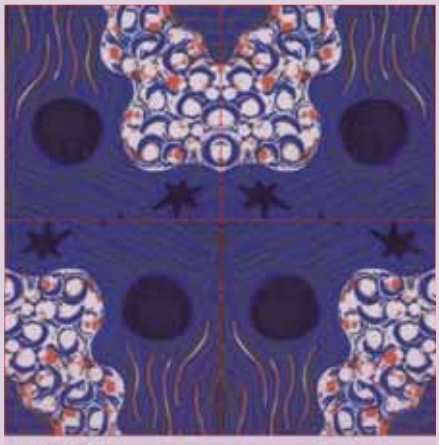
PROGRAMME AND ABSTRACT BOOK

The 6th Symposium & Workshop on Molecular Pathology and Histo(cyto)chemistry

The 94th Seminar of the Czech Division of the International Academy of Pathology


Jana Krejčová

The 2nd Olomouc Days of Histology Laboratory Technicians

APRIL 23–24, 2010
OLOMOUC

THE CZECH REPUBLIC

ORGANIZED BY

- The Czech Society of Pathologists CLS JEP
- The Molecular Pathology Working Group of the Czech Society of Pathologists and the European Society of Pathology
- The Czech Oncological Society CLS JEP
- The Czech Society for Histochemistry and Cytochemistry
- The Czech Society of Laboratory Technicians
- The Department of Pathology & the Laboratory of Molecular Pathology; The Laboratory of Experimental Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc
- University Hospital, Olomouc

ISBN 978-80-87327-07-4

The 6th Symposium & Workshop on Molecular Pathology and Histo(cyto)chemistry

The 94th Seminar of the Czech Division of the International Academy of Pathology

The 2nd Olomouc Days of Histology Laboratory Technicians

April 23–24, 2010, Olomouc, The Czech Republic

Under the auspices of

- Professor M. Mašláň, Ph.D., Rector of Palacký University, Olomouc
- Professor Z. Kolář, M.D., Ph.D., Dean of the Faculty of Medicine and Dentistry, Palacký University, Olomouc
- R. Maráček, M.D., Director of the University Hospital Olomouc

Chairman of the Congress:

Professor Zdeněk Kolář, M.D., Ph.D. / The Department of Pathology & The Laboratory of Molecular Pathology
Faculty of Medicine and Dentistry, Palacký University, Olomouc, Hněvotínská 3, Olomouc, 775 15
Tel: +420/585 632 451; Fax: +420/585 632 966; e-mail: kolarz@tunw.upol.cz

Organizing & Programme Commitee

Professor Jiří Ehrmann, M.D., Ph.D. / The Department of Pathology & The Laboratory of Molecular Pathology
Faculty of Medicine and Dentistry, Palacký University, Olomouc, Hněvotínská 3, Olomouc, 775 15
Tel: +420/585 632 466; Fax: +420/585 632 966; e-mail: info.lmp@seznam.cz

Associate professor Martin Tichý, M.D., Ph.D.
The Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc,
University Hospital, Hněvotínská 3, Olomouc, 775 15
Tel: +420/585 632 453; Fax: +420/585 632 966; e-mail: info.lmp@seznam.cz

Danuše Kvapilová
The Department of Pathology, University Hospital, Hněvotínská 3, Olomouc, 775 15
Tel: +420/585 632 465; Fax: +420/585 632 966; e-mail: d.kvapilova@post.cz

Jan Bouchal, Marie Geierová, Marián Hajdúch, Jana Holinková, Monika Levková, Tereza Muchová, Eva Pimrová, Lenka Prokopová, Jana Steigerová, Dana Šimková, Jozef Škarda, Michaela Šváchová, Ivo Überall

Venue:

Friday, April 23 – Regional Centre Olomouc (Jeremenkova 40B, 772 00 Olomouc)
Saturday, April 24 – Theoretical Institutes Building, Faculty of Medicine and Dentistry, Palacký University, Olomouc (Hněvotínská 3, 775 15 Olomouc)

Conference Language:

The 6th Symposium on Molecular Pathology and Histo(cyto)chemistry – English
The 94th Seminar of the Czech Division of the International Academy of Pathology – Czech
The 2nd Olomouc Days of Histology Laboratory Technicians – Czech

The 6th Symposium on Molecular Pathology and Histo(cyto)chemistry

FRIDAY, APRIL 23, 2010		
Time		**Venue / language**
9.00–9.30	Joint meeting ceremony	Regional Centre Centaurus Hall / English
Keynote lectures of invited speakers **Chairs: M. Hermanová (Brno), M. Elleder (Prague)**		
9.30–10.00	M. Elleder (Prague) Danon disease: rare or unrecognized entity in the Czech Republic	Regional Centre Perseus Hall / English
10.00–10.30	M. Hermanová (Brno) Molecular diagnostics of limb-girdle muscular dystrophies	Regional Centre Perseus Hall / English
10.30–11.00	T. Adam (Olomouc) On the pathophysiology of ADSL deficiency	Regional Centre Perseus Hall / English
11.00–11.30	Coffee break (Andromeda)	
ESP and pathology in the countries of the former Soviet Union **Chairs: G. Bevilacqua (Pisa), G. Burkadze (Tbilisi), J. Ehrmann (Olomouc)**		
11.30–12.00	G. Burkadze (Tbilisi) Pathological anatomy in Georgia: current reality and future prospects	Regional Centre Centaurus Hall / English
12.00–12.15	G. Bevilacqua (Pisa) Possibility of cooperation of European society of pathology with the national societies of pathology in the countries of the former Soviet Union	Regional Centre Centaurus Hall / English
12.15 –12.30	Discussion	Regional Centre Centaurus Hall / English
12.30–13.30	Lunch break (Andromeda) Poster Session	
Keynote lectures of invited speakers **Chairs: J. Mokrý (Hradec Králové), B. Dekel (Tel Aviv)**		
13.30–14.00	L. Lacina (Prague) The Myofibroblast – from the pathologist´s microscope to the nanomaterials development	Regional Centre Perseus Hall / English
14.00–14.30	B. Dekel (Tel Aviv) Identification and prospective isolation of novel human renal stem/progenitor populations from normal and cancerous kidney tissue	Regional Centre Perseus Hall / English
14.30–15.00	J. Mokrý (Hradec Králové) Telomere shortening after ex vivo expansion of tissue-specific stem cells	Regional Centre Perseus Hall / English
15.00–15.30	J. Bártek (Copenhagen) Searching for predictive markers and targets for personalized treatment of cancer	Regional Centre Perseus Hall / English
15.30–16.00	Coffee break (Andromeda)	
Keynote lectures of invited speakers **Chairs: P. Murray (Birmingham), J. Hejnar (Prague)**		
16.00–16.20	W. Doppler (Innsbruck) Signal transducers and activators of transcription (STATs) in breast cancer	Regional Centre Perseus Hall / English
16.20–16.40	P. Murray (Birmingham) Differential regulation of Blimp1 isoforms by the Epstein-Barr virus; implications for virus persistence and the pathogenesis of EBV-associated lymphomas	Regional Centre Perseus Hall / English
16.40–17.00	J. Hejnar (Prague) Tumor or rumor viruses? Newly emerging retrovirus infections in human	Regional Centre Perseus Hall / English

POSTERS

1 **Contribution of aberrant lipid signalling to the pathogenesis of Hodgkin's lymphoma**
Abdullah M.A.[1], Baumforth K.R.N.[1], Roberts C.[3], Wei W.[1], Woodman C.B.[1], Murray P.G.[1]
[1]School of Cancer Sciences, University of Birmingham, Vincent Drive, Edgbaston, Birmingham, United Kingdom;
[2]Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia;
[3]Department of Pathology, University Hospitals Birmingham NHS Foundation Trust, United Kingdom

2 **TOP2A gene status in "triple negative" breast cancer patients as a possible anthracycline response predictor**
Bouchalová K.[1], Čížková M.[1,2], Trojanec R.[1], Koudeláková V.[1], Mlčochová S.[1], Furstová J.[1], Radová L.[1], Dziechciarková M.[1], Melichar B.[2], Cwiertka K.[2], Kolář Z.[3], Hajdúch M.[1]
[1]Laboratory of Experimental Medicine, Department of Paediatrics, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic;
[2]Oncology Clinic, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic;
[3]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic

3 **Detection of Claudin-1 in tumors of the colon**
Brychtová S., Bezděková M., Sedláková E.
Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic

4 **Utilization of laser capture microdissection in diagnosis and prognosis of cancer disease**
Dziechciarková M.[1], Berkovcová J.[1], Trojanec R.[1], Čížková M.[1,2], Bouchalová K.[1], Srovnal J.[1], Hajdúch M.[1,2], Hostášková P.[1], Gilíková M.[1]
[1]Laboratory of Experimental Medicine, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital Olomouc, The Czech Republic;
[2]Oncology Clinic, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital in Olomouc, The Czech Republic

5 **Globotriaosylceramide expression in human placental fetal capillaries**
Hůlková H.[1], Elleder M.[1], Šmíd F.[2], Ledvinová J.[1], Kuchař L.[1]
[1]Institute of Inherited Metabolic Disorders, Charles University in Prague, First Faculty of Medicine;
[2]Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine and General University Hospital, Prague

6 **Immunohistochemical analysis of KLK11, KLK7, PSA and PSMA in cancerous and noncancerous prostatic tissues**
Jamaspishvili T.[1], Král M.[2], Khomeriki I.[2], Kurfürstová D.[1], Bouchal J.[1]
[1]Laboratory of Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;
[2]Department of Urology, Faculty Hospital, Olomouc, The Czech Republic

7 **Quantitative characterization of villous capillary branching in diabetic placentas**
Jirkovská M.[1], Kaláb J.[1], Jadrníček M.[1], Niedobová V.[1], Moravcová M.[2], Krejčí V.[2], Žižka Z.[2]
[1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, Prague, The Czech Republic;
[2]Department of Obstetrics and Gynecology of the First Faculty of Medicine and General University Hospital, Charles University in Prague, Prague, The Czech Republic

8 **Pericyte coverage of fetoplacental vessels in pregnancies complicated by DMI**
Kučera T.[1], Vyletěl I.[1], Moravcová M.[2], Krejčí V.[2], Žižka Z.[2], Jirkovská M.[1]
[1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague; The Czech Republic;
[2]Department of Obstetrics and Gynaecology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, The Czech Republic

9 **Modulation of function of MDR-associated ABC transporters by chemotherapeutic drugs**
Procházková J.[1], Sotolářová K.[1], Kozubík A.[1,2], Pachernik J.[1]
[1]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Brno, The Czech Republic;
[2]Department of Cytokinetics, Institute of Biophysics of Czech Academy of Science, Brno, The Czech Republic

10 **Analysis of the prognostic impact of nestin expression in non-small cell lung cancer**
Škarda J.[1], Kolář Z.[1], Janíková M.[1], Chmelová J.[2], Krejčí V.[1], Zapletalová J.[2], Langová K.[2], Klein J.[3], Grygárková I.[4], Kolek V.[4], Fridman E.[5], Kopolovic J.[6]
[1]Department of Pathology and Laboratory of Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;
[2]Department of Bioinformatics, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;
[3]1st Department of Surgery, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;
[4]Department of Tuberculosis and Respiratory Diseases, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;
[5]Department of Pathology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Israel;
[6]Department of Pathology, Hebrew University and Hadassah Medical School, Jerusalem, Israel

11 **Gene expression profiling in breast cancer after neoadjuvant treatment – preliminary report**
Tvrdík D.[1], Povýšil C.[1], Dundr P.[1], Velenská Z.[1], Melčáková Š.[1], Skálová H.[1], Petruželka L.[2]
[1]Institute of Pathology, First Faculty of Medicine, Charles University and General University Hospital in Prague, The Czech Republic;
[2]Department of Oncology, First Faculty of Medicine, Charles University and General University Hospital in Prague, The Czech Republic

12 **Chromosomal abnormalities of brain tumors – two years experience with cytogenetic analyses**
Urbanovská I.[1], Jalůvková M.[1], Uvírová M.[1], Dvořáčková J.[1,2], Buzrla P.[2], Paleček T.[3]
[1]CGB laboratory Inc., Ostrava, The Czech Republic;
[2]Department of Pathology, Faculty Hospital Ostrava, The Czech Republic;
[3]Clinic of Neurosurgery, Faculty Hospital Ostrava, The Czech Republic

13 **Immunohistochemical visualization of nestin coexpression with other antigens in vasculature of uterine horns of pregnant rats**
Vařejková M.[1], Hollerová H.[2], Kudláčková Z.[1], Mokrý J.[2]
[1]Department of Biological and Medical Sciences, Charles University Faculty of Pharmacy, Hradec Králové, The Czech Republic;
[2]Department of Histology and Embryology, Charles University Medical Faculty, Hradec Králové, The Czech Republic

SOCIAL PROGRAMME	
19.00–24.00	Concert in The Basilica Minor Church of the Visitation of the Virgin Mary – Svatý Kopeček Social evening in Archa restaurant – Svatý Kopeček Wine exhibition and tasting (Winery Polehňa, Blatnice pod Sv. Antonínkem)

