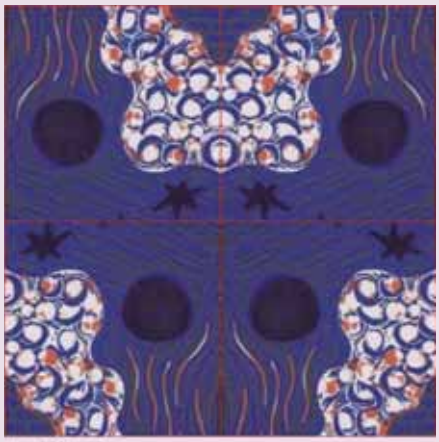
PROGRAMME AND ABSTRACT BOOK

The 5th Symposium & Workshop on Molecular Pathology and Histo(cyto)chemistry

The 92nd Olomouc Slide Seminar of the Czech Division of the International Academy of Pathology


Jana Krejčová

Congress of Histology Laboratory Technicians

APRIL 24–25, 2009
OLOMOUC

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 Division of the International Academy of Pathology
- The Czech Oncological Society CLS JEP
- The Czech Society for Histochemistry and Cytochemistry

- The Department of Pathology & the Laboratory of Molecular Pathology
- The Laboratory of Experimental Medicine

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The 5th Symposium & Workshop on Molecular Pathology and Histo(cyto)chemistry
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Congress of Histology Laboratory Technicians

April 24–25, 2009, Olomouc, Czech Republic

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- 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

Under the auspices of
- prof. RNDr. L. Dvořák, Ph.D. / Rector of Palacký University, Olomouc
- prof. MUDr. Z. Kolář, Ph.D. / Dean of the Faculty of Medicine and Dentistry, Palacký University, Olomouc
- prof. RNDr. J. Ševčík, Ph.D. / Dean of the Faculty of Science, Palacký University, Olomouc
- MUDr. R. Maráček / 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
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Venue:
Friday, April 24 – Regional Centre Olomouc (Jeremenkova 40B, 772 00 Olomouc)
Saturday, April 25 – Theoretical Institutes Building, Faculty of Medicine and Dentistry, Palacký University Olomouc
(Hněvotínská 3, 775 15 Olomouc)

Conference Language:
The 5th Symposium & Workshop on Molecular Pathology and Histo(cyto)chemistry – English
The 92nd Olomouc Slide Seminar of the Czech Division of the International Academy of Pathology – Czech
Congress of Histology Laboratory Technicians – Czech

The 5th Symposium on Molecular Pathology and Histo(cyto)chemistry

FRIDAY, APRIL 24		
Time		**Venue / language**
9:00–9:30	Joint meeting ceremony prof. RNDr. L. Dvořák, Ph.D., prof. RNDr. J. Ševčík, Ph.D., MUDr. R. Maráček, prof. MUDr. Z. Kolář, Ph.D.	Regional Centre Centaurus Hall / English, Czech
Keynote lectures of invited speakers **Chairman: K. Smetana (Praha), G. Turashvili (Vancouver)**		
9:30–10:00	K. Smetana (Prague) Tumor stromal fibroblasts as factor of tumor progression	Regional Centre Perseus Hall / English
10:00–10:30	Ch. Dinant (Copenhagen) Fluorescence microscopy and the DNA damage response	Regional Centre Perseus Hall / English
10:30–11:00	G. Turasvili (Vancouver) Columnar cell lesions of the breast: terminology, histologic classification, molecular pathology and clinical significance	Regional Centre Perseus Hall / English
11:00–11:30	Coffee break	Andromeda Hall
The role of the pathologist in the indication of the cancer treatment **Chairman: G. Bevilacqua (Pisa), A. Ryška (Hradec Králové)**		
11:30–11:45	G. Bevilacqua (Pisa) Rules and organisation for the future of molecular pathology	Regional Centre Centaurus Hall / English
11:45–12:00	A. Ryška (Hradec Králové) The role of the pathologist in tailored therapy	Regional Centre Centaurus Hall / English
12:00–12:15	J. Žaloudík (Brno) No ReStInG in surgical oncopathology	Regional Centre Centaurus Hall / English
12:15–12:30	M. Hajdúch (Olomouc) National experience with implementation of predictive medicine in oncology: Example of Her-2/neu (c-erbB-2) gene	Regional Centre Centaurus Hall / English
12:30–12:45	B. Melichar (Olomouc) Targeted therapy from the point of view of medical oncology	Regional Centre Centaurus Hall / English
12:45–13:30	Discussion	Regional Centre Centaurus Hall / English
13:30–14:00	Lunch break / Poster Session	Andromeda Hall
Keynote lectures of invited speakers **Chairman: A. Castiel (Tel Aviv), P. Knížetová (Copenhagen)**		
14:00–14:30	M. Puhr (Innsbruck) Down-regulation of suppressor of cytokine signaling-3 causes prostate cancer cell death through activation of the intrinsic apoptosis pathway	Regional Centre Perseus Hall / English
14:30–15:00	A. Castiel (Tel Aviv) The SIL (STIL) gene in mitosis and cancer	Regional Centre Perseus Hall / English
15:00–15:30	P. Knížetová (Copenhagen) DNA damage response activation in human brain tumors	Regional Centre Perseus Hall / English
15:30–16:00	Coffee break / Degustation of wine	Andromeda Hall
Selected presentations **Chairman: K. Vrzalíková (Birmingham), J. Škarda (Olomouc)**		
16:00–16:15	B. Dvořánková (Prague) HPV16 – transformed cells influence the phenotype of normal keratinocytes to stem cell-like character	Regional Centre Perseus Hall / English

