, Germany*

A number of viral, bacterial and parasitic diseases originate from transmissions of these infections by contacts with domestic animals or their products. Chronic, latent and persistent infections with several agents, prevalent in human populations are not or only rarely causing symptoms in the human host. Some of them, however, are relatively effective inducers of malignant tumors or of chronic neurological disease in rodents or New World primates, commonly under conditions not allowing their replication. This prompted the consideration whether similar agents exist in domestic animals (cattle), not tumorigenic in their native hosts, but potentially causing cancer in a non-permissive host, after infection of humans. Potentially oncogenic infectious factors were postulated in common dairy beef, probably interacting synergistically with some of the chemical carcinogens, produced in preparatory steps for consumption. This resulted in the analysis of sera from healthy cattle for the presence of infectious agents interacting with human cells. Eighteen novel types of episomally persisting single stranded DNAs have been isolated and analyzed, some of them also from commercially available cow milk and from patients with multiple sclerosis (MS). A concept of MS development will be presented.

THE ROLE OF INFLAMMASOME ACTIVATION IN CHRONIC INFLAMMATORY DISEASES

E. Latz[1,2]

[1] Institute of Innate Immunity, University of Bonn, Bonn, Germany

[2] University of Massachusetts Medical School, Division of Infectious Diseases & Immunology, Worcester, USA

The presentation will highlight the general concepts that explain how the innate immune system detects invading microbes and responds to danger situations using a set of germ-line encoded signaling receptors. Innate immune activation is important for infection control, the detection of cancerous cells and the repair of tissue damage. However, inappropriate or prolonged activation of innate immune receptors can lead to many acute and chronic inflammatory diseases. Therefore, a better understanding of innate immune activation mechanisms is important for the development of novel treatment strategies for inflammatory diseases.

An important innate signaling complex is called the inflammasome, which will be discussed in more detail. Inflammasomes recognize sterile tissue damage and danger signals that appear during cell stress. The inflammasome is activated by a broad range of cellular stressors and various substances that indicate metabolic derangements. We found that crystalline and aggregated material, which appears in several prevalent diseases, including atherosclerosis, diabetes and Alzheimer's disease, can damage lysosomes. We found that the induction of lysosomal damage by aggregates represents a common mechanism, by which NLRP3 inflammasome is activated. For example, we found that cholesterol crystals, which are found in atherosclerotic plaques, can cause inflammasome activation and that the NLRP3 inflammasome contributes to the development of atherosclerosis. Furthermore, we found that aggregated peptides, such as the aggregated amyloid beta peptides in brains of Alzheimer's disease patients can also trigger NLRP3 inflammasome activation. Importantly, lack of NLRP3 largely prevented the development of brain pathology and cognitive decline in murine Alzheimer's disease models. Based on our novel mechanistic understanding of disease pathogenesis we are currently developing new therapeutic approaches for the treatment of inflammatory diseases.

EXPERIMENTAL MODELS OF SEPTIC SHOCK

A. Poltorak[1,2]

[1]Tufts University, Boston, USA

[2]Petrozavodsk State University, Petrozavodsk, Republic of Karelia, Russia

Mechanisms of control of septic shock and human responses to G(-) infections and lipopolysaccharide (LPS), are poorly understood. One of the projects in my laboratory is concerned with identification of genes that confer resistance to LPS and its main effector TNF (tumor necrosis factor). The identification of these genes could lead to diagnostics or even open new opportunities for therapeutic intervention. Importantly, targeting the TNF-activation pathway, a relatively late event in the progression of septic shock, will likely be more beneficial than therapies currently explored by pharmaceutical companies. Here we describe a novel genetic model of resistance to lethality mediated by TNF receptor or Fas in the mice of so-called wild-derived strains. Specifically, we found mice of the genetically distinct MSM strain to be profoundly resistant to TNFR- and Fas-mediated lethality and liver injury. At the cellular level, MSM mice are responsive to both agonists thus providing further evidence against the possibility of a molecular defect in the receptors. To explain the trait, we generated our central hypothesis in that TNF-resistance in MSM mice is biased towards pro-survival as compared to cytotoxic signaling that leads to necrosis. We propose a model in which MSM mice are biased towards proliferative/inflammatory activation of TNFR and Fas. To identify genes that confer resistance to TNF, we employed a forward genetic analysis that combines serial backcrossing of the genome of the resistant to TNF mice onto “susceptible” background. Implementation of this approach provided highly significant association with four genomic loci. These findings present a new genetic model of resistance to Fas- and TNFR-mediated apoptosis, which will provide important insight on regulation of both pathways and will expand our understanding of biology of death receptor family members.

METABOLIC ACTIVATION OF INTRAHEPATIC CD8$^+$ AND NKT-CELLS CAUSES NONALCOHOLIC STEATOHEPATITIS AND HEPATOCELLULAR CARCINOMA VIA CROSS-TALK WITH HEPATOCYTES

M. Wolf[1], A. Adili[2], K. Piotrowitz[3], Z. Abdullah[4], Y. Boege[1], M. Ringelhan[2], N. Simonavicius[2], A. Lorentzen[2], P. Knolle[3,4,*], A. Weber[1,5,*] and M. Heikenwalder[2,5,*].

[1] Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland

[2] Institute of Virology, Technische Universität München/Helmholtz Zentrum München, Munich, Germany

[3] LIMES Life and Medical Sciences Institute, University of Bonn, Bonn, Germany

[4] Institutes of Molecular Medicine and Experimental Immunology, University of Bonn, Bonn, Germany

[5] These authors contributed equally to this work

*__Correspondence__:

heikenwaelder@helmholtz-muenchen.de

achim.weber@usz.ch

percy.knolle@tum.de

Hepatocellular carcinoma (HCC), the most severe outcome of nonalcoholic steatohepatitis (NASH), is the fastest-rising cancer in the USA and increasing in Europe. Mechanisms of NASH-induced HCC are largely unknown. By long-term choline-deficient high fat-diet we recapitulated central key-features of the human metabolic syndrome, NASH and NASH-induced HCC in mice. We identified an increase of activated intrahepatic CD8$^+$ T-cells, NKT-cells and associated cytokines, which was corroborated in NASH-patients. By analyzing *Rag1*$^{-/-}$, b*2m*$^{-/-}$, *Ikk*β^{Dhep}, *Lt*β*r*^{Dhep} and *Light*$^{-/-}$ mice we discovered that CD8$^+$ and NKT-cells promote NASH and HCC through interaction with hepatocytes. Immune-cell/hepatocyte co-culture revealed that NKT-cells enhance hepatocyte lipid-uptake via secreted LIGHT, while CD8$^+$ and NKT-cells cooperatively cause liver damage. Notably, hepatocellular-driven LTβR- and canonical NFκB-signaling facilitated NASH to HCC transition.

OBESITY PROMOTES CANCER DEVELOPMENT

Th. Wunderlich[1,2]

[1] Department of Mouse Genetics and Metabolism, Institute for Genetics, Center of Molecular Medicine, University of Cologne, Cologne, Germany

[2] Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Max Planck Institute for Neurological Research, Cologne, Germany

Obesity is a steadily increasing health burden that not only predisposes to classical obesity associated disorders but also increases the incidence of cancer. We and others have demonstrated that obesity induced inflammation such as IL6 promote HCC development. However a role of obesity in colorectal cancer development has not been addressed. Here we show that dietary and genetic obesity increase CRC development. Obesity polarizes macrophages that in turn attract gamma delta T cells thereby promoting cancerogenesis. Thus interfering with bodyweight regulation and insulin resistance might prevent obesity associated CRC development.

DETECTION OF METABOLIC TUMOR STATUS, USING GENETICALLY ENCODED SENSORS AND OPTICAL IMAGING

E. Zagainova[1,2], M. Shirmanova[1], I. Druzhkova[1], M. Kuznetsova[1,2], L. Snopova[1], N. Prodanetz[1], V. Belousov[1,3], S. Lukyanov[1,3]

[1] Nizhny Novgorod State Medical Academy, Russia

[2] Lobachevsky State University of Nizhny Novgorod, Russia

[3] Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

Introduction. The problem of all traditional chemotherapeutic drugs is the lack of specificity: they affect not only tumor cells, but the normal ones, first and foremost, the tissues with rapid proliferation. That is why a special attention should be paid to the search of medication with other mechanisms of action aimed at damaging the viability of a tumor cells. For example, fundamental differences of energy metabolism of a tumor cell from a normal one can be the base for the development of medications of a new generation aimed at the inhibition of glycolysis, damage of a structure or functions of mitochondria, system of pH regulation. It is supposed that the increased level of glycolysis in tumor cells is a relevant symptom of a malignant phenotype and a sign of invasiveness, and therefore it can be a prediction criterion of tumor development and its response to treatment(1). In the result of the increased glycolytic activity in tumors there is formed a reverse gradient of pH. Intracellular pH of tumor cells is more alkaline compared to normal cells of the organism (7.12-7.65 at the norm: 6.99-7.2), and extracellular pH of a tumor is more acidic (6.2-6.9 at the norm: 7.3-7.4) (2). The intracellular pH plays the main role in regulation of activity of glycolic enzymes and formation of the effect of multi-drug resistance. From the other hand, Co-factors of electron-transport chain – NADH and FAD are a relevant parameter of energy metabolism of a cell (3).