Workshop on Molecular Pathology

SATURDAY, APRIL 24, 2010		
Time	**Chairs: K. Souček (Brno), J. Bouchal (Olomouc)**	**Venue/language**
9.00–9.30	V. Horváth (Brno) Real-time cell analysis on xCELLigence instrument – principle of detection and applications	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech
9.30–10.00	L. Knopfová (Brno) c-Myb promotes motility and invasion of breast cancer cells	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech
10.00–10.30	K. Souček (Brno) TGF-β1 suppresses IL-6-induced STAT3 activation through regulation of Jak2 expression in prostate epithelial cells	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech
10.30–10.45	Coffee break (foyer)	
10.45–11.30	Practical demonstration – Roche Real time analysis of cell culture	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech

94. olomoucký meziregionální mezioborový diagnostický seminář Společnosti českých patologů a české sekce International Academy of Pathology

PÁTEK, 23. DUBNA 2010		
Čas	**Předsednictvo: M. Tichý, M. Geierová (Olomouc)**	**Místo konání/jazyk**
9.00–9.30	Slavnostní zahájení Diagnostický seminář Společnosti českých patologů a české sekce International Academy of Pathology	Regionální centrum sál Centaurus / anglicky
9.30–10.45	Diagnostický seminář – část I	Regionální centrum sál Hercules / česky
10.45–11.00	Ing. Z. Rous (Leica) Rychlý, spolehlivý a flexibilní způsob digitalizace sklíček – LEICA SCN 400	Regionální centrum sál Hercules / česky
11.00–11.30	Přestávka, občerstvení (Andromeda)	
ESP and pathology in the countries of the former Soviet Union Chairs: G. Bevilacqua (Pisa), G. Burkadze (Tbilisi), J. Ehrmann (Olomouc)		
11.30–12.00	G. Burkadze (Tbilisi) Pathological anatomy in Georgia: current reality and future prospects	Regional Centre Centaurus Hall / English
12.00–12.15	G. Bevilacqua (Pisa) Possibility of cooperation of European society of pathology with the national societies of pathology in the countries of the former Soviet Union	Regional Centre Centaurus Hall / English
12.15–12.30	Discussion	Regional Centre Centaurus Hall / English
12.30–13.30	Oběd (Andromeda) Sekce posterů	
13.30–13.45	M. Janíková, M. Tichý, J. Ehrmann, J. Škarda (Olomouc) Current methods in molecular pathology used in diagnosis of gastrointestinal stromal tumors	Regionální centrum sál Hercules / česky
13.45–15.30	Diagnostický seminář – část II	Regionální centrum sál Hercules / česky
15.30–16.00	Občerstvení (Andromeda)	

SPOLEČENSKÝ PROGRAM	
19.00–24.00	Koncert v bazilice Navštívení Panny Marie na Sv. Kopečku Společenský večer v restauraci Archa na Sv. Kopečku Degustace vín (Vinařství Polehňa, Blatnice pod Sv. Antonínkem)

Ve dnech 23.–25. 4. 2010 probíhá „Jarní Flora Olomouc“. Vřele doporučujeme!

Workshop molekulární patologie

SOBOTA, 24. DUBNA 2010		
Čas	**Předsednictvo: K. Souček (Brno), J. Bouchal (Olomouc)**	**Místo konání / jazyk**
9.00–9.30	V. Horváth (Brno) Analýza buněk v reálném čase na přístroji xCELLigence – princip, detekce a aplikace	Teoretické ústavy LF UP / česky
9.30–10.00	L. Knopfová (Brno) Protein c-Myb zvyšuje motilitu a invazivitu buněk prsního karcinomu	Teoretické ústavy LF UP / česky
10.00–10.30	K. Souček (Brno) Interakce signálních drah IL-6 a TGF-β1 u epitheliálních buněk prostaty	Teoretické ústavy LF UP / česky
10.30–10.45	Přestávka, občerstvení (foyer)	
10.45–11.30	Praktická ukázka – Roche Analýza buněčných kultur v reálném čase	Teoretické ústavy LF UP / česky

2. olomoucké dny histologických laborantů

PÁTEK, 23. DUBNA 2010		
Čas		**Místo konání / jazyk**
9.00–9.30	Slavnostní zahájení	Regionální centrum sál Centaurus / anglicky
Konference histologických laborantů I **Předsednictvo: J. Ehrmann, D. Kvapilová (Olomouc)**		
9.30–9.45	M. Šváchová, A. Lukášová (Olomouc) Molekulárně biologická analýza klonální přestavby IgH B-buněk a TCR T-buněk metodou PCR	Regionální centrum sál Centaurus / česky
9.45–10.00	P. Drápalová, M. Ondráková, P. Krystková (Brno) Přikrojení a zalití bioptického materiálu včera a dnes	Regionální centrum sál Centaurus / česky
10.00–10.15	R. Kopřiva (Medial s. r. o.) BRADY – Komplexní řešení laboratorního značení pro zajištění jakosti a bezpečnosti lidských tkání a buněk	Regionální centrum sál Centaurus / česky
10.15–10.30	Baria (Praha) „BARIA a BIOGENEX“	Regionální centrum sál Centaurus / česky
10.30–10.45	J. Škarda, T. Tichý, V. Krejčí, J. Šťastná (Olomouc) Využití imunohistochemických metodik k odhadu metastazování plicních nádorů	Regionální centrum sál Centaurus / česky
10.45–11.00	M. Ondráková (Brno) Informace z ČSHL	Regionální centrum sál Centaurus / česky
11.00–11.30	Přestávka, občerstvení (Andromeda)	
ESP and pathology in the countries of the former Soviet Union **Chairs: G. Bevilacqua (Pisa), G. Burkadze (Tbilisi), J. Ehrmann (Olomouc)**		
11.30–12.00	G. Burkadze (Tbilisi) Pathological anatomy in Georgia: current reality and future prospects	Regional Centre Centaurus Hall / English
12.00–12.15	G. Bevilacqua (Pisa) Possibility of cooperation of European society of pathology with the national societies of pathology in the countries of former Soviet Union	Regional Centre Centaurus Hall / English
12.15 –12.30	Discussion	Regional Centre Centaurus Hall / English
12.30–13.30	Oběd (Andromeda) Sekce posterů	
Konference histologických laborantů II **Předsednictvo: D. Kvapilová (Olomouc)**		
13.30–13.45	H. Hollerová (Hradec Králové) Průkaz aktivity acetylcholinesterázy podle Karnovsky-Rootse	Regionální centrum sál Centaurus / česky
13.45–14.00	Ing. Z. Rous (Leica) Nový servisní koncept LEICA Remote Care – kontrola na dálku	Regionální centrum sál Centaurus / česky
14.00–14.15	I. Rozkošný (Nikon s. r. o.) N-SIM and N-STORM super resolution microscopy and CellSurgeon and Tissue Surgeon Nanodisection technology	Regionální centrum sál Centaurus / česky
14.15–14.30	E. Sedláková (Olomouc) Kdy je Herceptin ten správný lék?	Regionální centrum sál Centaurus / česky
14.30–15.00	Diskuze	Regionální centrum sál Centaurus / česky
15.30–16.00	Občerstvení (Andromeda)	

POSTERY	
1	Gleason score punkční biopsie – porovnání s definitivním preparátem z radikální prostatektomie Němečková L.[1], Vodičková J.[1], Martinková L.[1], Navrátilová B.[1], Straka V.[1], Hafuda A.[2] [1]Oddělení patologické anatomie, Oblastní nemocnice Náchod, Česká republika; [2]Urologické oddělení, Oblastní nemocnice Náchod; Česká republika
2	New opportunities in monitoring mutations in patients with non-small cell lung cancer indicated for treatment with tyrosine kinases inhibitors Staněk L., Melčáková Š., Tvrdík D., Vítková I., Povýšil C. Ústav patologie, 1. lékařská fakulta, Karlova univerzita a Všeobecná fakultní nemocnice Praha, Česká republika

SPOLEČENSKÝ PROGRAM	
19.00–24.00	Koncert v bazilice Navštívení Panny Marie na Sv. Kopečku Společenský večer v restauraci Archa na Sv. Kopečku Degustace vín (Vinařství Polehňa, Blatnice pod Sv. Antonínkem)

Ve dnech 23.–25. 4. 2010 probíhá „Jarní Flora Olomouc". Vřele doporučujeme!

Workshop molekulární patologie

SOBOTA, 24. DUBNA 2010		
Čas	**Předsednictvo: K. Souček (Brno), J.Bouchal (Olomouc)**	**Místo konání / jazyk**
9.00–9.30	V. Horváth (Brno) Analýza buněk v reálném čase na přístroji xCELLigence – princip, detekce a aplikace	Teoretické ústavy LF UP / česky
9.30–10.00	L. Knopfová (Brno) Protein c-Myb zvyšuje motilitu a invazivitu buněk prsního karcinomu	Teoretické ústavy LF UP / česky
10.00–10.30	K. Souček (Brno) Interakce signálních drah IL-6 a TGF-β1 u epitheliálních buněk prostaty	Teoretické ústavy LF UP / česky
10.30–10.45	Přestávka, občerstvení (foyer)	
10.45–11.30	Praktická ukázka – Roche Analýza buněčných kultur v reálném čase	Teoretické ústavy LF UP / česky

Honorary guests

Generoso Bevilacqua, Professor, M.D., Ph.D.

CURICULUM VITAE

Generoso Bevilacqua currently holds the position of Professor of Pathology at the University of Pisa. He is a director of both BIOS – Research Doctorate School in BIOmolecular Sciences at the University of Pisa and Division of Surgical, Molecular and Ultrastructural Pathology at the University of Pisa and Pisa University Hospital. He gained his degree in medicine from the University of Pisa in 1971 and in pathology from the Catholic University in Rome in 1976, followed by extensive training at different institutions both in Europe and the U.S.A. He is currently chairman of two bodies (the Working Group for Molecular Pathology and Committee for Testing and Certification) of the European Society of Pathology. He is also a member of a number of important scientific and editorial boards. Professor Generoso Bevilacqua has published more than 200 reserach articles. Above all, he is the co-discoverer of the NM23 gene, the first gene found to be involved in tumor metastasis control. This is patented by the U.S.A. Government. He also participated in demonstrating the infectious pathway of the MMTV (murine mammary tumor virus) in the newborn mouse and demonstrating that MMTV exogenous sequences occurs in a high percentage of human breast carcinomas. He also uncovered the BRCA1 gene mutation considered typical of the Tuscany population.

Juri Kopolovic, Professor, M.D., Ph.D.

CURICULUM VITAE

Juri Kopolovic is currently Professor of Pathology at the Hebrew University – Hadassah Medical Center in Jerusalem. He completed his medical studies in 1972 at the Hebrew University – Hadassah School of Medicine. During residency at Hadassah Medical Center, he became interested in nephropathology and gynaecopathology. In 1986-1988 he continued his training with a clinical and research fellowship at the Pathology Department at the Beth Israel Hospital of Harvard School of Medicine in Boston, where he worked on morphological changes in the nephron in rat experimental model of hypoxia and reperfusion. Upon returning to Israel he continued research in the field of autoimmunity, atherosclerosis and extracellular matrix in malignancies. He showed that in an experimental model of mice SLE treatment with intravenous gamma globulin leads to a complete clinical and morphological remission of SLE. He studied the role of the extracellular matrix in local invasion, metastasis, angiogenesis and prognosis in female genital tract malignancies. He is presently the chairman of the Department of Pathology at the Hebrew University School of Medicine, Jerusalem, Israel.

Petr Dubový, Professor, D.Sc., Ph.D.

CURICULUM VITAE

Petr Dubový currently holds the position of Professor and Head of the Department of Anatomy at Masaryk University (MU) in Brno. He obtained his Master and Doctoral degree of Biology in the Faculty of Natural Science and degree of Professor of Human Anatomy in the Medical Faculty of MU.

He was a visiting scientist in the Department of Anatomy and Neuroscience, Karolinska Institutet, Stockholm (1991–1995). In the period of 2001–2009, he was chairman of the Czech Society for Histo- and Cytochemistry. He is interested mainly in the cellular and molecular biology of neuron-glial cell interactions during degeneration and regeneration of the nervous system, especially using in situ proteomics (immunohistochemistry). His recent scientific work focuses to study the role of cytokines, chemokines and their receptors in neuropathic pain induction as a simultaneous process of nerve regeneration. He has published over 100 peer-reviewed papers.

Invited guests

Milan Elleder, Professor of Pathology, M.D., D.Sc.