16:15–16:30	K. Vrzalíková (Birmingham) An investigation of the contribution of LMP1 to the differentiation of germinal centre B cells	Regional Centre Perseus Hall / English
16:30–16:45	L. Lacina (Prague) Biological activity of stromal fibroblasts in basal cell carcinoma	Regional Centre Perseus Hall / English
16:45–17:00	J. Škarda (Olomouc) N-cadherin as a potential predictor of metastases to central nervous system in NSCLC	Regional Centre Perseus Hall / English

POSTERS

1 Huntington's disease and postural stability impairment
Bezdičková M.[1], Filipčíková R.[1], Váverka P.[2], Laichman S.[1], Langová K.[3], David O.[1]
[1]Department of Anatomy, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic
[2]Czech Military Forces, Czech Republic
[3]Department of Biophysics, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

2 Estrogen receptor beta signalling is potentiated by coactivators p300 and CBP in PC3 prostate cancer cells
Bouchal J.[1], Neuwirt H.[2], R. Santer F.R.[2], Culig Z.[2]
[1]Laboratory of Molecular Pathology, Palacký University, Olomouc, Czech Republic
[2]Department of Urology, Innsbruck Medical University, Austria

3 Detection of phenotypic changes in malignant melanoma. Immunohistochemical study of c-Myc, PDGF, bFGF and nestin expression in benign and malignant cutaneous melanocytic lesions.
Brychtová S.[1], Bezděková M.[1], Tichý M. jr.[2], Brychta T.[3]
[1]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic
[2]Department of Dermatology and Venerology, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic
[3]Internal and Diabetology Ambulance, Dlouhá 34, Olomouc, Czech Republic

4 Triple negative breast cancer – cytogenetic changes assessment of EGFR (HER1) and TOP2A genes with clinical and histopathological data analysis
Čížková M. [1,2], Bouchalová K.[1], Cwiertka K.[2], Kolář Z.[3], Trojanec R.[1], Mlčochová S.[1], Fürstová J.[1], Radová L.[1], Hajdúch M.[1,2]
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[3]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Univeristy Hospital, Olomouc, Czech Republic

5 Extending Her-2/neu assessment using a larger spectrum of cytogenetic probes and monoclonal antibodies
Dziechciarková M.[1], Trojanec R.[1], Kolář Z.[2], Bouchalová K.[1], Braunerová B.[1]
[1]Laboratory of Experimental Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic
[2]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, University Hospitel, Olomouc, Czech Republic

6 Testing and control of postural stability of the human body
Filipčíková R.[1], Bezdičková M.[1], Váverka P.[2], Langová K.[3], David O.[1], Laichman S.[1]
[1]Department of Anatomy, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic
[2]Armed Forces of Czech Republic
[3]Department of Biophysics, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

7 Minimal residual disease as a new prognostic factor in pancreatic carcinoma patients
Kesselová M.[1], Srovnal J.[1], Resutíková L.[1], Havlík R.[2], Klos D.[2], Růžková V.[1], Radová L.[1], Hajdúch M.[1]
[1]Laboratory of Experimental Medicine, Department of Pediatrics, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic
[2]Department of Surgery, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic

8 Monitoring of metallothionein level in cancer tissue cultures exposed to cadmium
Křížková S.[1], Adam V.[1,2], Eckschlager T.[3], Kizek R.[1]
[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Brno, Czech Republic
[2]Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Brno, Czech Republic
[3]Department of Paediatric Haematology and Oncology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic

9 Collagen volume fraction, CTGF and TGF-beta expression in patients with end-stage heart failure and atrial fibrillation
Kučera T.[1], Hroudová H.[1], Aldhoon B.[2], Melenovský V.[2], Martínek J.[1], Kautzner J.[2]
[1]Institute of Histology and Embryology, 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
[2]Institute of Clinical and Experimental Medicine, Prague, Czech Republic