The intensive glycolysis (aerobic and anaerobic) typical of tumor cells causes changes in relative concentration of metabolic co-enzymes FAD and NADH. Due to the change of metabolism in tumor cells spatial distribution of FAD and NADH is also changed because other enzymes binding them are also involved (4). Materials and methods. *Analysis of intracellular pH value in cancer cells with ratiometric genetically encoded sensor.* To analyze intracellular pH there were used cancer cell lines stably expressing a genetically encoded sensor of cytozolic localization. The sensor has two excitation peaks of fluorescence (at about 420 and 500 nm) which allows ratiometrically calculate pH value. In alkaline medium there is a proportional decrease of the peak at 420 nm and an increase of the peak at 500 nm. The advantage of ratiometric sensors is that the ratio does not depend on the amount of the expressed protein, artifacts of cell motion and shape change. Ratiometric assessment of a sensor signal was performed by fluorescence microscopy and macroimaging. There were developed methods of assessment of intracellular pH level in tumor cells and animal's tumors with a genetically encoded ratiometric sensor.

Analysis of endogenous metabolic co-factors NADH and FAD in tumor cells based on fluorescence intensity and lifetime estimation. There were studied the change of fluorescence intensity and fluorescence lifetime of metabolic co-factors NADH and FAD in tumor cells. To achieve this there were developed the methods of visualization of metabolic co-factors of the respiratory chain in cancer cells *in vitro* and tumor tissue of animals by the method of two-photon fluorescence microscopy with temporal resolution. Results. In the in vitro system have been shown, that in more alkaline conditions excitation maxima of genetically encoded sensor at 420 nm decreased and at 500 nm increased, resulting in increase of the I500/I420 ratio. The cells were injected subcutaneously to nude mice to generate tumors, and wholebody fluorescence imaging was performed *in vivo* to visualize the indicator emission at 520 nm.

Calculation of the I500/I430 ratio showed that the signal of the sensor in HeLa tumor is highly heterogeneous, which indicates differences in pHi value in a tumor tissue. The data of the fluorescence imaging were confirmed by histopathological investigation and hypoxia analysis on the tumor tissue sections. The differences in the fluorescence intensity and fluorescence lifetime of metabolic co-factors NADH and FAD in tumor and normal cells *in vitro* have been shown. Also the specific features of NADH and FAD concentration have been found during tumor progression in living mice.

1. C.V. Dang, Links between metabolism and cancer // Genes Dev. 2012 26: 877-890.
2. L.E. Gerweck, K.Seetharaman. Cellular pH Gradient in Tumor versus Normal Tissue: Potential Exploitation for the Treatment of Cancer. Cancer research. 1996. 56. 1194-1198.
3. A.A. Heikal, Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies. Biomark Med. 2010 April; 4(2): 241-263.
4. M.C. Skala, K.M. Riching, A.Gendron-Fitzpatrick, J. Eickhoff, K.W. Eliceiri, J. G. White, and N. Ramanujam, In vivo multiphoton microscopy of NADH and FAD redox states, fluorescence lifetimes, and cellular morphology in precancerous epithelia. PNAS, 104(49), 19494-19499, 2007

FUNCTIONS OF TNF FROM DISTINCT CELLULAR SOURCES AND NEW APPROACH TO ANTI-TNF THERAPY

S. Nedospasov[1,2,3,4]

[1] Engelhardt Institute of Molecular Biology, Moscow, Russia

[2] Lomonosov Moscow State University, Moscow, Russia

[3] Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia

[4] German Rheumatism Reseach Center, a Leibniz Institute, Berlin, Germany

Tumor necrosis factor (TNF) contributes to pathogenesis of rheumatoid arthritis (RA) and its blockade is widely used as therapy. Using conditional targeting we dissected distinct contributions of TNF produced by various cell types in pathogenesis of collagen-induced arthritis (CIA) in mice.

Surprisingly, our data revealed that TNF produced by myeloid cells (M-TNF) and B cells (B-TNF) both mediate pathogenic effects in CIA, whereas T cell derived – TNF (T-TNF) is protective. Specifically, M-TNF contributes to arthritis induction, B-cell-derived TNF regulates disease severity via control of autoantibody production, and T-TNF limits autoreactive T cell development at systemic level. T-TNF also plays a unique protective role in Tb infection. Thus, distinct TNF-producing cells may differently and even oppositely contribute to disease development, suggesting that TNF inhibition from only restricted cellular sources may be superior to pan-anti-TNF therapy. We have generated such prototype inhibitors and established a system to evaluate them in mice.

LYMPHOTOXIN-DIRECTED MECHANISMS OF INTESTINAL IMMUNITY AND MICROBIOTA CONTROL

Andrey Kruglov[1,2]

[1]German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany

[2]Lomonosov Moscow State University, and Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow , Russia

Intestinal homeostasis depends on complex interactions between the microbiota, the intestinal epithelium and the host immune system. Production of immunoglobulin A (IgA) by intestinal plasma cells is one of the main mechanisms for regulation of intestinal microbiota composition and maintenance of homeostasis. However, molecular and cellular mechanisms governing IgA production still remain poorly defined. We have recently identified critical and distinct functions of soluble $LT\alpha_3$ and surface $LT\alpha_1\beta_2$ expressed by RORγt$^+$ innate lymphoid cells (RORγt$^+$ ILC) in controlling intestinal IgA production. In particular, our recent findings highlighted that two distinct pathways governed by transmembrane and soluble lymphotoxins: surface $LT\alpha_1\beta_2$ produced by RORγt$^+$ ILC is crucial for T cell-independent IgA induction in the small intestine by modulating INOS expression in gut dendritic cells, whereas RORγt$^+$ ILC-derived soluble $LT\alpha_3$ ($sLT\alpha_3$) regulates T-cell dependent IgA induction in the lamina propria. $sLT\alpha_3$ controls IgA induction via control of gut homing of T and B cells and acting via both TNFR1 and TNFR2 expressed by stromal cells. CD40 signaling triggered by T-cell-derived CD40L contributes to the T-cell dependent IgA production controlled by $sLT\alpha_3$. Furthermore, genetic ablation of LTα, but not LTβ, in RORγt$^+$ cells abrogates IgA production in the gut, significantly alters microbiota composition and finally leads to autoantibody production and metabolic changes.

Altogether, these data ascribe novel essential functions for soluble and membrane-bound lymphotoxins produced by RORγt$^+$ ILC in organizing adaptive immune responses in the gut, in the control of the commensal microbiota and subsequent changes in metabolic state and development of autoimmunity.

THE PRO- AND ANTI-INFLAMMATORY ACTIVITIES OF INTERLEUKIN-6: THERAPEUTIC CONSEQUENCES

S. Rose-John

Department of Biochemistry, University of Kiel, Kiel, Germany

Cytokines receptors exist in membrane bound and soluble form. The IL-6/soluble IL-6R complex stimulates target cells not stimulated by IL-6 alone, since they do not express the membrane bound IL-6R. We have named this process *'trans-signaling'*. The soluble IL-6R is generated via ectodomain shedding by the membrane bound metalloprotease ADAM17. Soluble gp130 is the natural inhibitor of IL-6/soluble IL-6R complex responses [1]. The dimerized recombinant soluble gp130Fc fusion protein is a molecular tool to discriminate between gp130 responses via membrane bound and soluble IL-6R responses.