CURICULUM VITAE

Milan Elleder was born in Prague and went to medical school at the Charles University in Prague, earning his medical degree in 1964 and immediately embarking upon a life long path of medical research. This path began at Hlava's Institute of Pathology in Prague (part of the First Faculty of Medicine of The Charles University), which was to be his professional home for thirty years (1964–1994). It was while working at Hlava's Institute that he was board-certified as a pathologist. In the research he pursued there, he investigated and developed histochemical techniques, particularly in the area of lipid histochemistry, focusing both on their theoretical aspects and on their use in diagnosing genetic disorders (especially lysosomal lipid-storage disorders). This research led to a CSc (a Czech title equivalent to a Ph.D.) thesis entitled "Lipid Histochemical Methodological Study and its Significance for Diagnosis of Inborn Errors of Lipid Metabolism", defended in 1981. The methods he had developed he then applied to the study of Niemann-Pick disease – the result being his doctoral (D.Sc.) thesis "Niemann-Pick disease: Heterogeneity of the Storage Process and Novel Phenotypical Variants", which he defended in 1988. When democracy returned in 1989, his academic carrier could take off. He served as Vice Dean for Research at the First Faculty of Medicine (1990–1996), during this time he succeeded in pushing through a common framework for Ph.D. programs in biomedicine that united the medical faculties, the Faculty of Natural Sciences of the Charles University and various institutes of the Czech Academy of Sciences. Moreover, in 1994 he founded a new research institute within the First Faculty of Medicine – the Institute of Inherited Metabolic Disorders. He served as its head until 2008 and continued to pursue research within its ranks. His life long research interest is cellular pathology of genetic disorders.

Milan Elleder is author of over 2000 articles. He is member of several national and international societies.

Danon disease: rare or unrecognized entity in the Czech Republic?

Milan Elleder
Institute of Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, and Teaching Hospital, Prague, The Czech Republic

Danon disease is an X-linked lysosomal genetic disorder caused by mutation of one of the lysosomal associated membrane proteins Lamp2. Its main manifestation is hypertrophic cardiomyopathy, which dominates the clinical picture, less expressed myopathy, hepatopathy and neurologic symptoms. It belongs to the group lysosomal disorders caused by mutation of noncatalytic proteins, the function of which is still not well understood. Consequences of these disorders are variable and differ from the classical lysosomal enzymopathies featured by intralysosomal accumulation of uncleaved enzyme substrates. Cell pathology of Lamp2 mutation is featured by altered macroautophagy which is increased but ineffective and by deficient chaperon mediate autophagy.

We applied diagnostic procedures relevant to recognize Lamp2 deficiency in endomyocardial biopsies together with a reliable screening procedure.

Endomyocardial biopsies and blood smears were used for detection of LAMP2 protein with a specific polyclonal antibody working in both unfixed and paraffin embedded samples.

The diagnosis was reached in one family by endomyocardial and skeletal muscle biopsies in a proband which displayed autophagic process and absence of LAMP2 protein either in situ or in cell lysates. Pathogenic mutation (new type) was defined by sequencing the responsible gene. Several affected members of the family were recognized additionally. Two of them are after successful heart transplantation. The findings in biopsies were correlated with findings in the blood smears, which showed absence of detectable Lamp2 in lysosomal, and lysosome related organelles in white blood cells.

Absence of Lamp2 protein should become part of differential diagnosis of cardiomyopathies, especially those types displaying signs of lysosomal affection. Screening procedure based on detection of Lamp2 protein in peripheral blood smears is highly recommended and is going to be applied in the future.

CURICULUM VITAE

Markéta Hermanová, Associate Professor, M.D., Ph.D.

Markéta Hermanová graduated from the Faculty of Medicine of Masaryk University Brno in 1993. She obtained her Ph.D. in pathology in 2001 and received an Associate Professorship in pathology in 2006. She is an active researcher and head of the First Department of Pathological Anatomy, St. Anne´s University Hospital Brno and Faculty of Medicine, Masaryk University Brno. Her research activities are focused on pathology of gastrointestinal tract with special interest in pancreatology. She is a member of a multidisciplinary team specialized in diagnostics of neuromuscular disorders. She is active in several Czech and international scientific societies and has published a number of outstanding research articles.

Molecular diagnostics of limb-girdle muscular dystrophies

Hermanová M.[1,2], Stehlíková K.[3], Vondráček P.[4], Zámečník J.[5], Fajkusová L.[3]

[1]First Department of Pathological Anatomy, St. Anne´s University Hospital Brno and Masaryk University, The Czech Republic
[2]Department of Pathology, University Hospital Brno and Masaryk University, The Czech Republic
[3]Centre of Molecular Biology and Gene Therapy, University Hospital Brno and Masaryk University, The Czech Republic
[4]Department of Pediatric Neurology, University Hospital Brno and Masaryk University, The Czech Republic
[5]Department of Pathology and Molecular Medicine, Charles University, Second Medical Faculty and University Hospital Motol, Prague, The Czech Republic

The muscular dystrophies represent a group of inherited disorders characterized by muscle wasting and weakness and sharing the common histological features of dystrophic muscle biopsy changes. Limb-girdle muscular dystrophies (LGMDs) represent a genetically and clinically heterogeneous group of muscular dystrophies preferentially affecting the large muscles of the pelvic and shoulder girdles. Till now, 14 forms of autosomal recesive (AR) LGMD and 8 forms of autosomal dominant (AD) LGMD have been identified and described. Mutations in many different genes encoding the sarcomeric, nuclear envelope, sarcolemmal and cytosolic proteins are responsible for the different forms of LGMDs. Several AR LGMDs are associated with mutation in genes encoding glycosyltranferases responsible for glycosylation of alpha-dystroglycan. Considering a large clinical and genetic heterogeneity, a precise diagnosis of LGMD requires a comprehensive clinical and laboratory approach.

To identify a study population with LGMD phenotype and to perform the mutational analysis of the expected most frequently mutated genes involved in pathogenesis of LGMD in the Czech Republic.

The study population consisted of 169 patients with LGMD phenotype. Based on the results of clinical assessment and histopathological examinations of muscle biopsy (including the evaluation of muscle proteins expression using immunohistochemistry and immunoblotting), the mutational analysis of potentially involved genes was performed.

DNA and/or mRNA mutational analysis of CAPN3 gene was performed in 169 patients, mutations in both alleles were revealed in 37 patients and diagnosis of LGMD2A was confirmed. DNA and/or mRNA mutational analysis of FKRP gene was performed in 110 patients, homozygous occurence of the mutation c.826C>A was detected in 5 patients and the diagnosis of LGMD2I was confirmed. The mRNA analysis of DYSF gene in 15 patients and the DNA analysis of CAV3 gene in 7 patients brought no positive result.

Accurate diagnoses of muscular dystrophies are essential for providing precise genetic counceling and effective clinical care in specific subgroups of patients. Moreover, an understanding of genetics and pathophysiology of LGMD will be helpful to identify future therapeutic targets and strategies.

The diagnostic process needs to integrate clinical analysis, protein analysis in muscle biopsies, and genetic testing in specialised centres where the multidiscilinary approach can be fully applied.

CURICULUM VITAE

Tomáš Adam, Associate Professor, Ph.D.

Tomáš Adam graduated from the Faculty of Natural Sciences of Palacký University in Olomouc in 1989. He obtained his Ph.D. in biochemistry in 1999 and received an Associate Professorship in medical genetics in 2005.

He is a founder and head of the Laboratory of Inherited Metabolic Disorders, Department of Paediatrics, Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc. His research activity focuses on nucleotide metabolism and most recent scientific work is directed to cellular metabolomics. His bibliography includes over 50 original papers and review articles and 2 chapters in books. He has successfully supervised many Ph.D. students and presented multiple lectures at international conferences.

He is a regular reviewer for peer-reviewed journals (e.g. Electrophoresis), member of a number of international scientific societies as well as editorial boards of scientific journals.

On the pathophysiology of ADSL deficiency

Žídková L.[1], Krijt J.[2], Hlobilková A.[3], Ehrmann J.[3], Zeman J.[2], Elleder M.[2], Adam T.[1]

[1]Laboratory for Inherited Metabolic Disorders, University Hospital and Medical Faculty, Palacký University, Olomouc, The Czech Republic

[2]Department of Paediatrics and Institute for Inherited Metabolic Disorders, Charles University, First Faculty of Medicine, Prague, The Czech Republic

[3]Department of Pathology, University Hospital and Medical Faculty, Palacký University, The Czech Republic

Purine nucleotides may be formed de novo, from small molecules, or by salvage, from preformed purines. Despite the essential functions of purine de novo synthesis (PDNS), salvage pathways recycle nucleotides to meet daily needs. In man, the main source of purines and the site of highest PDNS activity is considered to be the liver. A first disease described in PDNS – adenylosuccinate lyase (ADSL; EC 4.3.2.2.) deficiency is an autosomal-recessive disorder presenting extreme variability – from fatal neonatal type presented as encephalopathy to slight mental delay. Currently, no effective treatment is available for adenylosuccinate lyase deficiency, although several approaches have been tested.

Dephosphorylated substrates 5-amino-4-imidazole-N-succino-carboxamide (SAICA) riboside and succinyladenosine (SAdo) accumulate in body fluids of patients with ADSL deficiency. The content of succinylpurines in cerebrospinal fluid of patients exceeds 100 µmol/l and is two orders of magnitude higher in CSF compared to blood levels. It makes extracerebellar sources of the high concentration of the compounds in CSF unlikely. However, activity of PDNS in adult brain is considered minimal.

We report here results of studies using cultured oligodendroglia and direct biochemical analysis of white matter obtained at autopsy from a patients with severe form of ADSL deficiency. In vitro incorporation study revealed intracellular concentration of succinylpurines of up 100 µmol/l. The contribution of compounds newly synthesized was very high (up to 25%). The analysis of white mater from autopsy revealed extremely high concentration of SAdo and SAICAR (685 and 3124 µmol/g wet weight, respectively; ratio 0.18) exceeding concentration of sum of adenine nucleotides (49 µmol/g wet weight) by orders of magnitude.

Oligodendroglia is responsible for neurological symptoms of ADSL deficiency and provides useful model for development of treatment.

This work was supported by grant from Iceland, Liechtenstein and Norway through the EEA Financial Mechanism and the Norwegian Financial Mechanism A/CZ0046/2/0011 and MSM 0021620806.

CURICULUM VITAE

George Burkadze, Professor, M.D., Ph.D., M.Sc.D.

Dr. Burkadze currently holds the position of Professor of Pathology and Head of the Department of Pathological Anatomy, N. Kipshidze Central University Clinic, Tbilisi, Georgia. He is the vice-president of Georgian Association of Pathologists and Cytopathologists and works as a consultant cytopathologist for Georgian National Screening Program. Dr. Burkadze gained his M.D. degree from Tbilisi State Medical Institute in Georgia in 1983 and Ph.D. degree in Pathological Anatomy in 1986, followed by professional development including postgraduate training in pathology and cytopathology (Mashaw project), Tel Aviv University, Sackler Faculty of Medicine, Israel in 1997, and UICC (International Union Against Cancer) International Cancer Technology Transfer Fellowships to study the practice of modern pathology and molecular diagnostics in the Department of Pathology, Brigham & Women's hospital and Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, U.S.A. in 2008. Dr. Burkadze and his lab have been a pioneer in cervical cancer screening and Papanicolaou staining as well as implementing immunohistochemistry in diagnostic pathology in Georgia and educating health professionals about these techniques by means of workshops and continuing medical education courses. He is currently working on two research grants aiming to study BRCA1 expression and circulating tumor cells in breast cancer patients, awarded by Georgian National Science Foundation, Tbilisi, Georgia. Dr. Burkadze is a member of European Society of Pathology (ESP), College of American Pathologists (CAP), United States and Canadian Academy of Pathologists (USCAP), and the author of 54 original research articles, 38 presentations, and 7 textbooks and brochures.

Pathological anatomy in Georgia: current reality and future prospects

George Burkadze

Department of Pathological Anatomy, N. Kipshidze Central University Clinic, Tbilisi State Medical University, Tbilisi, Georgia

Georgia`s first pathological anatomy service was established in the Department of Pathological Anatomy of Tbilisi State Medical Institute in 1922. The major activities were autopsy and histological examination of surgical specimens. Autopsy was done in most cases aiming for quality control and improvement of patient health care. Cytopathology was separated from pathological anatomy and cytological diagnostic services were carried out by biologists in clinical laboratories. Since the 80`s, the volume of biopsies has increased in diagnostic pathology and intraoperative express diagnosis was introduced. Surgical and biopsy specimens were routinely embedded in celloidin which was the major obstacle to the use of special stains and archiving the blocks. The frequency of autopsies declined after a new autopsy law was introduced in the 90`s which did not allow autopsy without the consent of patient`s relatives. Autopsy is still being performed mostly by forensic pathologists to identify elements of the crime.

Papanicolaou staining was implemented in 2000. A significant number of cytopathologists and cytotechnologists have been trained and educational seminars have been organized in the Department of Pathological Anatomy, Tbilisi State Medical University. These activities led to the Georgian National Screening Program of Cervical and Breast Lesions which has been successfully working for the last three years under the patronage of Ms Sandra Roelofs, Georgia`s First Lady. Immunohistochemistry was first used for research in 1983, and for diagnostic purposes in 2000 which required celloidin to be replaced with paraffin. Most pathology laboratories now use paraffin embedding and four laboratories also offer immunohistochemistry.

There are nearly 60 licensed general pathologists in Georgia. There is no subspecialization, and most of them are above 60. Recruitment of young doctors in pathology has been a challenge not only due to the low salary but also limited educational resources. There is no continuing medical education system in pathology making the professional development difficult. In 2008, First Lady of Georgia supported Dutch and Georgian pathologists to propose a project for establishing the Georgian Federal Center of Pathology. Due to the recent political developments, this project was put on hold but not yet cancelled.