10	Polysomy of chromosome 17 in breast cancer patients and its impact to diagnosis and treatment Palková V.[1], Čížková M.[1,2], Trojanec R.[1], Radová L.[1], Melichar B.[2], Bouchalová K.[1], Mlčochová S.[1], Kolář Z.[3], Dziechciarková M.[1], Hajdúch M.[1] [1]Laboratory of Experimental Medicine, Department of Pediatrics, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic [2]Department of Oncology, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic [3]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Univeristy Hospital, Olomouc, Czech Republic
11	Induction of G1-phase cell cycle arrest and apoptosis in human breast and prostate cancer cells by natural brassinosteroids Steigerová J.[1], Oklešťková J.[2], Levková M.[1], Kolář Z.[1], Strnad M.[2] [1]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic [2]Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Olomouc, Czech Republic
12	Her-2/neu (c-erbB-2) gene evaluation in breast cancer; samples with ambiguous FISH results Trojanec R.[1], Palková V.[1], Kolář Z.[2], Berkovcová J.[1], Braunerová B.[1], Bouchalová K.[1], Tichý M.[2], Krejčí V.[2], Melichar B.[3], Dziechciarková M.[1], Cwiertka K.[3], Hajdúch M.[1] [1]Laboratory of Experimental Medicine, Department of Pediatrics, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic [2]Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Univeristy Hospital, Olomouc, Czech Republic [3]Department of Oncology, Faculty of Medicine and Dentistry, Palacký University, University Hospital, Olomouc, Czech Republic
13	Histone deacetylase inhibitors affect androgen receptor activity through corepressors Trtková K., Pašková L. Laboratory of Molecular Pathology, Department of Pathology, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic
14	Our experiences with molecular diagnosis of Ewing´s sarcoma in paraffin-embedded tissue Tvrdík D., Berková A., Melčáková Š., Matiášková L., Staněk L., Povýšil C. Institute of Pathology, 1st Faculty of Medicine, Charles University and General Faculty Hospital in Prague, Czech Republic
15	Molecular genetic investigations of breast carcinoma Urbanovská I.[1], Kubová B.[1], Skalíková R.[1], Uvírová M.[1], Dvořáčková J.[1,2] [1]CGB Laboratory Inc., Ostrava, Czech Republic [2]Department of Pathology, Faculty Hospital, Ostrava, Czech Republic

SOCIAL PROGRAMME	
19:00–24:00	Social evening in Archa restaurant at Svatý Kopeček Concert of Ivo Jahelka Night visit to the ZOO at Svatý Kopeček with small surprise

Workshop on Molecular Pathology – mi-RNA

SATURDAY, APRIL 25		
Time	**Chairman: A. Hlobilková (Olomouc), J. Bouchal (Olomouc),**	**Venue / language**
9:00–9:30	Applied Biosystems Introduction to mi-RNA world	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech
9:30–10:00	O. Slabý (Brno) Significance of microRNAs in predictive oncology: experimental and clinical observations in colorectal cancer, breast cancer and glioblastoma	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech
10:00–10:30	P. Murray (Birmingham) Alterations in cancer-causing lipids induced by Epstein-Barr virus in lymphoma	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / English
10:30–10:45	Coffee break	
10:45–13:00	Applied Biosystems Advanced methods for mi-RNA analysis	Theoretical Institutes Building Faculty of Medicine and Dentistry, Palacký University Olomouc / Czech

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

PÁTEK 24. DUBNA		
Čas		**Místo konání / jazyk**
9:00–9:30	Slavnostní zahájení prof. RNDr. L. Dvořák, CSc., prof. RNDr. J. Ševčík, Ph.D., MUDr. R. Maráček, prof. MUDr. Z. Kolář, CSc.	Regionální centrum sál Centaurus / anglicky, česky
Diagnostický seminář Společnosti českých patologů a české sekce International Academy of Pathology **Předsednictvo: M. Tichý, M. Geierová**		
9:30–11:00	Diagnostický seminář – část I	Regionální centrum sál Gemini / česky
11:00–11:30	Přestávka, občerstvení	sál Andromeda
Úloha patologa v indikaci terapie nádorů „šité na míru" **Předsednictvo: G. Bevilacqua (Pisa), A. Ryška (Hradec Králové)**		
11:30–11:45	G. Bevilacqua (Pisa) Introductory lecture	Regionální centrum sál Centaurus / anglicky
11:45–12:00	A. Ryška (Hradec Králové) The role of the pathologist in tailored therapy	Regionální centrum sál Centaurus / anglicky
12:00–12:15	J. Žaloudík (Brno) No ReStInG in surgical oncopathology	Regionální centrum sál Centaurus / anglicky
12:15–12:30	M. Hajdúch (Olomouc) National experience with implementation of predictive medicine in oncology: Example of Her-2/neu (c-erbB-2) gene	Regionální centrum sál Centaurus / anglicky
12:30–12:45	B. Melichar (Olomouc) Targeted therapy from the point of view of medical oncology	Regionální centrum sál Centaurus / anglicky
12:45–13:30	Diskuze	Regionální centrum sál Centaurus / anglicky
13:30–14:00	Oběd / Sekce posterů	sál Andromeda
14:00–15:30	Diagnostický seminář – část II	Regionální centrum sál Gemini / česky
15:30–16:00	Přestávka, občerstvení / Degustace vína	sál Andromeda
Vybraná sdělení **Předsednictvo: K. Vrzalíková (Birmingham), J. Škarda (Olomouc)**		
16:00–16:15	B. Dvořánková (Praha) HPV16 – transformed cells influence the phenotype of normal keratinocytes to stem cell-like character	Regionální centrum sál Perseus / anglicky
16:15–16:30	K. Vrzalíková (Birmingham) An investigation of the contribution of LMP1 to the differentiation of germinal centre B cells	Regionální centrum sál Perseus / anglicky
16:30–16:45	L. Lacina (Praha) Biological activity of stromal fibroblasts in basal cell carcinoma	Regionální centrum sál Perseus / anglicky
16:45–17:00	J. Škarda (Olomouc) N-cadherin as a potential predictor of metastases to central nervous system in NSCLC	Regionální centrum sál Perseus / anglicky