Interestingly, depending on the animal model used, global blockade of IL-6 signaling by neutralizing monoclonal antibodies and selective blockade of IL-6 trans-signaling can lead to different consequences. We used neutralizing monoclonal antibodies for global blockade of IL-6 signaling and the sgp130Fc protein for selective blockade of IL-6 trans-signaling in several animal models of human diseases. Inhibition of IL-6 trans-signaling but not global IL-6 blockade was beneficial in the cecal ligation and puncture sepsis model [2]. Defense against bacterial infections rely on the membrane bound IL-6R [3]. Acute pancreatitis often results in subsequent acute lung injury, which is an inflammatory disease with high mortality. IL-6 is necessary for the inflammatory process to reach the lung and blockade of IL-6 *trans-signaling* is sufficient to block the disease [4]. The extent of inflammation is controlled by *trans-signaling* via the soluble IL-6R. Using the sgp130Fc protein or sgp130Fc transgenic mice we demonstrate in animal models of inflammatory bowel disease, peritonitis, rheumatoid arthritis, atherosclerosis pancreatitis, colon cancer, ovarian cancer, pancreatic cancer and lupus erythematodes that IL-6 *trans-signaling* via the soluble IL-6R is the crucial step in the development and the progression of the disease [1-5]. Therefore, sgp130Fc is a novel therapeutic agent for the treatment of chronic inflammatory diseases and cancer and it underwent phase I clinical trials as an anti-inflammatory in 2013/2014.

1. Jones SA, Scheller J, Rose-John S (2011) Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. J Clin Invest 121: 3375-3383
2. Barkhausen T et al (2011) Selective blockade of IL-6 trans-signaling improves survival in a murine polymicrobial sepsis model. Crit Care Med 39: 1407-1413
3. Hoge J et al (2013) IL-6 controls the innate immune response against *Listeria monocytogenes* via classical IL-6 signaling. J Immunol 190: 703-711
4. Zhang H et al (2013) IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. J Clin Invest 123: 1019-1031
5. Scheller J, Garbers C and Rose-John S (2014) Interleukin-6: from Basic Biology to Selective Blockade of Pro-Inflammatory Activities. Sem Immunol 26: 2-12

SPECIFIC AND NON-HEPATOTOXIC DEGRADATION OF NUCLEAR HEPATITIS B VIRUS CCCDNA BY LYMPHOTOXIN-BETA RECEPTOR ACTIVATION

F. Reisinger[1], J. Lucifora[1,2,3], E. Dejardin[4], Y. Xia[1], K. Zhang[1], D. Stadler[1], X. Cheng[1], W.-M. Chou[1], C. Münk[5], J. Browning[6], M. Landthaler[7] U. Protzer[1,2], M. Heikenwalder[1]

[1] Institute of Virology, Technische Universität München–Helmholtz Zentrum München, Munich, Germany.

[2] German Center for Infection Research (DZIF), Munich and Hamburg sites, Germany.

[3] INSERM U1052, CNRS UMR 5286, Cancer Research Center of Lyon, University of Lyon, LabEx DEVweCAN, Lyon, France.

[4] GIGA-Research Laboratory of Molecular Immunology and Signal Transduction, University of Liège, 4000 Liège, Belgium.

[5] Clinic for Gastroenterology, Hepatology and Infectiology, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany.

[6] Department of Immunobiology, Biogen Idec, Cambridge, USA.

[7] Berlin Institute for Medical Systems Biology at the Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany

Current antiviral agents can control but not eliminate hepatitis B virus (HBV), because HBV establishes a stable nuclear covalently closed circular DNA (cccDNA). Interferon-α treatment can clear HBV but is limited by systemic side effects.

Looking for therapeutic alternatives for HBV infection we found that by triggering of the lymphotoxin-beta receptor (LTbR) signaling pathway, we can induce specific degradation of the nuclear viral DNA without hepatotoxicity. LTbR activation up-regulated and stabilized the expression of the cytidine deaminase APOBEC3B in HBV-infected, differentiated HepaRG cells and primary human hepatocytes resulting in cytidine deamination, apurinic/apyrimidinic site formation, and finally degradation of cccDNA. Hence, APOBEC3B actively prevented HBV reactivation(1).

The exact molecular mechanisms, how short-term LTbR activation leads to a long-lasting and stable up-regulation of APOBEC3B expression and finally to cccDNA degradation without affecting genomic DNA, are currently under investigation. Here I will present data that reveal some of the mechanistic underpinnings of APOBEC3B regulation and function.

1. Lucifora, J., et al. (2014). "Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA." Science 343(6176): 1221-1228

THE CONTRIBUTION OF T CELL-DERIVED TNF TO COLITIS

C. Winsauer[1], A. Kühl[2], and A. Kruglov[1, 3]

[1] German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany

[2] Charité - University Hospital Berlin, Berlin, Germany

[3] Lomonosov Moscow State University, and Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the mucosal immune system in the gastrointestinal tract characterized by enhanced proinflammatory cytokine production by T cell subsets. The systemic blockade of tumor necrosis factor (TNF) in IBD patients significantly ameliorates symptoms. However, the role of TNF during IBD remains not completely elucidated. In the current study we aimed to dissect the contribution of T cell-derived TNF in development and progression of autoimmune colitis. To achieve this, we have employed T cell transfer model of colitis in $Rag1^{-/-}$ animals. Adoptive transfer of naive wild type (WT) T cells resulted in wasting disease marked by weight loss and intestinal inflammation. Surprisingly, TNF-deficient naive T cells failed to induce colitis in $Rag1^{-/-}$ recipients due to crucial role of transmembrane TNF (tmTNF) in survival of T cells upon transfer into $Rag1^{-/-}$ recipients. Furthermore, death of $TNF^{-/-}$ T cells occured via CytC-dependent intrinsic apoptosis. Since tmTNF is known to induce reverse signaling, we next analyzed whether persistence of $TNF^{-/-}$ T cells under lymphopenic conditions requires reverse signaling. Indeed, overexpression of wild type tmTNF, but not of tmTNF lacking domains responsible for reverse signaling, in $TNF^{-/-}$ T cells rescued the survival of T cells in $Rag1^{-/-}$ mice, showing importance of reverse signaling in T cell survival during lymphopenic conditions.

In order to test contribution of T-TNF during established colitis, induction of colitis was performed by injecting naive T cells from human TNF knock-in (hTNF-KI) mice to $Rag1^{-/-}$ mice. Such mice express human TNF by donor T cells, whereas host cells produce murine TNF. Application of anti-human TNF drugs allows elucidating the role of T-TNF during established colitis. We found TNF from T cells is pathogenic during established colitis, since neutralizing T cell-derived TNF significantly ameliorates colitis, while TNF from non-T cells is dispensable and its blockade does not alter the course of disease.

Altogether, our data show an essential role of T cell-derived TNF in maintenance of T cells under lymphopenic conditions and describe a novel model for studying of effects of various anti-hTNF drugs during established colitis.

RIP1 KINASE-DEPENDENT INFLAMMATORY RESPONSE

V. Ilyuha, S. Schworer, A. Poltorak

Petrozavodsk State University, Petrozavodsk, Republic of Karelia, Russia

Receptor interacting protein (RIP) kinase 1 is a member of the family of serine/threonine kinases that is best characterized as mediator of stress responses to activate NF-κB, MAPK, apoptosis, and necrosis (1). The kinase activity of RIP1 is required exclusively for the receptor-mediated necroptosis whereas pro-inflammatory signaling is dependent on the scaffolding function of RIP1 (2,3). Multiple recent reports have identified that, in addition to its role in executing necroptosis, RIP1 kinase activity contributes to *in vivo* cytokines production (4) suggesting that the molecular components of this signaling pathway warrant elucidation. In this study, we have established an *in vitro* system in bone marrow-derived macrophages treated with the pan-caspase inhibitor ZVAD, which led to an increase in pro-inflammatory cytokine production that was inhibited by Necrostatin-1, a specific inhibitor of the kinase activity of RIP1. The effect of RIP1 kinase was compared in two genetically diverse mouse lines such as C57BL6 and MOLF. While ZVAD treatment led to an increase in TNF, CCL3, CXCL2, and IFNβ but not IL-6 in B6 and MOLF macrophages, though the MOLF response was more robust. Studies using chemical inhibitors identified a role for p38 in the high responses of MOLF and TAK1 was important for the RIP1 kinase dependent response in both strains. Additionally, IRAK2 may be an important contributor to the hyper-production of pro-inflammatory cytokines by MOLF in a RIP1 kinase dependent fashion, however whether this occurs through inducing p38 activation is not entirely clear. Using next generation sequencing (NGS) in expression analysis of up-regulated genes, we compared RIP1-mediated expression in C57BL6 and MOLF macrophages at the genome-wide level. Overall, the data establish a model in which TLR signaling in the presence of ZVAD introduced a RIP1 kinase-dependent arm to the production of pro-inflammatory cytokines.

1. Zhang, D., J. Lin, and J. Han. 2010. Receptor-interacting protein (RIP) kinase family. *Cell Mol Immunol* 7: 243–249.

2. Meylan, E., K. Burns, K. Hofmann, V. Blancheteau, F. Martinon, M. A. Kelliher, and J. Tschopp. 2004. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kB activation. *Nat Immunol* 5: 503–507.