The department has a strong commitment to teaching medical students as well as research activities. Two research projects funded by Georgian National Science Foundation (GNSF) are in progress: 1. Breast carcinoma and BRCA1 in Caucasian (Georgian) women, GNSF/ST07/6-223; 2. Detection of metastatic potential of circulating tumor cells (CTC) in patients with invasive ductal carcinoma of the breast, GNSF/ST08/6-461.

Although there is a lack of diagnostic textbooks available in Georgian, a number of textbooks have been published and intended for medical students, postgraduate fellows and residents.

1. G. Burkadze. Immunomorphology. 2001.
2. G. Burkadze. Gynecologic Cytopathology. 2002.
3. G. Burkadze, G. Turashvili. The basis of general pathology. 2005.
4. G. Burkadze, G. Turashvili. Pathology of organ systems. 2006.
5. G. Burkadze. Breast FNA cytopathology. 2008.
6. G. Burkadze, G. Turashvili. General pathology for dentists. 2008.
7. B. Kochlamazashvili, G. Burkadze. Dental pathology. 2010.
8. N. Museridze, G. Burkadze. A human papillomavirus (HPV) and cervical pathology. 2010.

CURICULUM VITAE

Jaroslav Mokrý , Professor, M.D., Ph.D.

Jaroslav Mokrý currently holds the position of Head of the Department of Histology and Embryology at Charles University Medical Faculty in Hradec Králové. He has been working in the Department of Histology and Embryology at Charles University Medical Faculty in Hradec Králové since 1985. He completed his M.D. degree in general medicine from Charles University in Prague in 1990. In 1993-1994 he was on his research stay in the Department of Human Anatomy, Oxford University, UK, where he was engaged in cultivation of fetal dopaminergic neurons. He defended his Ph.D. thesis in 1995 and was appointed Professor of Histology and Embryology in 2006. He was awarded Charles University Rector's Prizes in 1987 and 1990 and Young Histochemist Award from International Federation of Societies of Histochemistry and Cytochemistry in Kyoto, Japan, in 1996. He is president of Czech Society for Histo- and Cytochemistry and vice-president of Czech Anatomical Society. He published 130 research articles and presented over 300 lectures and posters. Main focus of his scientific activity covers the areas of stem cell biology, regenerative medicine, cell cultivation, cell transplantation, cell therapy, cell differentiation, angiogenesis, detection of molecules in situ and histology.

Telomere shortening after ex vivo expansion of tissue-specific stem cells

Mokrý J.[1], Soukup T.[1], Mičuda S.[2], Bouchal J.[3], Karbanová J.[1], Suchánek J.[4], Višek B.[1], Brčáková E.[2], Vokurková D.[5], Ivančáková R.[4]

[1]Department of Histology and Embryology, Charles University Medical Faculty, Hradec Králové, The Czech Republic
[2]Department of Pharmacology, Charles University Medical Faculty, Hradec Králové, The Czech Republic
[3]Laboratory of Molecular Pathology, Palacký University Medical Faculty, Olomouc, The Czech Republic
[4]Department of Dentistry, Charles University Medical Faculty, Hradec Králové, The Czech Republic
[5]Department of Clinical Immunology and Allergology, Charles University Medical Faculty, Hradec Králové, The Czech Republic

In adult multicellular organisms, population of tissue-specific stem cells is responsible for vital functions as tissue maintenance and tissue regeneration. To perform these functions for the entire lifespan, i.e. for more than seven decades in humans, stem cells have to be long living. Of many factors contributing to long-living functionality of tissue-specific stem cells, we focused on measurement of telomere length. After isolation of stem cells, these must be thoroughly characterized to confirm their unique biological properties and expression of stem cell markers.

For analysis, we utilized tissue-specific stem cells isolated from the dental pulp. To expand an initially small number of cells in vitro, stem cells were regularly passaged. We characterized the growth of stem cell lines by measurements of population doublings and doubling times. The cell viability, karyotype and phenotype were checked regularly. Telomere dynamics was examined with quantitative real-time PCR and results were verified by measurement of terminal restriction fragment length and flow FISH.

Dental pulp stem cells (DPSCs) expressed mesenchymal markers CD29, CD44, CD73, CD90, CD166, vimentin and STRO-1 at high levels while hemopoietic markers were negative (CD34, CD45). The cells expressed stem cell markers nanog, SOX-2, nestin, musashi-1, nucleostemin, VEGFR2 and CXCR4. DPSCs are multipotent as shown by their osteogenic and chondrogenic potential. Although these cells have a large proliferative capacity, they show a decline in their doubling time with prolonged cultivation. For the first 42 population doublings, doubling time was 27±6 hrs, however, at later passages doubling time slowed down to 47±7 hrs. All DPSC lines were able to grow beyond Hayflick's limit. The largest population doubling number reached in our lines was 81. Although measurement of telomere length revealed interindividual differences between the patients, we observed telomere attrition when samples of the same patients from different passages were compared. Comparison of telomere length between DPSCs and lymphocytes from the same patient showed that the latter had shorter telomeres than adult tissue-specific stem cells.

Our results document that ex vivo expansion contributes to telomere shortening which explains a retarded growth of DPSCs at later passages. Similar preliminary results were confirmed by measurement of telomere length in bone marrow mesenchymal stem cells and periodontal ligament stem cells. Although telomere loss in tissue-specific stem cells contributes to a tissue ageing, telomere length in stem cells still remained longer when compared with other somatic cells which likely contributes to extended lifespan of these unique cells.

Supported by MSM 0021620820, MSM 6198959216 and FP7 project 223298 – PurStem.

CURICULUM VITAE

Benjamin Dekel, M.D., Ph.D.

Benjamin Dekel graduated in general medicine in 1993 from Technion University in Haifa in Israel and defended his Ph.D. thesis "Human and porcine renal precursor cells as a new source for transplantation" in 1997 in Weizmann Institute of Science in Israel. Benjamin Dekel is currently Head of the Pediatric Stem Cell Research Institute of Safra Children's Hospital in Sheba Medical Center in Israel and Senior Lecturer of the Department of Pediatrics of the Sackler Faculty of Medicine, Tel Aviv University. His field of research is the study of normal and malignant stem cells in different types of malignancies, mainly renal malignant stem cells. He is a member of several Israel and American scientific societies, successfully supervised several Ph.D. students and presented multiple invited lecture at international conferences. He has successfully finished 13 research grants and published 47 original research papers and 7 review articles since 1996. He also published 4 chapters in books.

Identification and prospective isolation of novel human renal stem/progenitor populations from normal and cancerous kidney tissue

Benjamin Dekel

Department of Pediatrics, Pediatric Nephrology and the Pediatric Stem Cell Research Institute, Safra Children's Hospital, Sheba Medical Center, Tel Hashomer, Sackler School of Medicine, Tel Aviv University, Israel

The precursor of the adult mammalian kidney is called the metanephros and it appears at 5 weeks of human gestation, equivalent to embryonic day 11 in mice and day 12 in rats. At this stage, the organ consists of ureteric bud epithelium which becomes enveloped by renal mesenchyme/blastema: these tissues form collecting ducts and nephron tubules, respectively. In the developing human kidney, fresh stem cells are induced into the nephrogenic pathway to form nephrons until 34 weeks of gestation.

Others and we have shown that two pathologic situations strongly recapitulate this developmental program by activating specific transcription factors that mark the early renal progenitor population: (i) renal regeneration following episodes of acute injury, suggestive of the presence of adult kidney stem cells (ii) renal tumorigenesis in the form of a Wilms' tumor, a common pediatric kidney cancer, believed to arise from multipotent embryonic renal precursors of the renal mesenchyme/blastema. Thus, the renal progenitor pool is likely to be at the heart of all of these processes.

Recent molecular advances have much contributed to our understanding of the cell lineages in the developing kidney. Nevertheless, in contrast with other organs, such as the hematopoietic system, in which the identification of surface markers enabled purification of these cells, the lack of such in the kidney has hampered progress in identifying and isolating stem cells. Microarray experiments, cell selection strategies, clonal analysis and various in vivo assays of human cells derived from normal and cancerous kidney tissue have now afforded insights into relevant shared stem cell markers and accordingly to human renal stem/progenitor populations.

This may enable the potential application of renal stem cells in kidney repair and the treatment of kidney cancers.

CURICULUM VITAE

Jiří Bártek, Professor, M.D., Ph.D.

Jiří Bártek obtained his M.D. degree from the Palacký University in Olomouc, and Ph.D. at the Institute of Molecular Genetics, Czech Academy of Sciences, Prague in 1983. Jiří Bártek then worked at the Oncology Institute in Brno, the Imperial Cancer Research Fund Laboratories in London, the German Cancer Research Center in Heidelberg, and as a Head of Department at the Institute of Hematology in Prague. In 1992, he was appointed a senior scientist at the Danish Cancer Society in Copenhagen, Denmark, where he is (since 1997) the Head of the Department of Cell Cycle and Cancer, and since 2005 also the Deputy Director, Centre for Genotoxic Stress Research. Jiří Bártek is a member of EMBO and other scientific organizations, he published over 300 articles in international scientific journals, and he is the most frequently cited Czech scientist globally. His major achievements include insights into the roles of p53 and pRB tumor suppressors, discoveries of several checkpoint pathways, and identification of the DNA damage response machinery as anti-cancer barrier in early human tumorigenesis. His current interests focus on mechanisms of cell cycle control, genome integrity, involvement of these pathways in tumor development, and translational research into personalized treatment of cancer.

Searching for predictive markers and targets for personalized treatment of cancer

Jiří Bártek
Danish Cancer Society, Copenhagen, Denmark
Palacký University, Olomouc, The Czech Republic

Recent work in the field of DNA damage recognition, signaling and repair identified multiple protein modifications that are coordinated by the ATM/ATR-regulated DNA damage response (DDR) machinery. This lecture will briefly introduce our most recent published and unpublished data documenting the biological and pathophysiological roles of the phosphorylation-dephosphorylation, ubiquitylation-deubiquitylation cascades, and their interplay with sumoylation, chromatin-remodelling and methylation-mediated signaling of DNA damage, in orchestration of cell cycle checkpoints, DNA repair and cell death pathways in human cells. The examples will include our results from pan-genomic RNAi-based screens for novel DDR components, the first dynamic global phosphoproteomic analysis and mechanistic insights into the cooperation between such dynamic phosphorylations, ubiquitylation and protein-protein inter-actions in response to DNA double strand breaks. Furthermore, the emphasis will be on our recent data that extend our concept of DDR as a barrier in human cancer development and selection pressure to favor growth of tumor cells with DDR defects. Finally, our efforts to exploit germ-line or tumor-specific DDR alterations in human carcinomas as predictive markers to guide individualized chemotherapy or radiotherapy, as well as to provide targets of synthetic lethality for optimized combined treatments, will be discussed.

CURICULUM VITAE

Lukáš Lacina, M.D., Ph.D.

Lukáš Lacina graduated in general medicine at Charles University in Prague in 2004. In that year he also joined the research group of Professor Karel Smetana Jr., M.D., D.Sc., at the Institute on Anatomy of the First Faculty of Medicine and in 2008 obtained his Ph.D. in molecular biology and pathology of the cell with his thesis titled: "Glycobiology of Epidermis under Physiological and Pathological Conditions". He completed the board certification in dermatovenerology in 2009. His professional interests include the diagnostics and therapy of skin cancer with special emphasis on glycobiology and special methods, such as lectine immunohistochemistry and in vitro cell cultures and tissue modelling. He is also a researcher of the Center of Cell Therapy and Tissue Repair of the Second Faculty of Medicine with special interest in experimental oncology and cell therapy. His recent research activities are focusing on epithelial-mesenchymal interaction during the course of tumor development and spreading.

Lukáš Lacina was awarded by the Czech Academy of Dermatovenerology (Professor Šamberger´s Award) in 2007 for an outstanding dermatological publication. In 2008, Lukáš Lacina was awarded by Czech Histo and Cytochemical Society and is also by Award of Scientia Foundation.

The Myofibroblast – from the pathologist´s microscope to the nanomaterials development

Lukáš Lacina

Department of Dermatovenerology, First Faculty of Medicine, Charles University, The Czech Republic

The lectins are proteins or glykoproteins specifically recognizing carbohydrate motifs on the cell surfaces. They are widely distributed from viruses to humans. The lectins present in latter are called endogenous lectins. Galectins are members of endogenous lectin family. Galectins are multifunctional lectins participating in cell-matrix and cell-cell interactions as well as in immune recognition. Galectins influence regulation of cell proliferation, apoptosis and pre-mRNA splicing.

Adhesion/growth-regulatory tissue lectin galectin-1 in relation to angiogenesis/lymphocyte infiltration and prognostic relevance of stromal up-regulation in laryngeal carcinomas. Very strong accumulation of galectin-1 was described by us in wounded skin, in dermis of psoriatic plaques and in stroma of basal cell carcinomas and squamous cell carcinomas of head and neck region.

It is known, that malignant tumors share many structural and molecular features with healing wounds. Very general feature of either wounded skin or tumor stroma respectively are myofibroblasts. Myofibroblasts produce extracellular matrix and numerous soluble bioactive molecules supporting wound contraction and healing as well as tumor spreading respectively.