Meziregionální konference histologických laborantů

PÁTEK 24. DUBNA		
Čas		**Místo konání / jazyk**
9:00–9:30	Slavnostní zahájení prof. RNDr. L. Dvořák, CSc., prof. RNDr. J. Ševčík, Ph.D., MUDr. R. Maráček, prof. MUDr. Z. Kolář, CSc.	Regionální centrum sál Centaurus / anglicky, česky
Konference histologických laborantů I **Předsednictvo: J. Ehrmann, D. Kvapilová**		
9:30–10:00	P. Zdílna (Praha) Akreditace zdravotnických laboratoří – zkvalitnění lékařské péče	Regionální centrum sál Centaurus / česky
10:00–10:15	K. Klimeš (Brno) Úskalí zavádění systémů jakosti v laboratoři	Regionální centrum sál Centaurus / česky
10:15–10:30	J. Ehrmann (Olomouc) Zkušenosti manažera kvality – lékaře, při procesu akreditace zdravotnické laboratoře	Regionální centrum sál Centaurus / česky
10:30–11:00	Diskuze	Regionální centrum sál Centaurus / česky
11:00–11:30	Přestávka, občerstvení	sál Andromeda
Úloha patologa v indikaci terapie nádorů „šité na míru" **Předsednictvo: G. Bevilacqua (Pisa), A. Ryška (Olomouc)**		
11:30–11:45	G. Bevilacqua (Pisa) Rules and organisation for the future of molecular pathology	Regionální centrum sál Centaurus / anglicky
11:45–12:00	A. Ryška (Hradec Králové) The role of the pathologist in tailored therapy	Regionální centrum sál Centaurus / anglicky
12:00–12:15	J. Žaloudík (Brno) No ReStInG in surgical oncopathology	Regionální centrum sál Centaurus / anglicky
12:15–12:30	M. Hajdúch (Olomouc) National experience with implementation of predictive medicine in oncology: Example of Her-2/neu (c-erbB-2) gene	Regionální centrum sál Centaurus / anglicky
12:30–12:45	B. Melichar (Olomouc) Targeted therapy from the point of view of medical oncology	Regionální centrum sál Centaurus / anglicky
12:45–13:30	Diskuze	Regionální centrum sál Centaurus / anglicky
13:30–14:00	Oběd / Sekce posterů	sál Andromeda
Konference histologických laborantů II **Předsednictvo: K. Klimeš, D. Kvapilová**		
14:00–14:15	Z. Fejglová (Hradec Králové) Akreditace na FÚP FN HK	Regionální centrum sál Centaurus / česky
14:15–14:30	J. Vaculová (Ostrava) Jsme dostatečně standardní?	Regionální centrum sál Centaurus / česky
14:30–14:45	M. Petrová (Olomouc) Interní auditor laboratoře	Regionální centrum sál Centaurus / česky
14:45–15:15	Diskuze	Regionální centrum sál Centaurus / česky
15:30–16:00	Občerstvení / Degustace vína	sál Andromeda

POSTERY		
1	Historie patologie L. Hrabovská, A. Muchová Oddělení patologie NsP Havířov	

SPOLEČENSKÝ PROGRAM	
19:00–24:00	Společenský večer v restauraci Archa na Sv. Kopečku Koncert Ivo Jahelky Noční prohlídka ZOO s překvapením

Workshop molekulární patologie – mi-RNA

SOBOTA, 25. DUBNA		
Čas	**Předsedntictvo: A. Hlobilková (Olomouc), J. Bouchal (Olomouc),**	**Místo konání / jazyk**
9:00–9:30	Applied Biosystems Úvod do světa mi-RNA	Teoretické ústavy LF UP / česky
9:30–10:00	O. Slabý (Brno) Význam microRNA v prediktivní onkologii	Teoretické ústavy LF UP / česky
10:00–10:30	P. Murray (Birmingham) Alterations in cancer-causing lipids induced by Epstein-Barr virus in lymphoma	Teoretické ústavy LF UP / anglicky
10:30–10:45	Přestávka, občerstvení	
10:45–13:00	Applied Biosystems Metody analýzy mi-RNA	Teoretické ústavy LF UP / česky

Ve dnech 23.–26. 4. 2009 probíhá v Olomouci „Jarní Flora Olomouc". Vřele doporučujeme!