3. Lee, T. H., J. Shank, N. Cusson, and M. A. Kelliher. 2004. The Kinase Activity of Rip1 Is Not Required for Tumor Necrosis Factor-alpha-induced IkB Kinase or p38 MAP Kinase Activation or for the Ubiquitination of Rip1 by Traf2. *J. Biol. Chem.* 279: 33185–33191.

4. Christofferson, D. E., Y. Li, J. Hitomi, W. Zhou, C. Upperman, H. Zhu, S. A. Gerber, S. Gygi, and J. Yuan. 2012. A novel role for RIP1 kinase in mediating TNF-α production. *Cell Death Dis* 3: e320.

NOVEL RECOMBINANT ANTI-TNF ANTIBODY BASED FUSION PROTEINS FOR DIAGNOSIS AND TREATMENT.

G. Efimov[1]

[1] Engelhardt Institute of Molecular Biology RAS, Moscow, Russia

Tumor necrosis factor (TNF) is one of the key players in mounting the immune response to pathogens; at the meantime its overexpression plays an important part in pathogenesis of many autoimmune conditions. Therapeutic inhibition of TNF (mostly by recombinant anti-TNF antibodies) has proven to be beneficial for many diseases: rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, psoriasis and others. However in other diseases characterized by TNF overexpression anti-TNF therapy is inefficient or can even result in exacerbation of inflammation. Moreover disruption of TNF signaling can cause serious side effects including increased susceptibility to infections diseases and paradoxically secondary autoimmune pathology. This controversy may be related to recent experimental data suggesting that TNF produced by different cell types may play unequal physiological or pathological role. Depending on the context and probably on the kinetics of expressions the effects of TNF mediated signaling can be either pro- or anti-inflammatory. These finding suggest that systemic TNF-inhibition thought is very efficient in some diseases may not be the ideal strategy for others. Targeted approaches, which allow selective inhibition of TNF produced by particular cell types, may be efficient in conditions previously considered resistant to anti-TNF therapy. Moreover directed therapy is potentially less prone to side effects and may require smaller doses.

To test the idea of cell type specific pharmacological TNFinhibition we generated fusion proteins comprising of anti-TNF single-domain antibody and single chain antibody to cell surface protein, expressed on cells of monocyte/macrophage lineage.This bispecific antibody can simultaneously bind both its targets and can retain TNF produced by macrophages on their surface, preventing its systemic release. *In vivo* experiments showed that this targeted TNF-inhibitor is more efficient than control systemic TNF-inhibitor with the same properties.

Besides the vast amount of experimental data on role of TNF in autoimmunity, precise knowledge of patterns of expression of TNF in inflamed tissues during the course of the disease and its treatment remains elusive. This is partially due to the fact, that current methods of quantification of TNF in tissues in experimental diseases on laboratory animal are invasive and thus will itself influence the course of the disease. There is a need for molecular tool, which allows studying TNF expression i*n vivo* during the course of the disease without affecting it.

To address this problem we generated fluorescent sensor of TNF, which is a fusion protein comprising anti-TNF recombinant single domain antibody and the far-red fluorescent protein. This fusion protein binds TNF with high affinity but does not interfere with simultaneous TNF receptor binding and thus does not inhibit TNF biological activity. This sensor specifically accumulates at the sites of its expression and can be detected *in vivo*. We have successfully tested this fusion protein in several autoimmune disease models. This fluorescent TNF sensor can be used to highlight sites of TNF expression in model diseases without affecting its course, and to reveal how the patterns of TNF expression change during the onset, acute phase of disease and during its treatment. Such sensor may help advance TNF research and ultimately benefit patients with autoimmune disorders by helping to further optimize clinical protocols.

STUDY OF IMMUNOGENICITY OF THE KILLER-RED EXPRESSING TUMORS

M. Shirmanova[1], D. Yuzhakova[1], I. Druzhkova[1], L. Snopova[1], E. Serebrovskaya[2], S. Lukyanov[1, 2] and E. Zagaynova[1]

[1] Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russia

[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia

Establishment of highly immunogenic tumor models is relevant for development of tumor therapies based on activation of the antitumor immunity. There are some reports that enhanced *green fluorescent protein* (EGFP) is immunogenic when expressed in mouse tumors [1, 2]. The purpose of the present work was to assess the immunogenicity of the CT26 tumor expressing phototoxic red fluorescent protein KillerRed (KR).

The study was performed on the immunocompetent Balb/c mice with mouse colon carcinoma CT26 and CT26-KR. Mice that had their subcutaneous (s.c.) tumors surgically removed on the day 9 were rechallenged by a second s.c. or i.v. injection of CT26-KR cells. The tumor growth rate, fluorescence and the number of lung metastases were estimated.

Challenge doses of 0.5 x 10^6 CT26-KR cells s.c. resulted in palpable tumors in 75% of the animals by day 6, followed by progressive tumor growth in 100% of the animals. The same dose of CT26 cells displayed tumor formation in 100% of the animals. Resection of the primary CT26-KR tumors and rechallenge with 0.5 x 10^6 CT26-KR cells s.c. on the contralateral side, demonstrate a protection against tumor challenge in 4 of 7 mice (57%). The growth rate of the rechallenged CT26-KR was significantly slower than the growth rate of naive CT26-KR tumors. Intravenous injection of the surgically cured mice with the mixture of CT26 and CT26-KR (1:1) resulted in formation of lung metastases of CT26 cells in 100 % animals.

These results showed that KillerRed can act as a foreign model tumor antigen in immunocompetent mice. Our future work will be focused on the combination of the immunogenic properties of KillerRed with photodynamic and low-dose cyclophosphamide therapy.

This work has been financially supported by the Ministry of Education and Science of the Russian Federation (project No. 11.G 34. 31.0017), and by the Russian Foundation for Basic Research (grants No. 14-02-00860).

1. A Gambotto et al. // Gene Therapy (2000) 7 (23), 2036-2040
2. AP Castano et al. // British Journal of Cancer (2006) 94, 391 – 397

IRF4 BUT NOT RORγt IS INDISPENSABLE FOR IL17A AND GM-CSF PRODUCTION *IN VIVO*.

I. Mufazalov[1], C. Hackenbruch[1], J. Kuschmann[1], C. Schelmbauer[1], T. Regen[1], N. Yogev[1], U. Klein[3], D. Littman[4], T. Bopp[2] and A. Waisman[1].

[1] Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

[2] Institute for Immunology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

[3] Herbert Irving Comprehensive Cancer Center, Columbia University, New York, USA

[4] Kimmel Center for Biology and Medicine at the Skirball Institute, Department of Pathology, Howard Hughes Medical Institute, New York University, New York, USA

The transcription factors IRF4 and RORγt are both known to play a crucial role in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of human multiple sclerosis. In fact, RORγt knock-out animals develop only mild disease, while mice lacking IRF4 show complete resistance to EAE induction. However, both null knock-out models display certain systemic consequences as a result of gene disruption. Of special importance, RORγt deficient animals do not have lymph nodes, while IRF4 is essential for the development and function of the variety immune cell types.

To overcome these effects we deleted IRF4 or RORγt specifically in T cells. For that purpose we crossed mice carrying *Irf4* and *Rorc* conditional alleles to the *CD4*-Cre transgenic mouse strain. The resulting *Irf4*$^{\Delta T}$ and *Rorc*$^{\Delta T}$ mice are characterized by a T cell-specific loss of function of the indicated factors.

Whereas *Irf4*$^{\Delta T}$ mice present a normal thymic development of T cells, in *Rorc*$^{\Delta T}$ mice CD4$^+$ T cells are underrepresented. Both mouse models show an increase in Th1 and Treg cell populations compared to wild type controls. As a result of *Irf4* deletion, T cells fail to differentiate to Th17 cells under *in vitro* polarizing conditions and after MOG immunization of *Irf4*$^{\Delta T}$ mice. Consequently, *Irf4*$^{\Delta T}$ mice are fully resistant to the induction of EAE. In contrast, T cells lacking RORγt expression can be triggered towards Th17 cells by *in vitro* polarization involving the cytokines TGFβ, IL-6 and IL-23. Moreover, *Rorc*$^{\Delta T}$ mice are susceptible to EAE induction, although the disease severity is reduced compared to wild type mice. At the peak of disease CNS-infiltrating CD4$^+$IL17A$^+$ cells can be found in *Rorc*$^{\Delta T}$ mice, pointing to the pathogenic potential of RORγt-deficient T cells. Importantly, most of the MOG-specific T cells within the inflamed CNS express GM-CSF.