The presented invention is based on the surprising observation that some galectins induce fibroblast-to-myofibroblast transition and therefrom arising three-dimensional nanofibrous extracellular matrix scaffolds. We claim that the object of the invention is utilization of at least one recombinant mammalian, with special advantage human, selected galectin (selected galectins are galectin-1, galectin-3, galectin-4 or galectin-7, respectively) as inductors of fibroblasts to myofibroblasts transition and/or production of extracellular matrix nanofibers along. The three-dimensional extracellular matrix scaffold is suitable for purposes of cell cultivation. These nanofibrous matrices can be used for basic research, biotechnological purposes or for therapeutically purposes during the transplantation in cell and tissue therapy. For example the nanofibrous scaffolds colonized by keratinocytes can be used as wound and ulcer dressing facilitating the healing process of.

CURICULUM VITAE

Wolfgang Doppler, Professor, M.D., Ph.D.

Wolfgang Doppler holds the position as Professor in Biochemistry at the Biocenter, Innsbruck Medical University. He serves there as co-ordinator of the Ph.D. program "Molecular Oncology". He got his M.D. and Ph.D. from the University of Innsbruck in 1982 and 1984, respectively. His interest on the molecular mechanisms of hormone action (steroid hormones, prolactin, insulin like growth factors) in the normal mammary gland and in mammary cancer was initiated in 1986 when he was working as Research Scientist at the Ludwig Institute for Cancer Research in Bern, and later at the Friedrich Miescher Institute in Basel. In 1989, his work on the transcriptional regulation of milk protein genes led to the discovery of the signal transducers and activators of transcription 5 (STAT5) as a mediator of the prolactin response. Since then, his major focus is on the role of the STAT factors STAT1, STAT3 and STAT5 to regulate the balance between cell proliferation and cell death in the normal mammary gland as well as in mammary carcinomas. Current research is on the role of STAT1 in tumor biology, immunosurveillance, and response to chemotherapeutics.

Signal transducers and activators of transcription (STATs) in breast cancer

Wolfgang Doppler
Medical Biochemistry, Biocenter, Innsbruck Medical University, Austria

STAT proteins are activated during mammary gland development and in a variety of tumors. STAT5 is required for alveolar differentiation and the response to lactogenic hormones. In contrast to tumors of the hematopoietic system, where STAT5 has been implicated in tumor formation and progression, in mammary carcinomas STAT5 activation and expression of the STAT5 target gene SOCS2 are linked to good prognosis. STAT3 is a key factor in the initiation of mammary gland involution, however its role in breast cancer remains unclear. STAT1 is considered to act as an anti-oncogene and linked to cell cycle arrest and apoptosis. Furthermore it is considered to be a key factor in tumor immunosurveillance and the response to chemotherapeutics. By studying STAT1 DNA binding activity and tyrosine phosphorylation as markers for STAT1 activation, we have observed a link between STAT1 activity and good prognosis in primary human breast cancer. One hypothesis to explain these findings is that impaired STAT1 function can promote resistance to chemotherapeutics. A causal relationship between STAT1 and resistance to chemotherapy was tested in an animal model for erbB2 positive breast cancer, the MMTVneu (N) tumor mice, which develop adenocarcinomas. STAT1 proficient and deficient animals in FVB/N background were generated. Tumor formation and response to the chemotherapeutic agent doxorubicin, which is frequently used in the therapy of erbB2 positive human breast cancer, was investigated. We observe a shorter tumor latency in STAT1 deficient mice. Furthermore, STAT1 deficient mice exhibited an impaired response to doxorubicin treatment in vivo. In accordance, the effect of doxorubicin on activation of p53, induction of apoptosis, and formation of DNA strand breaks as judged by gH2AX phosphorylation was diminished in tumor explant cultures derived from STAT1 deficient mice. Our experiments indicate that in erbB2 positive mammary carcinoma, loss of STAT1 leads to an accelerated tumor development and to resistance against doxorubicin treatment, suggesting that the determination of the STAT1 activation status in breast cancer can serve as an important parameter to predict response to chemotherapy.

CURICULUM VITAE

Paul G. Murray, Professor, M.Sc., Ph.D.

Paul G. Murray is currently Professor of Molecular Pathology at the School of Cancer Sciences of the College of Medical and Dental Studies, University of Birmingham. The major focus of his research group is to provide a better understanding of the molecular events leading to the development of Hodgkin´s lymphoma and especially to study the role of the Epstein-Barr virus in this process. He has succesfully finished 38 research grants and published 78 original research papers and 14 review articles since 1989. He also published 7 books and 3 textbooks. Paul G. Murray is a regular reviewer for over 20 peer-reviewed journals including Blood, American Journal of Pathology, Journal of Pathology, Oncogene and Cancer Research. Last but not least, he has succesfully supervised many Ph.D. students, presented multiple invited lecture at international conferences and is a member of several editorial boards and scientific societies.

Differential regulation of Blimp1 isoforms by the Epstein-Barr virus; implications for virus persistence and the pathogenesis of EBV-associated lymphomas

Vrzalíková K.[2], Vockerodt M.[1], Leonard S.[1], Kolář Z.[2], Woodman C.[1], Murray P.G.[1]
[1]School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom
[2]Laboratory of Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic

The Epstein-Barr virus (EBV) is associated with several types of B cell lymphoma, which include Burkitt's lymphoma (BL) and Hodgkin's lymphoma (HL). An important pathogenic event in these cancers is the suppression of virus replication which would otherwise result in tumour cell apoptosis. Because the induction of virus replication in EBV-infected non-malignant B cells is intimately linked to their differentiation to plasma cells, we have asked if the physiological signals which drive normal B cell differentiation are absent in EBV-infected lymphoma cells. We have focussed on BLIMP1, a transcription factor which exists as two major isoforms; BLIMP1α, which is required for normal plasma cell differentiation, and BLIMP1β which has impaired ability to repress gene transcription and is highly expressed in myeloma cell lines. We have shown that BLIMP1α expression: is low in EBV-infected BL and HL cells; can be down-regulated by EBV infection of primary B cells; and, when introduced ectopically into EBV-infected primary B cells or BL cells, leads to induction of the virus replicative cycle. We have also shown that EBV infection up-regulates BLIMP1β expression, an effect that is associated with hypo-methylation of the BLIMP1β-specific promoter. Taken together our results support an important role for the differential regulation of the BLIMP1 isoforms in the pathogenesis of EBV-associated lymphomas.

CURICULUM VITAE

Jiří Hejnar, D.Sc., Ph.D.

Jiří Hejnar obtained Master degree in Genetics at the Purkinje´s University in Brno and Ph.D. degree in Virology at the Institute of Molecular Genetics, Academy of Sciences of the Czech Republic in Prague. Here, he started his research in the lab of Prof. Jan Svoboda. After spending a short period of postdoctoral research at Beatson Institute in Glasgow under the supervision of Prof. John Wyke, he returned to Prague to become a head of Department of Viral and Cellular Genetics at the Institute of Molecular Genetics. Since 1996, the research in his group focuses on retroviruses, retrotransposons, and epigenetics. He published more than 40 papers concerning regulation of transcription by DNA methylation, regulation of retroviral expression by the host cell, mechanisms of retroviral latency and persistence, human endogenous retroviruses, retrotransposons, retroviral vectors, receptors for retroviruses, integration preference of retroviruses, chicken genomics and transgenesis. To learn more about the current projects and people in the lab, please visit the lab web page (http://www.img.cas.cz/public/skupiny/Hejnar.html).

Tumor or rumor viruses? Newly emerging retrovirus infections in human

Jiří Hejnar
Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, The Czech Republic

The current pandemic of acquired immunodeficiency syndrome is, in fact, a zoonosis: simian immunodeficiency viruses (SIV) crossed the interspecific barriers between chimps and humans and developed into what we call now human immunodeficiency viruses type 1 and 2 (HIV-1 and -2). The enormous variability of HIVs is caused, in part, by multiple and repeated infections with different SIV types. Far from being a historical oddity, the transmission of retroviruses from non-human primates to humans is a regular and ongoing process. For example, SIVs, SIV-related viruses, simian T-lymphotropic viruses, and simian foamy viruses were detected in bush-meat hunters from Cameroon and primate keepers in zoos and primate centers worldwide.

Another example of newly emerging retroviral infections to be discussed is the XMRV, a human retrovirus related to the xenotropic murine leukemia virus. In several but not all recent studies, XMRV was documented to be present in patients with certain type of prostatic carcinoma and even in cohorts of patients with chronic fatigue syndrome (CFS). It remains to be understood whether XMRV plays a role in the patogenesis of prostatic carcinoma and CFS. Similarly, HMTV, retrovirus related to the mouse mammary tumor virus is associated with autoimmune primary biliary cirrhosis and certain types of mammary carcinoma.

A possible source of a new zoonosis might be the xenotransplantation of porcine organs or tissues. Porcine endogenous retroviruses (PERV), particularly recombinant PERV-A-C, infect human cells in vitro and impose a potential risc on therapeutic xenotransplantation. Although PERV transmission to the grafted humans or experimental baboons was not detected so far in vivo, the use of genetically modified GALKO pigs will require new prospective studies. Thanks to the integration polymorphism, some PERV copies can be eliminated during the breeding of donor pigs and, therefore, the identification of highly expressed and recombinogenic PERV-A and PERV-C copies is urgently needed.

Last but not least, infectious retroviruses might emanate even from human endogenous retroviruses (HERV) integrated in the human genome, which form 8% of our DNA. This is the case of multiple sclerosis-related virus (MSRV) similar to HERV type W. MSRV is considered as a proinflammatory agent in several human neurological disorders like optic neuritis, multiple sclerosis, and CFS. The diagnostic and prognostic value of XMRV, HMTV, and MSRV detection in blood and cerebrospinal fluid will be discussed.

This abstract refers to the research supported by the grant No. 301/09/2031 awarded by the Czech Science Foundation and Framework Program 6 project XENOME by the European Committee.

CURICULUM VITAE

Lucia Knopfová, M.Sc.

Lucia Knopfová has graduated from Faculty of Science of Masaryk University in Brno in 2007. She has master degree in Molecular Biology and Genetics focused on molecular mechanisms of hematopoietic cells transformation. Currently finishing Ph.D. study in Molecular Cell Biology in Laboratory of Cell Differentiation, Institute of Experimental Biology, Faculty of Science, Masaryk University under the supervision of Professor Jan Šmarda. She is awarded by Sigma Young Scientist Award 2009 and obtained financial support for her research from Masaryk University in 2006 and 2009. The main focus of her research is a control of breast and colon cancer cells invasion, motility and apoptosis.

c-Myb promotes motility and invasion of breast cancer cells

Knopfová L., Trčka F., Beneš P., Šmarda J.
Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, The Czech Republic

The c-Myb gene codes for transcription factor that is essential for regulation of hematopoiesis in vertebrates. Deregulated expression and/or mutation of c-Myb can result in leukemias. In addition to hematopoietic malignancies, the role of c-Myb in development of solid tumors has been documented as well. c-Myb was shown to promote proliferation and inhibit differentiation/apoptosis of various cancer cells. While the role of c-Myb in control of these processes has been extensively studied, there are only a few indications that c-Myb can be involved in cancer cell invasion and metastatic spread. The aim of this study was to assess the role of the c-Myb protein in control of invasion of breast cancer cells.

MDA-MB-231 breast cancer cells were transfected with the c-Myb coding cDNA to prepare MDA-MB-231MYBup derivatives. The effects of c-Myb overexpression on migration and invasion capacity of these cells were assessed using Cultrex Cell invasion assay (RnD Systems). MDA-MB-231MYBup cells were significantly more active in both motility and invasion than controls as determined by this assay. In order to reveal dynamics of these processes, we performed real-time analysis of cell migration and invasion using the xCELLigence RT-CA system (Roche). This system is based on non-invasive impedance-based monitoring of the transition of cells through the microporous membrane in real time. This real-time analysis of cell migration/invasion clearly confirmed increased invasive capacity of MDA-MB-231MYBup cells compared to controls. To address the mechanism of c-Myb-enhanced breast cancer cell invasion, we examined the role of c-Myb in control of expression and activity of some of the proteases involved in degradation of extracellular matrix. We found that c-Myb increased production of cathepsin D and matrix metalloproteinases in MDA-MB-231MYBup cells.

The results of this study showed that c-Myb promotes motility and invasive capacity of breast cancer MDA-MB-231 cells and this effect at least partially results from deregulation of expression/activity of cathepsin D and some matrix metalloproteinases. These results suggest a novel role of c-Myb protein in control of tumor invasion and metastatic progression.

This work was supported by grants 301/09/1115 of GACR, IAA501630801 of GAAV CR, MUNI/C/0099/2009 of Masaryk University and MSM 0021622415.

CURICULUM VITAE

Karel Souček, Ph.D.

Karel Souček obtained his Ph.D. degree in animal physiology at Masaryk University in Brno in 2003. He is author and co-author of more than 40 original research articles published in peer-reviewed journals. Currently he is associate scientist and group leader at Department of Cytokinetics, Institute of Biophysics, Academy of Sciences, Brno. Primary interest of his group is to investigate the role of tissue microenvironment in development and progression of cancer diseases. Group is focused on study of role of crucial signaling pathways such as TGF-β and IL-6/gp130 and their interactions affecting cellular fate, particularly pathological plasticity of adult epithelial cells. One of the main interests is aimed to the elucidation of signal transduction and physiological role of divergent member of TGF–β family – Growth Differentiation Factor-15.