Koncert Ivo Jahelky

Pátek 24. dubna od 19.00 hod.
v restauraci Archa na Sv. Kopečku

CURICULUM VITAE

Karel Smetana, Professor, M.D., D.Sc.

Upon completion of his M.D. degree in general medicine from Charles University (CU) in Prague in 1983, Dr. Smetana joined the Institute of Anatomy, 1st Faculty of Medicine of CU where he is currently Professor of Anatomy. He is also a member of the Center of Cell Therapy and Tissue Repair of the 2nd Faculty of Medicine at CU. His focus is cell and developmental biology and experimental morphology including cell therapy. He is interested predominantly in the biology of squamous epithelia under normal and pathological conditions such as cancer. His research focuses on glycobiology and in particular on the galectin family. His most recent scientific work is directed to the tumor microenvironment, especially the role of cancer associated fibroblasts in the biology of basal/squamous cell carcinoma. He is the co-founder of a new cell-based treatment for skin defects employing epidermal stem cells. This achievement was given the National Scientific Award of the Czech Republic in 2002. Professor Smetana is an executive editor of Folia Biologica and a member of the editorial boards of Recent Patents in Biomedical Engineering and the Open Anatomy Journal.

Tumor stromal fibroblasts as a factor of tumor progression

Karel Smetana, Jr.
Charles University, 1st Faculty of Medicine, Institute of Anatomy, 2nd Faculty of Medicine, Center of Cell Therapy and Tissue Repair, Prague, Czech Republic

Malignant tumors are widely occurring in humans and they form a serious medical, economical and social problem. The aging of the population may be related to the increased incidence of such malignancies. However, despite the progress in cancer treatment, the prognosis for many patients is not optimistic. Remarkable achievements in stem cell research have delineated new horizons for possible future improvement in cancer treatment. A paradigm of the existence of cancer stem cells has been established for solid tumors where it is based on a parallel between tissue stem cells and a population of cancer cells responsible for tumor spread. Normal tissue stem cells require a highly specialized microenvironment, a so-called niche, necessary for the maintenance of their stemness. The positive role of tumor stroma in the course of the vascularisation of the tumor bed has already been well-described. The tumor stroma includes a large number of cell types (fibroblasts, leukocytes, endothelial cells). The evidence to date is that cancer stromal fibroblasts have an important role in cancer progression. Phenotype and functional differences between cancer stroma and normal tissue fibroblasts have been established in e.g. tumors of breast, pancreas, colon, prostatic gland, skin and oral cavity. We isolated stromal fibroblasts from basal and squamous cell carcinoma and compared them with normal human fibroblasts. When normal keratinocytes are co-cultured with basal/squamous cell carcinoma associated fibroblasts, the phenotype of keratinocytes is heavily altered to resemble epidermal stem cells and/or cancer cells in comparison to keratinocytes co-cultured with normal human fibroblasts. Modern analytic technologies such as DNA microarray analysis of transcriptoma (Illumina) used as screening method reveal differences in expression of genes encoding production of regulatory factors/cytokines/chemokines that are significant in the biological activity of cancer associated fibroblasts. The nature of these fibroblasts is not well-understood, but principally they can originate in local mesenchyme under the control of cancer cells or from tumor cells undergoing epithelial-mesenchymal transition. The participation of mesenchymal stem cells is also possible. Summarizing these data, like in embryonic development, mesenchymal-epithelial interaction can play an important role in tumor progression. Experiments involving blocking the activity of selected regulatory factorsare enabling us to understand the role of the stroma in tumor biology. The management of the tumor microenvironment may be a promising future anti-cancer therapeutic approach.

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CURICULUM VITAE

Christoffel Dinant, M.Sc., Ph.D.

Christoffel Dinant is currently working as a postdoctoral fellow in the laboratory of Professor Jiří Lukáš at the Centre for Genotoxic Stress Research, Institute of Cancer Biology, Danish Cancer Society, Copenhagen. He got his Ph.D. from Erasmus University Rotterdam in 2008 where he defended his thesis A Microscopic Study of the DNA Damage Response under the supervision of Professors Hoeijmakers, Dr. Vermeulen and Dr. Houtsmuller. His master's studies in biology were succesfully completed under the supervision of Prof. van Driel of the University of Amsterdam and Dr. Jackson of the University of Manchester. Christoffel Dinant is working in the field of the DNA damage response and analysis of chromatin structure where he has published a number of outstanding research articles. He is also a reviewer of for a number of peer-reviewed journals, in particular for Nucleic Acids Research.