In summary, we show that IRF4 is absolutely required for the development of T cells expressing IL-17A or GM-CSF (two major cytokines associated with T cell encephalitogenicity *in vivo*) and is therefore indispensable for EAE induction. Although IRF4-deficient T cells can produce IFNγ, these Th1 cells are not pathogenic due to impaired MOG-specific T cell generation. In contrast, RORγt is partially dispensable for Th17 cell development and is redundant for GM-CSF production. Thus, we could show that IRF4 and RORγt, although both essential for Th17 cell development are not equally contributing to their pathogenicity.

SOLUBLE FORMS OF MEMBRANE DIFFERENTIATION MOLECULES IN MONITORING OF ONCOLOGICAL DISEASES

K. Shakhova[1,2], E. Kontorshchikova[2], V. Novikov[1]

[1] N.I. Lobachevsky State University of Nizhni Novgorod, *Russia*

[2] Nizhni Novgorod State Medical Academy, *Russia*

Soluble forms of membrane molecules on immunocytes may modulate immune responses. In this study, total and oligomeric fractions of soluble CD8, CD25, CD38, CD50, CD54, CD18, CD95 molecules, as well as CD18-CD54 and CD18-CD50 complexes were quantified by ELISA in patients with various oncological diseases. Serum levels of soluble CD38 and CD95 molecules were elevated in breast cancer and may serve as prognostic factors for response to polychemotherapy. The resistance of breast cancer patients to chemotherapy was associated with high CD38 and CD95 serum levels, while the level of oligomeric CD95 molecules was decreased. Serum levels of soluble *CD38, CD50 and CD54* were elevated in patients with myoma of uterus. The higher CD50 level was associated with the size and the number of tumor nodes. Endometrial cancer patients showed higher levels of soluble CD54 and CD18-CD54 complexes. In summary, soluble CD molecules may be used as useful molecular markers for monitoring clinical course and for response to treatment.

ADAM17 AS PROTEOLYTIC GATEKEEPER OF INFLAMMATION AND CANCER

A. Chalaris-Rißmann[1], S. Schmidt[1], P. Arnold[2] and S. Rose-John[1]

[1] *Institute for Biochemistry, University of Kiel, Kiel, Germany*

[2] *Institute forAnatomy, University of Kiel, Kiel, Germany*

As a member of the ADAM (A Disintegrin And Metalloprotease) family of metalloproteases, ADAM17 was originally identified as a protease responsible for the ectodomain shedding of the membrane-bound precursor of TNF-α. ADAM17 is a type-I transmembrane protein and is expressed in all organs, with high levels of expression in the heart, skeletal muscle, lung, placenta, testis and ovary. Because of the perinatal lethality of ADAM17-deficient mice, radiation-chimeric mice reconstituted with ADAM17-deficient bone marrow cells were used in several studies to evaluate the function of ADAM17 in the hematopoietic system. However, the consequence of inactivation of ADAM17 in nonhematopoietic cells in adult animals remained poorly understood. In this study, we develop a new strategy to generate hypomorphic ADAM17 mice, which show a dramatic reduction of ADAM17 levels.

Intriguingly ADAM17 is a key modulator for clinically important signaling pathways, namely, the TNF-α / TNF receptor, the IL-6 / IL-6R and the epidermal growth factor receptor (EGFR) ligands / EGFR signaling pathways. IL-6 is one of the most crucial modulators of host defense and of the pathogenesis of various inflammatory disorders, such as endotoxin shock, inflammatory bowel disease and rheumatoid arthritis. Moreover IL-6 signaling is dysregulated in many cancers and ADAM17 is essential for shedding of the membrane-bound IL-6R. In recent experiments we investigated the function of ADAM17 in colon cancer development. Colitis associated cancer (CAC) induced by the intraperitoneal injection of the carcinogen azoxymethane (AOM), followed by multiple rounds of inflammation and leukocyte infiltration caused by administration of DSS. We found a moderate decrease in tumor formation in the $ADAM17^{ex/ex}$ mice coupled with increased intestinal inflammation indicating that depletion of ADAM17 interferes with tumor progression rather than tumor initiation. Moreover, sporadic intestinal cancer development was significantly reduced in APC^{Min} / $ADAM17^{ex/ex}$ compared to their APC^{Min} / $ADAM17^{wt/wt}$ littermate controls. Collectively, our work describes novel functions for the metalloprotease ADAM17 in tissue homeostasis, regenerative processes, neoplasia and inflammation and highlight the remarkable feature of this enzyme to modulate various signal transduction pathways by proteolytic cleavage of hormones, cytokines and their cognate receptors.

THE CHAINS OF DEATH: A NEW VIEW ON CASPASE-8 ACTIVATION AT THE DISC

I. Lavrik

Institute of Experimental Internal Medicine, Otto von Guericke University, Magdeburg, Germany

Apoptosis is a programme of cell death, which is essential to all multicellular organisms. There are two signaling pathways of apoptosis: intrinsic that is mediated by mitochondria and extrinsic, that is mediated by a family of death receptors. CD95 (APO-1/Fas) is a member of the death receptor family. The CD95 death-inducing signaling complex (DISC), comprising CD95, FADD, procaspase-8, procaspase-10, and c-FLIP, plays a key role in apoptosis induction. Recently, it was demonstrated that procaspase-8 activation is driven by death effector domain (DED) chains at the DISC. However, their molecular architecture was poorly defined. We used quantitative mass spectrometry, biochemistry and mathematical modeling to further unravel the molecular composition, dynamics and function of the DED chains. Our recent findings will be presented and discussed. These findings provide new insights into apoptosis initiation and the role of c-FLIP/caspase-8/10.

NEURODEGENERATIVE VERSUS CARCINOGENIC MOLECULAR-GENETIC PATHWAYS

E. Rogaev[1,2,3], I. Kuznetsova[2], A. Grigorenko[1,2,3]

[1]Department of Psychiatry, Brudnick Neuropsychiatric Research Institute, University of Massachusetts Medical School, Worcester, USA

[2] Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia

[3] Center for Brain Neurobiology and Neurogenetics, Institute of Cytology and Genetics, RAS, Novosibirsk, Russia

We summarize and discuss here the data on potential interaction of neurodegenerative and cancerogenic molecular pathways. Recent epidemiological association studies have shown inverse correlation between cancer instances and neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease. In contrast, several particular types of cancer, such as melanoma, as well as prostate cancer or breast cancer, have been reported to develop at higher rates in patients with specific neuronal disorders (PD and schizophrenia, respectively). These data may point to the existence of a shared links between cancer and neurodegenerative disorders. Key genes involved in the inherited form of AD participate in apoptotic pathways essential for both degeneration and cancer. Fine mechanisms must be elucidated that force the cell to decide whether to apoptose (cell death and degeneration) or continue growth and proliferation (carcinogenic way). One of the important contributing pathways coordinating such decision involves intra-mitochondrial perturbations. The major genes associated with PD (PARK1, PINK1, LRRK2, parkin) encode proteins known to function in mitochondria. Amyloid precursor protein (APP) and its derivate Aβ peptide, playing the central role in AD development, interacts with mitochondria and Aβ contributes to mitochondrial dysfunction. The linkage between cancer and neurodegeneration is most pronounced if one considers MAPK signaling which regulates mitochondrial regulated apoptosis. This pathway is of particular interest in view of its key role in melanoma development, which in turn shows positive association with PD. The MAPK signaling may act as a double-edged sword, either being protective or cancer-promoting depending on the specific context. We also must further consider that a malfunction may occur on direct dysregulation of components of signal transduction pathways (e.g., such as Notch- and Wnt- signaling) via genetic and epigenetic mechanisms. The role of intramembrane aspartic proteases (PS1/PS2 and SPP/IMPAS) may be of particular interest. For example, PS1 and PS2 are essential for both APP and Notch- processing. The gain of function mutations in PS1/PS2 is a major cause of familial early-onset AD. The mutations lead to increased production of Aβ and to accumulation of amyloid plaques- the hallmark of AD. In contrast, the loss-of function mutations in presenilins may lead to skin cancer (in mouse). Moreover, upregulation of Notch signaling, caused by somatic mutations, contribute to T-cell acute lymphoblastic leukemias. Therefore, it is predicted that certain drug compounds perspective for AD (gamma-secretase inhibitors) reducing proteolytic activity of presenilins may have

therapeutic effects for certain types of cancer (immune cell and breast cancers), but may, potentially, produce also undesirable effects on skin cells. To monitor a function of presenilins on APP, critical in AD degeneration, and on Notch 1, essential in cancerogenic pathways, we generated multiple mutant forms of PS1 and tested them in parallel in proteolytic APP and Notch1 assays. A comprehensive study of molecular-genetic interactions between cancer and neurodegenerative process is of utmost importance for development of safe and targeted therapeutic strategies for both cancer and neurodegenerative disorders.