TGF-β1 suppresses IL-6-induced STAT3 activation through regulation of Jak2 expression in prostate epithelial cells

Staršíchová A.[1,3], Lincová E.[1,2], Pernicová Z.[1,2], Kozubík A.[1,2], Souček K.[1,2]

[1]Department of Cytokinetics, Institute of Biophysics AVCR, Brno, The Czech Republic

[2]Department of Experimental Biology, Faculty of Sciences, Masaryk University, Brno, The Czech Republic

[3]current address: European Institute for Molecular Imaging, University of Münster, Germany

Chronic inflammation plays an important role in initiation and progression of various human diseases including benign prostatic hyperplasia or prostate cancer. Here, we show that proinflammatory cytokine interleukin-6 (IL-6) has prosurvival effects and chronically activates Jak2/STAT3 signaling pathway in a model of benign prostatic hyperplasia. We demonstrate that anti-inflammatory cytokine transforming growth factor β1 (TGF-β1), which also permanently activates its canonical signaling pathway through SMAD proteins in BPH-1 cells, affects the effects of IL-6 on cell proliferation. Moreover and importantly, TGF-β1 inhibits IL-6 signal transduction (during longtime action of both cytokines) by decreasing phosphorylation levels of STAT3 and decreases nuclear localization of phosphorylated STAT3 as well. This is associated with reduced expression of Jak2 at both mRNA and protein levels. In conclusion, our data show that TGF-β1 reverts prosurvival effects of IL-6 through regulation of Jak2 expression in prostate epithelial cells.

Contribution of aberrant lipid signalling to the pathogenesis of Hodgkin's lymphoma

Abdullah M.A.[1], Baumforth K.R.N.[1], Roberts C.[3], Wei W.[1], Woodman C.B.[1], Murray P.G.[1]

[1]*School of Cancer Sciences, University of Birmingham, Vincent Drive, Edgbaston, Birmingham, United Kingdom;*
[2]*Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia;*
[3]*Department of Pathology, University Hospitals Birmingham NHS Foundation Trust, United Kingdom*

Introduction: Sphingosine-1-phosphate (S1P) is a pleiotropic lipid mediator which is produced by phosphorylation of sphingosine, a reaction catalysed by the enzyme Sphingosine kinase-1 (SPHK1). S1P has been shown to regulate cell growth, survival, invasion, and angiogenesis, and is up-regulated in many solid tumors such as lung, breast, melanoma, and ovarian cancers where its expression has been shown to modulate multi-drug resistance.

Objectives: These include: profiling the expression of lipid signalling molecules in HL cell lines and primary HL tissue; investigating the contribution of SPHK1 to the generation of S1P in HL; and determining whether the S1P inhibitor, Sphingomab, can reverse the malignant phenotype of HL cell lines.

Results: It has been shown that the expression of SPHK1 is increased in both HL cell lines and in primary HRS cells, and that its inhibition by Dimethylsphingosine and SPHK1 Inhibitor II reduces the proliferation and viability of HL cells. It has been confirmed a marked reduction of SPHK1 RNA expression following treatment with SPHK1 siRNA. Using gene expression profiling, that treatment of the HL cell line, L591, with the S1P inhibitor, Sphingomab, is followed by reversal of many of the transcriptional changes previously described in HL.

Conclusion: S1P/SPHK1 pathway plays a significant role in the pathogenesis of Hodgkin's lymphoma. Thus direct targeting of important lipid signalling molecules is a novel strategy for development of new types of cancer treatments that could be useful in combination with other treatment strategies.

TOP2A gene status in "triple negative" breast cancer patients as a possible anthracycline response predictor

Bouchalová K.[1], Čížková M.[1,2], Trojanec R.[1], Koudeláková V.[1], Mlčochová S.[1], Furstová J.[1], Radová L.[1], Dziechciarková M.[1], Melichar B.[2], Cwiertka K.[2], Kolář Z.[3], Hajdúch M.[1]

[1]*Laboratory of Experimental Medicine, Department of Paediatrics, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic;*
[2]*Oncology Clinic, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic;*
[3]*Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic*

Introduction and Aim: "Triple negative" breast cancer (TNBC), oestrogen (ER), progesterone (PR) and HER2 receptor negative is an aggressive form of breast cancer (BC) with poor prognosis. Further, TNBC patients cannot benefit from treatment based on ER, PR and HER2. Other markers are sought. TOP2A gene amplification is considered an anthracycline treatment response predictor and TOP2A aberrations (amplifications, deletions) are found in HER2 amplified BC. Recently, TOP2A amplification has been described in HER2 non-amplified BC. We investigated this further.

Materials and methods: Retrospective tumor samples from 68 TNBC patients diagnosed with clinical stage I and II were assessed by fluorescent in situ hybridization (FISH). The TOP2A/chromosome 17 ratio was counted. Histopathological and clinical data were analyzed with the cytogenetic results using standard statistical methods.

Results: TOP2A gene amplification was found in 10.3% of cases. Rare deletion was detected in 4.4%. TOP2A gene changes were found only in tumors with HER1 non-amplified status. Anthracycline therapy was used in 18 patients. The 2 anthracycline-treated patients displaying TOP2A amplification are alive and disease-free.

Statistical analysis showed association between patient age at diagnosis and histopathological diagnosis ($p=0.0165$). Tumors with normal TOP2A FISH findings had higher bcl-2 expression ($p=0.0364$). Associations between TOP2A and HER2 gene copy numbers ($p<0.00001$) and between TOP2A gene copy number and HER2/chromosome 17 centromere ratio ($p=0.0183$) were also found.

Conclusion: TOP2A amplification, a predictive factor for anthracycline therapy response, is usually connected to HER2 amplification. Here we present TOP2A gene changes in HER2 non-amplified tumors, a result which supports some previously published data. These findings suggest the possibility of targeted treatment with anthracyclines in TNBC. Association between TOP2A and HER2 gene copy numbers, even in the absence of HER2 amplification as in TNBC, confirms a relationship of both genes in the process of cytogenetic

changes. The clinical application of the rare 4.4% TOP2A deletion found, however is unclear.

Supported by grants: IGA NS10286–3, MSM 6198959216, LC07017 a EEA/Norway Financial Mechanisms (CZ0099).

Detection of Claudin-1 in tumors of the colon

Brychtová S., Bezděková M., Sedláková E.
Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic

Introduction: The hallmark of cancer cell invasion is disruption of cell-cell junction leading to changes in the expression of junctional proteins. The role of the adherens junction (AJ) proteins has been studied extensively, but the role of tight junction (TJ) proteins has not been clearly defined. TJs form a network of anastomosing strands within the cell membranes, serving as an important barrier in epithelial and endothelial cells against the paracellular passage of macromolecules and to separate the plasma membrane into apical and basolateral domains. TJ proteins are believed to be critical in the neoplastic process via their roles as couplers of the extracellular compartment to intracellullar signalling pathways and the cytoskeleton. Alteration of TJ proteins may also allow increased diffusion of nutrients and growth factors critical for tumor growth and survival. In addition, loss of TJ integrity is important for the development of the metastatic phenotype.

Claudins are a family of proteins integral to the structure and function of TJ and their altered expression has been detected in several types of cancer.

Aim: The aim of the study was to determine the role of Claudin-1 expression in carcinomas and adenomas of the colon.

Material and Methods: Altogether 40 cases including adenocarcinomas and adenomas with various degrees of dysplasia were analyzed by indirect immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections using mouse monoclonal anti-Claudin-1 primary antibody, sc-81796 diluted 1:500 (Santa Cruz Biotechnology, INC.). Protein detection was performed in the staining automaton Ventana Benchmark. Evaluation was done by H-score (percent of positive cell × intensity of staining).

Results: In normal colonic mucosa, Claudin-1 showed only weak membranous staining. Marked increase of Claudin-1 expression was detected in adenomas, even in those without any dysplastic changes. Similarly, adenocarcinomas overexpressed the protein, and the differences between adenomas and carcinomas were not significant. However, we demonstrated different protein location. Claudin-1 remained located in cytoplasmic membranes in adenomas without or with mild degree of dysplasia, whereas the shift to the cytoplasma with only cytoplasmic or membranous/cytoplasmic expression was observed in severe dysplasias and in carcinomas.

Increased expression of Claudin-1 was observed in histologically normal mucosa close to the neoplastic areas, when compared to distant areas.

Conclusion: We demonstrated that Claudin-1 deregulation is involved in early stage of colon cancerogenesis and it substantially may contribute to the neoplastic transformation.

This work was supported by grants GACR P304/10/1070 and MSM 6198959216.

Utilization of laser capture microdissection in diagnosis and prognosis of cancer disease

Dziechciarková M.[1], Berkovcová J.[1], Trojanec R.[1], Čížková M.[1,2], Bouchalová K.[1], Srovnal J.[1], Hajdúch M.[1,2], Hostášková P.[1], Gilíková M.[1]
[1]*Laboratory of Experimental Medicine, Faculty of Medicine and Dentistry, Palacký University and Faculty hospital Olomouc, The Czech Republic;*
[2]*Oncology clinic, Faculty of Medicine and Dentistry, Palacký University and Faculty Hospital, Olomouc, The Czech Republic*

Laser capture microdissection (LCM) is a rapid, reliable method for obtaining pure populations of targeted cells from specific microscopic regions of tissue sections for subsequent analysis. LCM is based on the adherence of visually selected cells to a thermoplastic membrane, which overlies the dehydrated tissue section and is focally melted by the triggering of a low energy infrared laser pulse. Tissue sections are mounted on standard glass slides, and a transparent thermoplastic membrane is then placed over the dry section. The laser provides enough energy to transiently melt this thermoplastic film into the target cells. Several systems are available for LCM, and these vary in cell-capture method, system configuration and applications. LCM was applied to a wide range of cell and tissue preparations including frozen samples, formalin-fixed paraffin-embedded tissue or cytology smear. Depending on the starting material, DNA, good quality mRNA, and proteins can by extracted successfully from captured tissue fragments, down to the single cell level. In combination with another techniques like expression library construction and cDNA array hybridisation, LCM will allow the establishment of new diagnostic and prognostic markers. This approach could help in establishing individualised a tailored to the molecular profile of a tumor.

This project was supported by grants MSM 6198959216, IGA NR/9076 and the MPO 1H-PK/45.

Globotriaosylceramide expression in human placental fetal capillaries

Hůlková H.[1], Elleder M.[1], Šmíd F.[2], Ledvinová J.[1], Kuchař L.[1]

[1]*Institute of Inherited Metabolic Disorders, Charles University in Prague, First Faculty of Medicine, The Czech Republic;*
[2]*Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine and General University Hospital, Prague, The Czech Republic*

Introduction: Globotriaosylceramide (Gn3Cer) is the main stored lipid in Fabry disease (deficiency of alpha galactosidase). There is dearth of knowledge on its distribution in normal tissues.

Aim: We had access to placentas examined for prenatal diagnosis of Fabry disease. Having effective commercially available antibody against Gb3Cer we extended the standard examination of placentas with this disorder beyond the usual electronmicroscopy (used for detection of lysosomal lipid storage) and we were surprised by the presence of Gb3Cer in the capillary endothelium unrelated to storage. This led us to examine healthy control placentas.

Material and Methods: A series of placentas at term were examined using imunohistochemical in situ analysis of Gb3Cer, lactosylceramide, and GM1 ganglioside (with cholera toxin-B subunit). The results of in situ analyses were correlated with extra situ biochemical analysis and with tandem mass spectrometry.

Results: Immunohistochemical study of a series of placentas at term showed uniform distinct staining of the apical membrane of the capillary endothelial cells for globotriaosylceramide (Gb3Cer) using monoclonal antibody. The lipid was not detectable in endothelial cells of arterial and umbilical veins and in capillaries in somatic structures (heart, skin, skeletal muscle) of neonates available. Gb3Cer was also detected in heparinocytes and in stromal cells of placental villi. Both cyto- and synciciotrophoblast, smooth muscle cells and mesothelial cells were negative. In situ correlation of Gb3Cer with other glycosphingolipids showed coexistence with GM1 ganglioside only in endothelial cells of placental capillaries. Lactosylceramide was restricted to Hoffbauer cells.

Conclusion: The findings point to specific glycolipid microdomains of endothelial cells of placental fetal capillaries which may reflect specific regulation.

Immunohistochemical analysis of KLK11, KLK7, PSA and PSMA in cancerous and noncancerous prostatic tissues

Jamaspishvili T.[1], Král M.[2], Khomeriki I.[2], Kurfürstová D.[1], Bouchal J.[1]

[1]*Laboratory of Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;*
[2]*Department of Urology, Faculty Hospital, Olomouc, The Czech Republic*

Introduction and aims: Kallikreins (KLK) 7 and 11 share a high degree of structural similarity with PSA and other KLKs and may serve as new biomarkers for prostate cancer. The aim of this study was to evaluate differences of KLK11/KLK7 expression in the paired cancer/benign prostate foci of the same patient and to define any association with clinicopathological parameters.