Fluorescence microscopy and the DNA damage response

Christoffel Dinant
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Some of the main causes of cancer development are mutations in DNA caused by un- or misrepaired DNA damage. DNA can be damaged by many different sources, including ionizing radiation, UV-C light and certain endogenous metabolites. Cells respond to DNA damage with a variety of mechanisms, together known as the DNA damage response (DDR). The DDR consists of activation of cell cycle checkpoints initiated by phosphorylation of histone variant H2AX by ATM or ATR, a transcriptional response and DNA repair. We use fluorescence microscopy techniques to study the cellular response to DNA damage. For this purpose, DDR proteins are either visualized by immunofluorescence, or they are genetically tagged with GFP or a spectral variant of GFP to enable their visualization in living cells. To study the behavior of DDR proteins, we use a number of techniques to induce DNA damage in living cells, including ionizing radiation and UV lasers. Many DDR proteins accumulate in small nuclear foci in response to DNA damage induction. These foci are thought to be the locations where DNA repair takes place. Interestingly, there appear to be at least two different types of foci, each containing a subset of DDR proteins. The smaller foci consist of proteins directly involved in DNA repair, while the larger foci have a role in cell cycle signaling, which involves changes in chromatin structure. We are interested in these changes in chromatin structure following DNA damage induction and in the communication between the chromatin domain and the sites of DNA repair. Techniques we use to study these processes include the above-mentioned laser-assisted DNA damage induction, confocal microscopy photobleaching techniques and high-throughput siRNA screens.

CURICULUM VITAE

Gulisa Turashvili, M.D., Ph.D.

Dr. Gulisa Turashvili obtained her medical degree from Tbilisi State Medical University, Georgia, in 2002. She subsequently entered a residency training program in pathology in Tbilisi, Georgia, and a Ph.D. program at the Laboratory of Molecular Pathology, Department of Pathology, Palacký University, Olomouc, Czech Republic (supervisor Professor Zdeněk Kolář). Her Ph.D. involved microarray analysis of microdissected invasive breast carcinomas and normal mammary epithelium in collaboration with the Institute of Cancer Studies, University of Birmingham, UK. Her postgraduate training also included the European School of Pathology, Turin, Italy, in 2002 and 2003, and a research stay at the Department of Pathology, University of Birmingham in 2006. She defended her Ph.D. in April 2007 and certification in pathology in Georgia in the following month. Dr. Turashvili was awarded a Canadian Institute of Health Research (CIHR) fellowship starting in June 2007. She is working with Professor Samuel Aparicio and Professor Peter Watson at the Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, BC, Canada. Her current research activities include work on METABRIC (MolEcular TAxonomy of BReast cancer International Consortium) project as well as columnar cell lesions (CCLs) and early breast cancer risk. METABRIC is an Anglo-Canadian International study aimed at examining several thousands of breast tumors, using a combination of high resolution array-CGH, gene expression profiling, miRNA profiling and tissue microarray (TMA) analysis, and correlating the molecular profiles obtained with the clinical outcome. The CCL project aims to characterize the molecular features of CCLs in relation to in situ and invasive breast carcinomas, to conduct a retrospective analysis to determine the frequency of CCLs and their epidemiology in relation to other breast cancer risk factors, and to improve the current clinical-pathological definition of CCLs.

Columnar cell lesions of the breast: terminology, histologic classification, molecular pathology and clinical significance

Gulisa Turashvili
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Columnar cell lesions (CCLs) are one of the most common abnormalities in the adult female human breast and comprise a spectrum of histologic changes characterized by the presence of one to several layers of columnar-shaped epithelial cells in enlarged terminal duct lobular units. CCLs are being seen increasingly in core biopsies taken for non-palpable calcifications identified by screening mammography. The increased incidence may reflect improved recognition of CCLs by pathologists or a true increase in incidence related to biological and/or environmental factors. We identified CCLs in 17 % of normal breast tissue samples obtained from a forensic autopsy series, 4.2 % of reduction mammoplasty specimens and 44 % of breast cancer patients.

CCLs may have been first recognized by Warren in 1905 as an abnormal pattern of involution. They have since been described under a variety of names such as blunt duct adenosis, clinging carcinoma, columnar alteration with prominent apical snouts and secretions (CAPSS), enlarged lobular units with columnar alteration (ELUCA), hyperplastic enlarged lobular units (HELUs), monomorphic epithelial proliferation (MEP), columnar cell change (CCC), columnar cell hyperplasia (CCH) with or without atypia, and flat epithelial atypia, originally designated as ductal intraepithelial neoplasia, type 1 b (DIN1b), and later as DIN1a. The lack of standardized terminology has been a serious obstacle to wide recognition of CCLs by practicing pathologists and clinicians.