The authors were supported, in part, by NIH/NIA R01 AG029360 and by the Government of the Russian Federation (No 14.B25.31.0033).

MECHANISMS OF AGING AND STEM CELL DERIVED CANCER: ESSENTIAL ROLE OF TELOMERASE IN ANEUPLOIDY-INDUCED TRANSFORMATION OF HUMAN CELLS

L. Rudolph[1]

[1] Leibniz Institute for Age Research, Fritz-Lipmann-Institute e.v. (FLI), Germany

The risk of cancer development is low at young age but shows an exponential increase during aging. The molecular mechanisms for the aging-associated increase in cancer development are still poorly understood. There is experimental and clinical evidence that adult tissue stem cells exhibit an aging associated increase in mutations and impairments in functionality to maintain tissue homeostasis and regeneration. In addition, adult tissue stem cells were shown to represent the cell type of origin in some types of cancer. During my talk I will present novel molecular mechanisms and checkpoints that contribute to maintenance of genome stability in tissue stem cells and I will present experimental data indicating how the failure of these systems can contribute to cancer development.

HUMAN ARTIFICIAL CHROMOSOMES FOR REGENERATIVE MEDICINE AND GENE THERAPY

M. Liskovykh[1,2], V. Larionov[3], N. Kouprina[3], S. Ponomatrsev[1,2], N. Alenina[4], M. Bader[4], A. Tomilin[1,2].

[1] Institute of Cytology, Russian Academy of Sciences (St.-Petersburg, Russia)

[2] CREI Stem Cells, SkolTech (Russia)

[3] National Institute of Health, National Cancer Institute (Bethesda, USA)

[4] Max-Delbruck Center of Molecular Medicine (Berlin, Germany)

Human artificial chromosomes (HACs) are a powerful DNA vector system developed recently to introduce large chromosomal fragments, genes and regulatory elements into cultured mammalian cells without affecting the host genome. This approach is devoid of known problems of viral or other vector tools such as insertional mutagenesis and unstable expression. However the delivery of HACs directly into cells of living organism is feasible so far only via cultured cells that are first to be targeted with HACs, and then incorporated into desired tissues and organs. Pluripotent stem cells such as embryo-derived embryonic stem (ES) cells and autologous induced pluripotent stem (iPS) cells seem to be an ideal choice for HAC delivery via tissue-replacement because they possess the capacity for unlimited self-renewal *ex vivo* and can differentiate into virtually any cell type of the organism both *in vivo* and *in vitro*.

As initial steps of the envisioned approach, we set to create and functionally evaluate mouse ES cells carrying HAC. To this end, the alphoid^{tetO}-HAC, the newest generation of in vitro assembled HACs, was transferred into mouse ES cells. Autonomous maintenance of this HAC was checked via FISH analysis of chromosome spreads. We also assessed pluripotent state of these ES cells by verifying the expression of pluripotency markers (Oct4, Nanog, and SSEA1) and by examining differentiation capacities of these cells in vitro and in teratoma-formation tests. Furthermore, we observed that HAC-bearing ES cells could also broadly contribute to various tissues of chimeric mice following their injection into blastocysts. Taken together our data suggest that alphoid^{tetO}-HACs can be stably maintained and expressed in pluripotent and derived thereof differentiated cells without any detrimental effects on developmental processes. The result thus serves as a paradigm for the development of further HAC-based strategies of tackling a broad range of hereditary recessive diseases in human.

This work was supported by the Skolkovo Foundation, International Academy of Life Science (IALS) grant, Boehringer Ingelheim Fonds (BIF) grant, President's stipend for young scientists (СП-3805.2013.4), and the Program "Molecular and Cellular Biology" of the Presidium of Russian Academy of Sciences.

COMPARISON OF ISOGENIC HUMAN ES AND IPS CELL LINES REVEALS NO SPECIFIC TRACES OF THE REPROGRAMMING PROCESS

M. Lagarkova[1,2]

[1] Vavilov Institute of General Genetics RAS, Moscow, Russia

[2] Scientific Research Institute of Physical-Chemical Medicine, Moscow, Russia

Human pluripotent stem cell (PSC) lines can be cultured and indefinitely expanded in vitro while maintaining their capacity to differentiate into a variety of cell types. Of the two types of hPSCs—human embryonic (hE)SCs and induced (i)PSCs—the latter are patient-specific; before reprogramming technology can be applied to disease modeling or regenerative medicine, the question of how many independent clones/cell lines should be established and how these should be analyzed to accurately represent patients' cells of interest must be resolved. Also unclear is the extent of similarity between iPSCs and hESCs, which are the gold standard of pluripotency. To obtain comprehensive data on the transcriptional and epigenetic variations that are inherent to the reprogramming process, iPSC lines generated from different somatic cell types that were differentiated from hESCs were compared in two genome-wide assays that analyzed the methylation and expression patterns in these cells. The results revealed the fundamental similarity between hiPSCs and hESCs by two methods: first, by proving that hiPSC lines do not have a specific signature even with the same genetic background; and second, by showing that each act of reprogramming leads to the acquisition of a slightly different pluripotent status that reflects the source of heterogeneity of hiPSCs as well as hESCs.

THERAPEUTIC VACCINATION AGAINST TUMORS: THE TÜBINGEN EXPERIENCE

C. Gouttefangeas[1], K. Laske[1], M. Löffler[1,2], J. Bedke[3], S. Stevanović[1], A. Stenzl[3], H.-G. Rammensee[1]

[1] Institute for Cell Biology, Department of Immunology, Eberhard Karls University Tuebingen and DKFZ partner site Tuebingen,Germany

[2] Department of General, Visceral and Transplant Surgery, Eberhard Karls University, Tuebingen, Germany

[3] Clinic for Urology, Eberhard Karls University, Tuebingen, Germany

Significant advances have recently been achieved in active immunotherapy of cancer, including approval of the first therapeutic treatments. Among the strategies that can be followed, such as adoptive T cell transfer, checkpoint blocking antibodies or vaccines in different formats, our work focuses on the development of therapeutic multipeptide vaccines.

We have established a combined approach to describe novel T-cell target antigens for a number of different tumour types. This approach relies on the identification of tumours MHC-ligands using mass spectrometry, complemented by gene expression analysis and database search. In vitro immunomonitoring is then performed to assess peptide immunogenicity and functional attributes of CD4 and CD8 T cells in vaccinated patients. The results of two phase I/II studies for prostate and renal cell carcinomas that apply multipeptide vaccines together with various adjuvants will be presented. To further improve vaccine efficacy, our current strategy aims at developing patient-tailored cancer vaccines and includes efforts to identify peptides originating from unique tumor-specific genetic alterations.

IMMUNE SYSTEM CONTRIBUTES TO THE EFFICACY OF CANCER CHEMOTHERAPY AND OUTCOME

N. Cherdyntseva[1,2,3], M. Stakheyeva[1], N. Litviakov[1,2], M. Zavjalova[1,2,3], Ya. Kukharev[1], J. Kzhyshkowska[2]

[1] *Cancer Research Institute of Siberian Branch of the Russian Academy of Medical Sciences, Tomsk, Russian Federation*

[2] *Laboratory for translational cellular and molecular biomedicine, Tomsk State University, Tomsk, Russian Federation*

[3] *Siberian State Medical University, Tomsk, Russian Federation*

Introduction. Metastasis of malignant tumors is the main cause of death of cancer patients. Risk of metastasis formation is provided be both tumor cell biological characteristics and the microenvironment features within the primary tumor along with local and systemic conditions for metastatic niche formation. Strong impact of the inflammatory infiltration on tumor progression has been confirmed both by studies of large patient cohorts as well as by using of modern techniques of system biology. Immunosuppressive factors in the tumor microenvironment may impair not only local immune responses but also disturb systemic immunity. L.Zitvogel et al. proposed that understanding the mechanisms governing the immunogenicity of cell death will have a profound impact on the design of anticancer therapies. However, only few immune parameters have been validated as tumor progression associated biomarkers, and one of them was a chemotherapy predictive marker. It is also likely that specific features of tumor cells may influence the potential of immune response. Identification of immune factors and mechanisms promoting tumor dissemination is necessary to identify new prognostic markers to predict metastatic process and optimize anti-metastatic therapeutic strategy at early stages of the disease.

Objective. To study the impact of immune system on clinical response to neoadjuvant chemotherapy and metastasis -free survival in breast cancer patients.