Methods: Seventy archived paraffin-embedded tissues obtained from radical prostatectomy were used for immunohistochemical analysis (IHC) and stained for KLK7, KLK11, PSA and PSMA. Intensity of IHC staining and percentage of positive tumor cells were determined for both BPH (benign prostatic hyperplasia) and CaP (prostate carcinoma) foci in each patient. Statistical analysis was done with SPSS using Wilcoxon, Spearman and Kruskal-Wallis non-parametric tests.

Results: We found significant differences for all studied proteins between BPH and CaP foci. Both KLK7 ($p=0.026$) and KLK11 ($p<0.001$) expressions were decreased in prostate cancer compared with its benign counterparts. The same difference was found for PSA ($p<0.001$) while reverse one for PSMA expression ($p<0.001$). Positive correlations were found for both KLK7 ($Rs=0.74$) and KLK11 ($Rs=0.35$) in CaP and BPH. No correlation or significant differences were found with respect to Gleason score, tumor grade, pT stage or serum PSA values. We only found slight upregulation of KLK11 in advanced cases when compared with localized ones ($p=0.026$).

Conclusion: For the first time, we report lower IHC expression of KLK11 in CaP than in BPH and also its slight upregulation in advanced tumors accord compared to localized ones. In concordance with the literature, we confirm both higher expression of PSMA and lower expression of KLK7 and PSA in CaP compared with BPH.

This work was supported by grants NS 9940–4 and MSM 6198959216. Tamara Jamaspishvili was also supported by GACR 303/09/H048.

Quantitative characterization of villous capillary branching in diabetic placentas

Jirkovská M.[1], Kaláb J.[1], Jadrníček M.[1], Niedobová V.[1], Moravcová M.[2], Krejčí V.[2], Žižka Z.[2]

[1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, Prague, The Czech Republic;

[2]Department of Obstetrics and Gynecology of the First Faculty of Medicine and General University Hospital, Charles University in Prague, Prague, The Czech Republic

Introduction: Maternal diabetes mellitus is associated with adverse consequences to mother and baby. In the placenta, the only organ mediating the effects of a changed maternal environment on the fetus, maternal diabetes manifests itself by changed placental structure. The microscopic picture encompasses among other great variability in peripheral villous vascularity showing both normal vascularization, hypovascular as well as strikingly hypervascular villi. Stereological studies of total capillary volumes and surface areas have shown discrepant results. Studies on the spatial arrangement of villous vascular bed are sparse and inconsistent.

Aim: To investigate differences in spatial organization and density of the capillary bed in peripheral villi, normal and diabetic placentas were analyzed.

Material and Methods: Placental specimens were collected by systematic random sampling from 14 normal, 11 GDM (= gestational diabetes) and 16 DMI (= type I diabetes) placentas. Tissue samples were fixed in formalin with admixture of eosin, and embedded in paraffin. Sections cut at 120 µm from randomly chosen seven blocks per placenta were analyzed by a confocal laser scanning microscope Leica SPE. Fifteen fields of view per section were sampled in a systematic uniform random manner and examined in well-developed terminal villi which were lying completely inside the thick section. The topology of capillaries was analyzed by the method of topological schemes. The frequency of variable types of capillary bed found in studied groups was statistically analyzed. Stacks of optical sections recorded by a confocal microscope were used for both the visualization of villous capillaries by 3D reconstruction, and visualization of mutual relationships of villi in the intervillous space by the maximal projection method.

Results: These two simplest forms of villous capillary bed were U-like loops and three longitudinally oriented capillary segments confluent near the tip of villus. Beds consisting of four or more longitudinal capillaries occurred rarely, but were more frequent in diabetic villi. Some villi had capillary segments interconnected with one or more "redundant connections" (RC), i.e. capillaries that could be removed without disconnecting the capillary bed. The analysis of topological schemes showed that the proportion of villous capillary beds without RCs was higher in normal placentas (80%) than in GDM (61%) and DMI (66%) placentas respectively. In both groups of diabetic placentas the mean number of RCs per villus was significantly higher (0.49 in GDM group, 0.43 in DM I group) than in the control group (0.23). In some GDM placentas, but in particular in the DMI placentas were observed abnormal villi of conspicuously larger size with edematous stroma and few capillaries. In addition, the villi in some DMI placentas displayed chorangiosis. 3D reconstruction of those villi showed a markedly waved capillaries of highly variable diameter forming dense capillary bed. As shown by the maximal projection method, the arrangement of capillaries in such villi influences their size and shape, which results in changes in the shape and dimensions of the adjoining parts of the intervillous space.

Conclusions: In order to compensate maternal metabolic disorder, the diabetic placenta manifests more intense placental angiogenesis, which results in both a denser villous capillary bed and abnormalities in spatial arrangement. The consequent changes in the shape and size of the villi may influence the microrheological conditions of the maternal blood flow in the intervillous space.

Supported by the GACR 304/09/0733.

Pericyte coverage of fetoplacental vessels in pregnancies complicated by DMI

Kučera T.[1], Vyletěl I.[1], Moravcová M.[2], Krejčí V.[2], Žižka Z.[2], Jirkovská M.[1]

[1]Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, The Czech Republic;

[2]Department of Obstetrics and Gynaecology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, The Czech Republic

Introduction: Diabetes mellitus type I (DMI) in pregnant women is a risk factor for impairing the physiological intrauterine development of the fetus. Consistent with the morphological abnormalities found in the placentas of women with DMI, the capillary bed in the terminal villae is more branched in the placentas from pregnancies with gestational diabetes mellitus. Angiogenesis occures in the placenta until delivery, as demonstrated by expression of markers for neovasculature in the placenta at term. Perivascular cells such as smooth muscle cells and pericytes participate in the vascular wall formation in neovessels. According to some authors, neovessels are covered by perivascular cells to a lesser extent than mature vessels and, in the placentas, this situation occurs in pregnancies in high altitudes. This might reflect more active angiogenesis or an adaptation of the microvascular wall morphology to lower oxygen.

Aim: Here we studied the extent of pericyte coverage in microvessels of normal pregnancies and pregnancies complicated by DMI. In addition we characterized the phenotype of pericytes in normal and DMI pregnancies.

Material and Methods: The placentas from normal pregnancies (n=8) and placentas from mothers with DMI

(n=18) were obtained at the time of delivery. The specimens were collected using unbiased systematic random sampling. They were fixed with formaldehyde and embedded in paraffin. Immunohistochemical detection was performed using standard procedures. Images for quantification were collected using Leica SPE CLSM and analyzed in ImageJ.

Results: Pericytes of capillaries in terminal and intermediate villi were immunoreactive for smooth muscle actin (SMA), but they were negative for intermediate filament desmin. We thus decided to use SMA as a marker for quantitation of pericyte coverage in placental microvessels. The extent of pericyte coverage was quite variable. However SMA+ pericytes regularly avoided segments of capillary wall forming vasculosyncytial membranes. The proportion of capillaries covered with SMA+ pericytes (microvessel pericyte coverage index) was 84±13% in normal vs. 79,5±13% in DMI pregnancies. The extent of pericyte coverage around the vessel circumference was 38±11% in normal vs. 33±10% in DMI pregnancies. Diabetic women were also grouped according to their compensation reflected by levels of glycated hemoglobin (GlyHb). Extent of pericyte coverage around the vessel circumference was 35±7% in the group with normal GlyHb vs. 32±12% in the group with elevated GlyHb.

Conclusion: Immunohistochemical phenotyping of perivascular cells in human fetoplacental vessels showed that pericytes surrounding capillaries in terminal and intermediate villi are SMA+/desmin- perivascular cells. There is further great variability of these cells in different organs. The phenotype of pericytes in normal pregnancies and in pregnancies complicated with DMI was virtually identical. No statistically significant difference in the extent of pericyte coverage around the vessel circumference between DMI and normal pregnancies was found. The morphology of angiogenic fetoplacental vessels in DMI is thus different from the placental microvasculature developing under low oxygen pressures such as in high altitudes.

The work was supported by the Research Project MSM 0021620807 and Grant GACR 304/09/0733.

Modulation of function of MDR-associated ABC transporters by chemotherapeutic drugs

Procházková J.[1], Sotolářová K.[1], Kozubík A.[1,2], Pacherník J.[1]

[1]Department of Animal Physiology and Immunology, Institute of Experimental Biology, Masaryk University, Brno, The Czech Republic;

[2]Department of Cytokinetics, Institute of Biophysics of Czech Academy of Science, Brno, The Czech Republic

The major cause of chemotherapy failure is acquired resistance of cancer cells not only to the prime drug but also to other unrelated ones. There are three main types of ABC transporters in mammals that have been found to cause multidrug resistance (MDR) of cancer cells when over-expressed – MDR1, MRP1, and BCRP (reviewed in Li et al., 2007). Recently it has been reported that expression of ABC transporters could be regulated by mitogen activated protein kinases (MAPKs), namely p38 and ERK subtype (Katayama et al. 2007), and also PI3K/Akt pathway (Liang et al. 2009). Those kinases were found to be able to modulate the activity of some transcription factors that bind into ABC transporters gene promoters, i.e. NFkappaB, AP1, or p53 (Scotto et al. 2003). We aim to connect effects of selected chemotherapeutics on function of ABC transporters with activity of particular kinase and its targets.

First, we selected chemotherapeutic drugs for their specificity using WST-1 cytotoxicity assay and cell lines over-expressing studied transporters (MDR1, MRP1 and BCRP). In concentrations we further used Roscovitin is a substrate of MDR1 protein, Camptothecin and Actinomycin D are substrates of MRP1 protein, Valinomycin of BCRP protein, and Doxorubicin and Geldanamycin are general substrates of all the studied ABC transporters. We evaluated their effect on function and expression of ABC transporters in A549 cells using "Dye Exclusion Assays" by means of flow cytometry (JC1 was used as a fluorescent substrate of MDR1, Calcein AM for MRP1 and Bodipy-prazosin for BCRP). Results were correlated with Immunocytochemistry and qRT-PCR. The selected cytotoxic drugs were shown to activate studied kinases to the different degrees. One of the most effective activators of stress kinases (JNK, p38 MAPK) was Roscovitin, which was also able to modulate function and expression of the studied ABC transporters. Further we used pharmacologic inhibitors of these kinases to assess how they affect the up-regulated function of ABC transporters. Those inhibitors either potentiated the effect of the chemotherapeutic drug or had not any effects. In the next step we aim to assess the role of the involved transcription factors.

Project is supported by GACR 301/08/0717, AVOZ50040507, and AVOZ50040702 of Academy of Science.

Analysis of the prognostic impact of nestin expression in non-small cell lung cancer

Škarda J.[1], Kolář Z.[1], Janíková M.[1], Chmelová J.[2], Krejčí V.[1], Zapletalová J.[2], Langová K.[2], Klein J.[3], Grygárková I.[4], Kolek V.[4], Fridman E.[5], Kopolovic J.[6]

[1]*Department of Pathology and Laboratory of Molecular Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;*

[2]*Department of Bioinformatics, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;*

[3]*1st Department of Surgery, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;*

[4]*Department of Tuberculosis and Respiratory Diseases, Faculty of Medicine and Dentistry, Palacký University, Olomouc, The Czech Republic;*

[5]*Department of Pathology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University, Israel;*

[6]*Department of Pathology, Hebrew University and Hadassah Medical School, Jerusalem, Israel*

Background: Nestin expression in tumor cells may be associated with the degree of tumor differentiation, biological potential of tumor and degree of neoangiogenesis.

Methods: Immunohistochemical detection of nestin was performed by indirect immunohistochemistry using available anti-bodies on tissue microarrays constructed from 115 formalin-fixed paraffin-embedded non-small cell lung cancer samples and 35 brain metastasis. The H-score and degree of nestin positive vascularisation evaluated semi-quantitatively in areas of the most prominent vascularisation were determined. The parameters were correlated with each other and with disease-free and overall survival.

Results: Significantly higher expression of nestin was found in brain metastases of squamous cell carcinomas compared to brain metastases of adenocarcinomas (p=0.003). In sqamous cell carcinomas and adenocarcinomas a significantly higher expression of nestin was found in brain metastases compared to primary tumors (p<0.0001, p=0.034). There was significantly higher occurrence of nestin positive vascularisation in brain metastases of adenocarcinomas than primary adenocarcinomas (p=0.044). In brain metastasis, there was significantly higher occurrence of nestin positive vascularisation in poorly differentiated adenocarcinomas than in well-differentiated adenocarcinomas (p=0.927) or poorly differentiated primary adenocarcinomas (p = 0.008).

Kaplan-Meier curves of disease-free and overall survival showed no significant correlation with nestin expression except for better overall survival of patients with brain metastasis and high nestin expression than patients with brain metastasis and low nestin expression (p=0,043).