The cellular origin of CCLs, and their possible relationship to either expansion or metaplasia of a pre-existing normal cell phenotype remains unclear. Although columnar cells lack mature luminal or basal/myoepithelial and apocrine markers, they are often positive for estrogen receptor-α, progesterone receptors, Bcl-2 and FASN. It is likely that the development of CCLs is influenced by steroid hormones and/or growth factors. CCLs are frequently associated with lobular and ductal in-situ tumors, and invasive lobular and tubular carcinomas. The genetic alterations in CCLs are not well characterized. A few studies have reported changes in ERBB and amphiregulin genes by DNA microarray, allelic imbalance targeting loci at 9q, 10q, 17p and 17q by PCR, and recurrent 16q loss by comparative genomic hybridization. After age and BRCA1/2 genetic predisposition, high mammographic density is the third largest population risk factor

for subsequent development of in-situ or invasive carcinoma. We reported an association between CCLs and breast tissue composition, including mammographic density.

Both histological and molecular genetic studies suggest that CCLs represent the earliest histologically identifiable, non-obligate precursor of low-grade breast cancer. Although the follow-up data show that the risk of local recurrence and progression of most CCLs to invasive cancer is low, we believe that there is sufficient evidence to recommend regular follow-up when CCLs are diagnosed in women participating in mammographic screening programs. Whether further excision should be recommended for CCLs detected in core biopsies remains controversial. As the natural history of CCLs is currently uncertain, further detailed phenotype and genotype studies across the spectrum of CCLs and in the context of normal and other pre-neoplastic breast lesions will be required to delineate the biology and better define the clinical implications of CCLs.

CURICULUM VITAE

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

Generoso Bevilacqua currently holds the position of Professor of Pathology at the University of Pisa. He is 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 USA. 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 that 200 research articles. In particular, he is the co-discoverer of the NM23 gene, the first gene found to be involved in tumor metastasis control. This is patented by USA Government. He was also involved in demonstrating the infectious pathway of the MMTV (murine mammary tumor virus) in the newborn mouse and demonstrating that MMTV exogenous sequences occur in a high percentage of human breast carcinomas. He also uncovered the first BRCA1 gene mutation considered typical of the Tuscany population and demonstrated that in human breast cancer progression, neoangiogenesis is a very early event, induced by epithelial hyperplasia.

Rules and organisation for the future of molecular pathology

Generoso Bevilaqua
University of Pisa, Italy

Understanding of the molecular mechanisms of disease constantly increases. As a result, the concept of pathology is changing. Today, as a discipline, pathology is the study of the etiology and pathogenesis of disease through analysis of both the morphological and molecular modifications of cells and tissues, and as a profession it involves using the knowledge of the etiopathogenesis of the disease to make a diagnosis and provide prognostic and therapeutic information. Molecular Pathology (MP) is today universally acknowledged as the modern face of Pathology and more frequently it is defined as the Pathology of the future. In addition to covering the two classic areas of diagnosis and prognosis, MP has also allowed Pathologists to be involved in the therapeutic process. It is considered a relevant component of Molecular Medicine and of the so-called Personalized Medicine. The development of the two main branches of molecular biology, genomics and proteomics, has given strong input to immunohistochemistry (IHC), which is today an integral part of Molecular Pathology. All the methods capable of studying genes and proteins are considered tools of MP and these can be divided into two: 1) Molecular Biology, where nucleic acids and proteins are analyzed after their extraction, 2) Molecular Morphology, where they are studied in situ, with techniques such as IHC, FISH or SISH, etc. Opportunely, IHC can be considered as in situ proteomics. The ever more extensive use of Molecular Pathology and its current role in predicting response to specific drugs and requires high standard laboratories and very qualified personnel. As a consequence, the organization of the laboratories and "who has to do what" is an important topics of discussion.

The involvement of Pathologist in the therapeutic process requires the development of a biomolecular culture and intense collaboration with Clinical Oncologists. The Pathologist has to know exactly what information to ask for, which techniques are used, how to interpret the molecular data and to include them in the final report. Moreover, the "microenvironment" of a Pathology Department will change, in relation to various types of professional experiences: pathology, molecular genetics, proteomics, etc. intense educational programmes all over Europe are necessary for the achievement of this goal.

Aleš Ryška, Professor, M.D., Ph.D.

CURICULUM VITAE

Aleš Ryška graduated in general medicine in 1994 and defended his Ph.D. thesis „New approaches in morphological diagnostics of breast carcinoma" in 2001 both from the Medical Faculty of Charles University in Hradec Králové. He completed the board certification in pathology (1st degree 1997, 2nd degree 2000) and he has 15 years teaching experience in the Fingerland's Department of Pathology, Charles University in Hradec Králové. At present, he is Deputy Dean for Student Affairs at the Medical Faculty of Charles University in Hradec Králové and head of the Fingerland´s Department of Pathology. He is also President of the Czech Division of the International Academy of Pathology and chairman of the Society of Czech Pathologists. His professional interests and research activities include the diagnostics of breast, thyroid and salivary gland lesions and, special methods, such as immunohistochemistry, molecular biology, etc. He is the author/co-author of 80 original research articles in peer-reviewed journals, 43 articles in journals with IF. His publication „Ryška A, Seifert G. Adenolymphoma (Warthin's tumor) with multiple sarcoid-like granulomas. Path Res Pract, 1999; 195: 835–839" was awarded the Fingerland prize in 2000.