Material and methods. 350 patients with newly diagnosed invasive breast cancer at the age range from 18 to 50 years, treated with preoperative (neoadjuvant) chemotherapy (NAC) were enrolled into the study. The procedures were made in accordance with the Helsinki Declaration. Clinical response to chemotherapy, the 5-year metastasis-free survival and all major clinical and morphological parameters were determined by standard methods. The original method of multidimensional data visualization was applied to present the immune system state as integral entirety in visual image for classification of breast cancer patients with different risk of metastasis (NovoSpark Corporation, Canada). Copy number aberrations (CNA) of cytokine gene regions in tumor specimens were tested using high-density microarray platform CytoScanTM HD Array (Affymetrix, USA). Cytokine gene polymorphism was analyzed with Restriction Fragment Lenth Polymorphism PCR. Numerous parameters of immune system in peripheral blood were evaluated (cell subpopulations, spontaneous and stimulated cytokine production by blood mononuclear cells, their proliferative activity and apoptosis). Subpopulations of lymphocytes and macrophages were determined within the primary tumors from NAC free patients by immunohistochemistry method.

Results. We found, that favorable clinical immediate response to preoperative chemotherapy was related to the high levels of IL-1 β, TNF-alpha and IL-10 production

by peripheral mononuclear cells before the treatment. This correlation was further confirmed by data from the study on association between cytokine gene functional polymorphism and response to NAC. Complete tumor regression after NAC was related to IL-10 high activity polymorphism (IL10 592AA), while low genotypes of IL-1β (IL-1β 3954CC) and TNF-alpha (TNFA 308 GG) were frequently observed in patients with no response to therapy. The increase in soluble Fas receptor, known to protect tumor cells from apoptosis, has been shown in patients with no clinical response to therapy.

We used NovoSpark Corporation visualization approach allowing representation the immune system state as integral unit and to discriminate breast cancer patients with high and low risk of haematogeneic metastasis. When estimated before cancer treatment, 95 % of breast cancer patients had risk of metastasis. The neoadjuvant chemotherapy and surgical tumor removal reduced the risk of tumor progression to 62-71%. However, in a year after adjuvant chemo- and radiotherapy have been completed, the patient group with high risk of metastases increased to 81% again. Thus, the cancer treatment can change the primarily estimated outcome prognosis in breast cancer patients, and the evaluation of the parameters of immune system is a promising approach to predict the risk of cancer progression or resistance to the therapy of the patient.

We have also examined the immune cell infiltration in breast tumor microenvironment in relation to different morphological structures of tumor parenchyma that displays the phenomenon of intratumor heterogeneity providing tumor progression and chemoresistance. The connection between tumor cell expression profile and inflammatory elements has been revealed, indicating the difference in tumor-stromal interaction in various tumor sites. Immune cell infiltration outside the tumor appeared to be associated with hematogenic metastasis.

It is known that tumor has a significant effect on the activation of immune cell where cytokines play a key regulatory role. Cytokine gene expression may be influenced by the chromosome anomalies (CNA - Copy Number Aberration) - deletion and amplification – of cytokine gene loci in tumor cells. The association of cytokine related CNA with both efficacy of NAC and patients' survival has been investigated. We found the close relation between the clinical response to NAC and gain of function of IL-10 and CHI3L1 (YKL40) genes. In contrast, loss of TNF-alpha and IL-17 gene function due to corresponding CNA was associated with good response to NAC. Metastasis- free survival of breast cancer patients was shown to be closely related to CNA affected cytokine gene activity.

Conclusion. The parameters of the activation of systemic and intra-tumoral immune system by growing tumor have to be validated in order to identify the new prognostic markers. It is necessary to take into account the phenomenon of intra-tumor heterogeneity and inflammatory infiltration heterogeneity in order to improve the strategy for tumor-associated immune biomarkers. This heterogeneity is responsible for the patient-specific tumor-stroma interactions and formation of individual tumor microenvironment. It is important to perform systematic and multicomponent analysis of the biological properties of tumor cell affecting their metastatic potentials as well as the analysis of the specific local (tumor microenvironment) and systemic conditions, including immune system, responsible for mobilization of niche-forming component and supporting the metastasis in breast cancer patients.

EXPLORING THE AUTOANTIGENIC REPERTOIRE OF THYROID TUMORS FOR DIFFERENTIAL DIAGNOSTICS

P. Belousov[1], E. Gubernatorova[1], A. Abrosimov[2], A. Kopylov[3], A. Bogolyubova[1,4], Y. Kim[1], K. Lanshchakov[5], V. Vanushko[5], D. Kuprash[1,6]

[1]Department of Immunology, Biological Faculty, M.V. Lomonosov Moscow State University

[2]Laboratory of Pathomorphology and [5] Department of Surgery, Endocrinology Research Center

[3]Laboratory of Systemic Biology, V.N. Orehovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences

[4]Laboratory of Molecular Mechanisms of Immunity and [6]Laboratory of Intracellular Signaling in Health and Disease, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

Serum autoantibodies against tumor-associated antigens (TAA) is a promising class of cancer biomarkers that has been extensively studied for all common human neoplasms. However, protein targets of humoral immune response in patients with endocrine tumors and thyroid neoplasms in particular remain poorly explored. We focused our research efforts on encapsulated follicular-patterned thyroid tumors of different malignant potential, with an emphasis on stratification of patients based on fine histopathological analysis. We found that both the number of autoantigens recognized by serum immunoglobulins from individual patients and frequency of autoantibodies against individual TAA gradually increase from benign through borderline and further to overtly malignant tumors. We identified a number of diagnostically relevant TAA represented by proteins involved in metabolism of fatty acids, steroids and ketone bodies, as well as RNA splicing and protein folding. Our data improve our understanding of immune response to endocrine tumors and provide the basis for a novel non-invasive strategy of differential diagnosis of thyroid neoplasms.

The work is supported by research grant 13-04-01265-a from Russian Foundation for Basic Research and by «Basic Sciences for Medicine» program of the Russian Academy of Sciences.

PARTICIPANTS

A

Achterberg Jörn
DFG Office Russia/CIS
Moscow, Russia
joern.achterberg@dfg.de

Astrakhantseva Irina
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
astrakhantsevairina@gmail.com

B

Belousov Pavel
Lomonosov Moscow State University
Moscow, Russia
belous_p@rambler.ru

Bogolyubova Apollinariya
Lomonosov Moscow State University
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
apollinariya.bogolyubova@gmail.com

C

Chalaris-Rißmann Athena
Institute for Biochemistry,
Christian-Albrechts University of Kiel
Kiel, Germany
achalaris@biochem.uni-kiel.de

Cherdyntseva Nadezhda
Cancer Research Institute of Siberian Branch RAMS
Tomsk, Russia
nvch@oncology.tomsk.ru

D

Drutskaya Marina
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
marinadru@gmail.com

E

Efimov Grigory
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
grigory@efimov.info

Eremenko Elena
Moscow Office Helmholtz Association
German-Russian House
Moscow, Russia
elena.eremenko@helmholtz.de

G

Gouttefangeas Cécile
Institute for Cell Biology
Eberhard Karls University
Tuebingen, Germany
cecile.gouttefangeas@uni-tuebingen.de

H

Heikenwälder Matthias
Institute of Virology,
Technical University of Munich
Helmholtz Zentrum München
Munich, Germany
heikenwaelder@helmholtz-muenchen.de

I

Ilyukha Vladimir
Petrozavodsk State University
Petrozavodsk, Russia
karax911@mail.ru

K

Karaulov Alexander
Sechenov First Moscow State Medical University
Moscow, Russia
drkaraulov@mail.ru

Kazaryan Rafael
Lomonosov Moscow State University
Moscow, Russia
kazaryan@yandex.ru

Kisselev Fedor
Russian Oncological Research Center
Moscow, Russia

Korneev Kirill
Lomonosov Moscow State University
Moscow, Russia
kirkorneev@yandex.ru

Kravchenko Galina
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
kravchukgala@mail.ru

Krispin Martin
German Houses of Science and Innovation (DWIH)
Moscow, Russia
krispin@daad.ru

Kruglov Andrey
Belozersky Institute
of Physico-Chemical Biology MSU
Moscow, Russia
German Rheumatism Research Center (DRFZ)
Berlin, Germany
andrey_krugloff@mail.ru

Kuprash Dmitry
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
kuprash@gmail.com

L

Lagarkova Maria
Vavilov Institute of General Genetics RAS
Moscow, Russia
maryalag@yahoo.com

Latz Eicke
Institute of Innate Immunity
Friedrich-Wilhelms University of Bonn
Bonn, Germany
eicke.latz@uni-bonn.de