Conclusion (s): Our results suggest that the immunohistochemical expression of nestin may be an important diagnostic marker

Gene expression profiling in breast cancer after neoadjuvant treatment – preliminary report

Tvrdík D.[1], Povýšil C.[1], Dundr P.[1], Velenská Z.[1], Melčáková Š.[1], Skálová H.[1], Petruželka L.[2]

[1]*Institute of Pathology, First Faculty of Medicine, Charles University and General University Hospital in Prague, The Czech Republic;*

[2]*Department of Oncology, First Faculty of Medicine, Charles University and General University Hospital in Prague, The Czech Republic*

Introduction: Breast cancer is the most common malignant disease in women, occurring more frequently in developed countries. Neoadjuvant chemotherapy, also called preoperative chemotherapy, is now widely used in the treatment of breast carcinoma. It has several potential advantages compared with the traditional strategy of surgery followed by adjuvant chemotherapy. Neoadjuvant chemotherapy substantially reduces the size of the primary tumor and lymph node metastasis in more than 80% of cases, increasing the probability that breast-conserving surgery can be performed instead of mastectomy. Neoadjuvant chemotherapy is also a valuable research tool for investigating the mechanism of chemotherapy-induced cell death and mechanisms of chemotherapy resistance.

Aim: The study aimed at investigation of the mechanism of neoadjuvant chemotherapy-induced cell death in patients with breast carcinoma and study of the biomarkers in predicting pathologic response to the therapy.

Material and Methods: Transcriptional profile of the biomarkers were determined by DNA microarray technology (SABiosciences) with biochips including 84 human genes associated with apoptosis. The quantification of the changes in expression of individual genes due to the treatment was performed by Real-Time PCR assay (SABiosciences).

Results: This preliminary study included 4 patients with histologically proven invasive breast cancer, that are primarily indicated for breast conserving surgery or mastectomy but who were treated by neoadjuvant anti-EGFR chemotherapy (Herceptin). In each cases, analysis of the transcription profile was performed before and after the treatment, respectively.

The analysis showed that the expression of caspase 14 was significantly up-regulated at the RNA level after the treatment. In contrast, the expression of BCL2-related gene MCL1 was down-regulated due to the treatment.

Conclusion: Breast cancer is a clinically heterogeneous disease, and existing histopathological classifications do not fully capture the varied clinical course of this disease. However, little progress has been made with regards to new molecular prognostic markers that can assist oncologists in treatment decision-making for breast cancer.

We showed, gene expression profiling using microarray or Real-Time PCR assay is a valuable research tool for investigation of molecular markers and prognostic factors which may better

reflect tumor biology and treatment response. Our preliminary data showed that over-expression of caspase 14 together with under-expression of MCL1 gene correlated well with regression of tumor after neoadjuvant anti-EGFR chemotherapy.

However, more patients, more tumors, more genes have to be investigated in correlation with findings of routine histopathology and with clinical outcome before any conclusions can be made.

This work was supported by Grant IGA NS10575–3/2009.

Chromosomal abnormalities of brain tumors – two years experience with cytogenetic analyses

Urbanovská I.[1], Jalůvková M.[1], Uvírová M.[1], Dvořáčková J.[1,2], Buzrla P.[2], Paleček T.[3]

[1]CGB laboratory Inc., Ostrava, The Czech Republic

[2]Department of Pathology, Faculty Hospital Ostrava, The Czech Republic;

[3]Clinic of Neurosurgery, Faculty Hospital Ostrava, The Czech Republic

Primary brain tumors comprise about 1–2% of all tumors. Optimal therapeutic approach that significantly affects the prognosis of patients has not been found yet. It is expected that the therapy would be based among others on the knowledge of genetic changes in individual types of tumor by monitoring the chromosomal abnormalities.

Molecular cytogenetic studies have identified some of the genetic changes that underlie the pathologic differences among astrocytic tumors; progression in tumor grade is associated with an accumulation of aberrations.

We examined 136 primary brain tumor samples, 43 samples were glioblastomas, 25 samples were low grade astrocytomas, 6 tumor samples were anaplastic astrocytomas. The most frequent abnormalities include amplification of EGFR gene, polysomy of chromosome 7 and monosomy of chromosome 10. Other frequent chromosomal changes were deletions of tumor suppressor genes RB1 on chromosome 13, TP53 on chromosome 17 and CDKN2 on chromosome 9.

Then we examined 42 meningeomas and the most frequent abnormalities were deletions of 22q and 1p36 and then monosomy 14. The combination of deletion 1p36 and monosomy 14 is associated with higher risk of tumor recurrence and with worse prognosis.

In most cases, the results were in accordance with the clinical and histological findings and confirmed the original diagnosis. Some of the aberrations have predictive and prognostic value. In addition, the interdisciplinary co-operation of the brain surgeons, pathologists and geneticists is necessary to ensure that the material is sampled properly, the diagnosis is made correctly and quickly and that the risk of false negative findings is reduced.

Immunohistochemical visualization of nestin coexpression with other antigens in vasculature of uterine horns of pregnant rats

Vařejková M.[1], Hollerová H.[2], Kudláčková Z.[1], Mokrý J.[2]

[1]Department of Biological and Medical Sciences, Charles University Faculty of Pharmacy, Hradec Králové, The Czech Republic;

[2]Department of Histology and Embryology, Charles University Medical Faculty, Hradec Králové, The Czech Republic

Standard immunohistochemical detection methods make visualization of only two or three antigens in a histological section. However, this approach does not allow us to exploit the full potential of scarce archived paraffin-embedded sections. We utilized a recently described sequential immunoperoxidase labelling and erasing (SIMPLE) method (Glass et al. J Histochem Cytochem 2009, 57: 899–905) for simultaneous visualization of intermediate filament nestin with other markers within a single tissue section to investigate multiple antigen expression in the vasculature of the uterine horns of pregnant rats.

Paraffin-embedded sections containing the structures of a interest were deparaffinised in xylen and rehydrated in series of graded alcohols to distilled water. Sections were exposed to microwaves to revitalize epitopes in tissue fixed with 4% paraformaldehyde and pretreated with normal donkey serum. Then the first antigen, intermediate filament protein nestin, was detected with Rat 401 antibody. After washing, donkey anti-mouse biotinylated antibody was applied followed by incubation with streptavidin conjugated with horse radish peroxidase. After washing, the signal was developed using aminoethylcarbasole and sections were mounted in buffered glycerol.

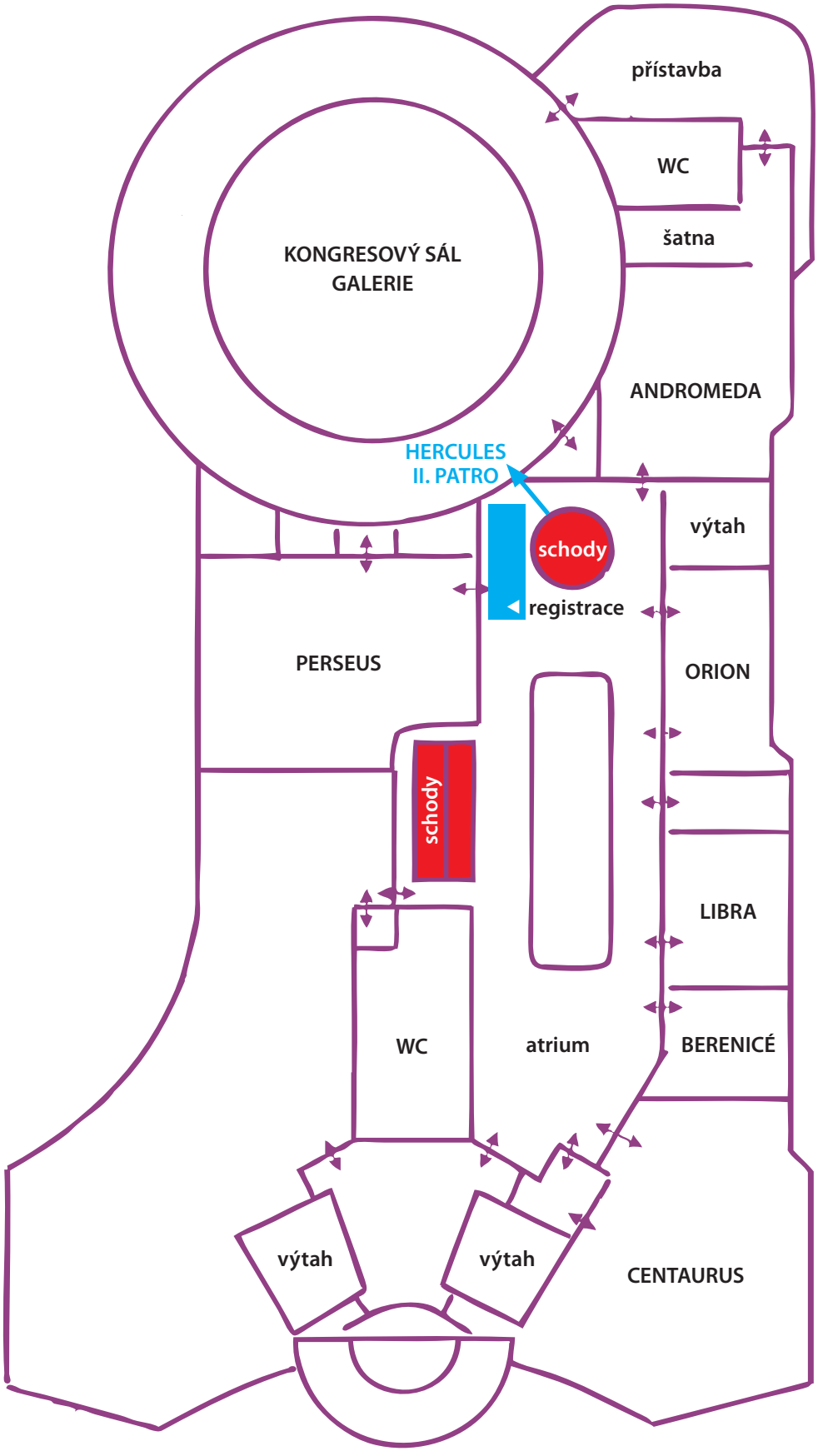
Following microphotography of nestin-immunoreactive structures with DP71 digital camera, the slides were decoverslipped, the red specific signal was washed away by ethanol and antigen-antibody complex was removed in an elution solution. The immunostaining process was then repeated in subsequent steps using the same protocol with distinct primary antibodies: anti-PCNA was used in the second round of immunostaining, anti-desmin in the third and anti-BrdU in the fourth round. Each time the immunopositive signals were examined with BX51 microscope and digitalized images were archived. We also modified the original SIMPLE method to test whether its principles is applicable to immunophosphatase labelling. In this modification, we utilized streptavidin conjugated with alkaline phosphatase and specific signal was developed with Fast Red TR/naphthol AS-MX tablets. To create multicolour composite images, digital snapshots of identical sites with distinct signals obtained in different rounds of the SIMPLE method were aligned as separate layers using Adobe Photoshop software and red colour of immunoreactive structures was replaced with the distinct pseudocolour.

Immunohistochemical staining provided evidence of nestin expression in endothelium, smooth muscle cells and interstitial

myofibroblasts. In endothelial cells, nestin was intensely expressed in the arterial endothelial lining while it was mostly absent from lumina of adjacent veins. Endothelium of blood capillaries was also immunoreactive for nestin. Coexpression of nestin with proliferative markers PCNA an BrdU was observed in endothelial cells; its coexpression with desmin was typically observed in myocytes.

Our results provide evidence for different expression of nestin in arterial and venous endothelium which may be associated with remodelling of blood vasculature during pregnancy and the different functional demands on arteries and veins. We demonstrated the feasibility of SIMPLE method in investigation of multiple antigens expression in the remodelling of blood vessels. The SIMPLE method for immunoperoxidase proved to be less problematic than immunophosphatase staining due to formation of precipitate after antibody elution.

Study was supported by MSM 0021620820.


přístavba
WC
šatna
KONGRESOVÝ SÁL
GALERIE
ANDROMEDA
HERCULES
II. PATRO
schody
registrace
výtah
PERSEUS
ORION
schody
LIBRA
WC
atrium
BERENICÉ
výtah
výtah
CENTAURUS

LINEQ
Liquid Nitrogen – Equipment & Cryobanking

www.lineq.cz

Společnost Lineq se specializuje na prodej a servis v oblasti kryogenních aplikací.

Lineq nabízí:

- Dewarovy nádoby Taylor-Wharton HARSCO
- **dodávky tekutého dusíku**
- různé vestavby a příslušenství k Dewarovým nádobám
- programovatelné zamrazovače Sy-Lab
- projekci, realizaci a údržbu kryobank
- transport vzorků v tekutém dusíku (LIN) nebo jeho parách
- zapůjčení Dewarových nádob
- skladování vzorků v LIN nebo jeho parách
- zálohování vzorků při poruchách
- monitorování Dewarových nádob a prostoru kryoskladu

Lineq, s.r.o.
V Horce 178
252 28 Černošice

tel./fax: +420 251 642 390
mobil: +420 602 311 893
e-mail: lineq@lineq.cz

SPONZOŘI


BARJA


DispoLab


ita


KRD
molecular technologies


Leica
MICROSYSTEMS


MERCI
laboratoř jak má být


scintila


eppendorf
Czech & Slovakia


LAB MARK


MEDIAL
LÉKAŘSKÁ TECHNIKA A SPECIÁLNÍ ZDRAVOTNICKÝ MATERIÁL


Roche


OLYMPUS


bamed
www.bamed.cz


Nikon


NOVARTIS


SIGMA-ALDRICH


Vitrum


VWR


YBUX