Lavrik Inna
Institute of Experimental Internal Medicine,
Otto von Guericke University
Magdeburg, Germany
ilav3103@gmail.com

M

Mufazalov Ilgiz
Institute for Molecular Medicine,
University Medical Center
Johannes Gutenberg University
Mainz, Germany
mufazalo@uni-mainz.de

N

Nedospasov Sergei
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
sergei.nedospasov@gmail.com

Novikov Dmitry
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
novikov.dv75@mail.ru

Novikov Viktor
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
mbre@mail.ru

P

Panov Mikhail
Belozersky Institute
of Physico-Chemical Biology MSU
Moscow, Russia
panovm@ya.ru

Poltorak Alexander
Petrozavodsk State University
Petrozavodsk, Russia
Boston, USA
Alexander.Poltorak@tufts.edu

R

Reisinger Florian
Institute of Virology,
Technical University of Munich
Helmholtz Zentrum München
Munich, Germany
florian.reisinger@helmholtz-muenchen.de

Rogaev Evgeny
Vavilov Insitute of General Genetics RAS
Moscow, Russia
Worcester, USA
Evgeny.Rogaev@umassmed.edu

Rose-John Stefan
Department of Biochemistry
Christian-Albrechts University
Kiel, Germany
rosejohn@biochem.uni-kiel.de

Rudolph Karl Lenhard
Leibniz Institute for Age Research,
Fritz-Lipmann-Institute e.v. (FLI)
Jena, Germany
klrudolph@fli-leibniz.de

S

Shakhova Ksenia
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
kka84@yandex.ru

Shirmanova Marina
Nizhny Novgorod State Medical Academy
Nizhny Novgorod, Russia
shirmanovam@mail.ru

Shvarev Dmitry
Lomonosov Moscow State University
Moscow, Russia
d-shvarev@yandex.ru

T

Tomilin Alexey
Institute of Cytology RAS
Saint Petersburg, Russia
antom111@googlemail.com

Tumanov Alexei
Trudeau Institute
Saranac Lake, USA
atumanov@trudeauinstitute.org

W

Winsauer Caroline
German Rheumatism Research Center (DRFZ),
a Leibniz Institute
Berlin, Germany
Caroline.Winsauer@drfz.de

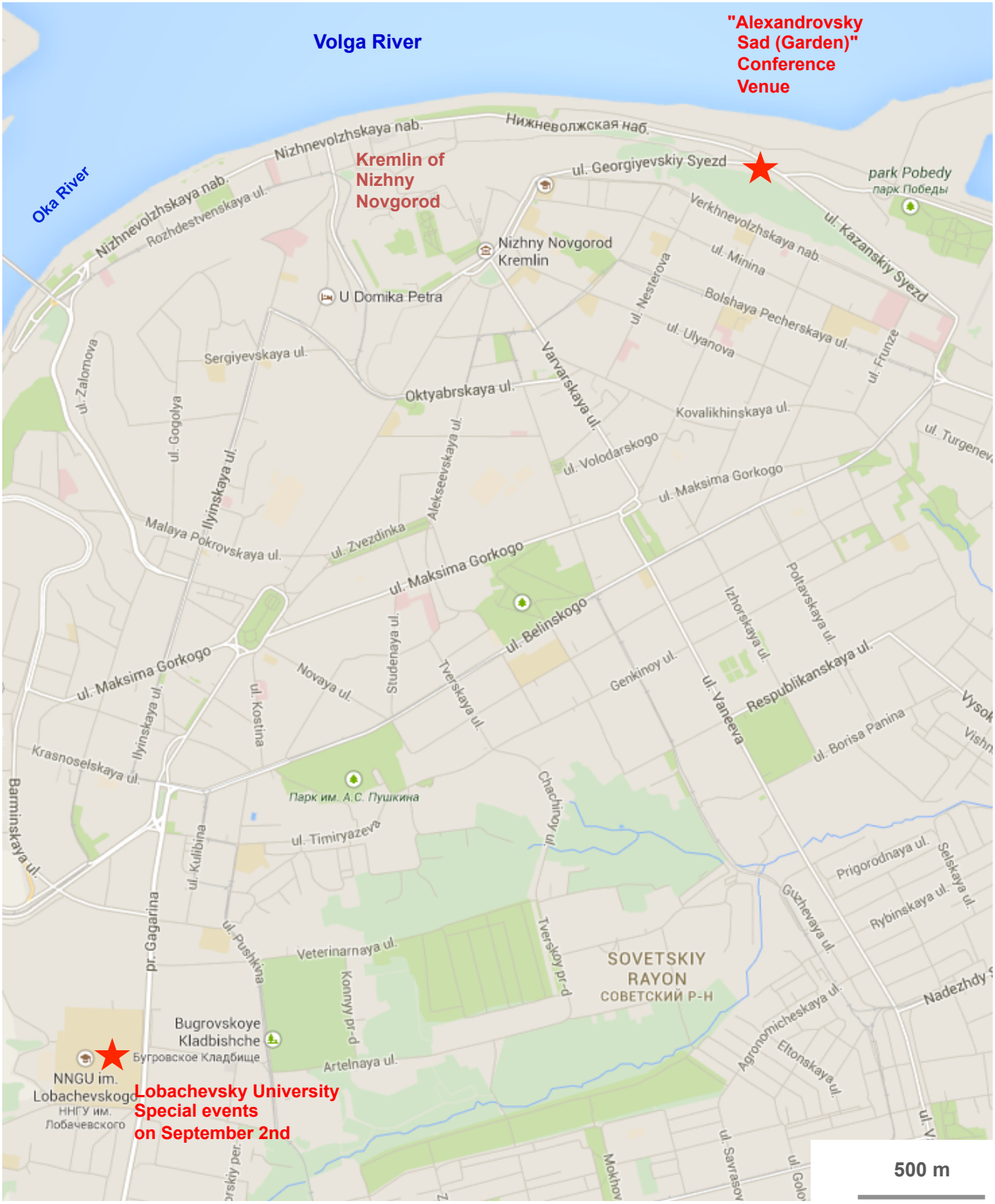
Wunderlich Thomas
Center of Molecular Medicine,
University of Cologne
Cologne, Germany
Thomas.Wunderlich@uni-koeln.de

Z

Zagaynova Elena
Lobachevsky State University of Nizhny Novgorod
Nizhny Novgorod, Russia
ezagaynova@gmail.com

zur Hausen Harald
German Cancer Research Center
Heidelberg, Germany
zurhausen@dkfz.de

Zvartsev Ruslan
Engelhardt Institute of Molecular Biology RAS
Moscow, Russia
r.zvartsev@gmail.com


Volga River
"Alexandrovsky Sad (Garden)" Conference Venue
Oka River
Kremlin of Nizhny Novgorod
Nizhnevolzhskaya nab.
Нижневолжская наб.
ul. Georgiyevskiy Syezd
park Pobedy
парк Победы
Rozhdestvenskaya ul.
Nizhny Novgorod Kremlin
U Domika Petra
Verkhnevolzhskaya nab.
ul. Kazanskiy Syezd
ul. Minina
ul. Nesterova
Bolshaya Pecherskaya ul.
ul. Ulyanova
ul. Frunze
ul. Zalomova
Sergiyevskaya ul.
ul. Gogolya
Oktyabrskaya ul.
Varvarskaya ul.
Kovalikhinskaya ul.
Ilyinskaya ul.
Alekseevskaya ul.
ul. Volodarskogo
ul. Turgeneva
ul. Maksima Gorkogo
Malaya Pokrovskaya ul.
ul. Zvezdinka
ul. Belinskogo
Poltavskaya ul.
Izhorskaya ul.
Novaya ul.
Studenaya ul.
Tverskaya ul.
Genkinoy ul.
ul. Vaneeva
Respublikanskaya ul.
ul. Borisa Panina
Krasnoselskaya ul.
Парк им. А.С. Пушкина
Chachinoy ul.
Barminskaya ul.
ul. Kostina
ul. Timiryazeva
Prigorodnaya ul.
Selskaya ul.
Rybinskaya ul.
pr. Gagarina
ul. Kulibina
ul. Pushkina
Veterinarnaya ul.
Tverskoy pr-d
SOVETSKIY RAYON
СОВЕТСКИЙ Р-Н
Gukheveya ul.
Nadezhdy
Konnyy pr-d
Bugrovskoye Kladbishche
Бугровское Кладбище
Agronomicheskaya ul.
Eltonskaya ul.
Artelnaya ul.
NNGU im. Lobachevskogo
ННГУ им. Лобачевского
Lobachevsky University Special events on September 2nd
ul. Savrasova
500 m

NOTES

NOTES

NOTES