The role of the pathologist in tailored therapy

Aleš Ryška
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The role of the pathologist has dramatically changed in recent decades. Originally, the main task of the (histo) pathologist was to establish the diagnosis of a tumor – including precise type of lesion, its biological potential, grade and stage. However, in certain cases the morphological classification of lesions did not correlate well with the biological potential of the tumor. Thus, the modern classification of tumors is based on a combination of morphology and the genetic profile of the neoplasm – this is also mirrored in the title of the new edition of WHO classification of tumors (Pathology and genetics of tumors).

Since the introduction of modern therapeutic options (biological treatment, individualized „tailored" therapy), the original data supplied by the pathologist are not sufficient for clinicians (namely oncologists). They need and expect significantly more precise typing of the tumor, including detection of the expression of various molecules serving as targets for this advanced treatment.

Breast carcinoma may serve as a classical example – initially only detection of hormonal (estrogen and progesterone) receptors was needed for choice of appropriate treatment and estimation of prognosis. In recent years, the detection of HER-2/neu has been introduced and is now considered to be an integral part of pathological rating of such a tumor. In the course of time a system for external quality control for these examinations has been established, as validity and reproducibility are absolutely crucial for effective treatment.

New approaches to the treatment of colorectal cancer can serve as another example. After the introduction of anti-EGFR therapypathologists began to detect immunohistochemically EGFR in tumor cells. It was predicted that its expression would correlate with the effect of treatment as it had been observed in anti-HER-2/neu treatment of HER2 positive breast carcinomas. However, multiple studies have shown a lack of correlation of anti-EGFR therapy with immunohistochemical EGFR positivity. A similar lack of correlation has also been found in the detection of a number of EGFR gene copies by fluorescent in situ hybridization (FISH). The proportion of patients who benefitted from treatment was virtually identical in EGFR positive and EGFR negative subgroups of patients with colorectal carcinoma. Recently, activating mutation of k-ras gene (observed in about 30 % of all colorectal cancers) has been shown to be strongly associated with failure of the anti-EGFR therapy and molecular analysis of tumor DNA (search for most frequent mutations of k-ras gene) is now needed before starting this treatment.

These examples illustrate the need for deeper understanding of the molecular pathways involved in the etiopathogenesis of cancer as well as in the mechanism of biological treatment. Thus, the pathologists will be more and more involved in the detection at various critical points (expression of proteins, mutations or rearrangements of genes, etc.) which may be responsible for success/failure of cancer treatment.

Jan Žaloudík, Professor, M.D., Ph.D.

Graduated from the Faculty of Medicine in Brno in 1979. Surgical oncologist at the Masaryk Memorial Cancer Institute (MMCI) in Brno since 1980. Visiting scientist at the Paterson Institute for Cancer Research in Manchester, UK, in 1985 and 1987 (cancer immunology and DNA flow cytometry) and at the Wistar Institute and Pennsylvania University, Philadelphia, USA 1990–1992 (cancer vaccines). Chief executive officer (2000–2001) and Director for Research and Development (2001–2008) at the MMCI. Dean of the Faculty of Medicine of Masaryk University in Brno since 2003. Chairman of the Czech Section of the World Federation of Surgical Oncology Societies and member of the National Board of the Czech Cancer Society. Author and co-author of more than 170 papers in scientific journals, over 300 presentations and invited lectures.

CURICULUM VITAE

No ReStInG in surgical oncopathology

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Molecular oncology and prediction have become popular directions in the management of cancer which is now claimed to follow a personalized direction. However, in order to connect „this new roof with an older basement" it is necessary to check using main standard parameters in practice. No single receptor, mutated gene or signaling pathway can restore disorder in standard clinicopathological data. There still remains a lot of space for no resting or „No ReStInG" of surgical oncologists and pathologists. Examination of nodes (No), resection margins (Re), clinical stage (St) and investigation of grade (InG) remain crucial factors in treatment strategies and prognosis. The accuracy of these parameters influence clinical outcomes more than any modification in chemotherapy, biotherapy or introduction of any new marker. Controlled trials of any therapeutic regimen may be easily distorted in case of errors or absence of any of the four principal „No ReStInG" parameters. In order to describe the current situation in the Czech Republic which may not be far from other European countries, I checked the reports on these four parameters for several frequently occurring cancers in the nationwidepopulation-based ***Czech National Cancer Registry (functional since 1977, almost 1.5 million reported cancer cases)*** for years 2004 and 2005:

A) Negative lymph node (No) status varied widely in 14 regionsranging from 12–33 % in the case of colon carcinoma and 3–13 % in the case of malignant melanoma. It is unlikely that the proportions of stages I-II and III are so different between regions with comparable numbers of cases and without any organized selection. In both diagnoses the shift from stage II (with N0) to stage III (with N+) indicates adjuvant therapy.

B) Completeness of resection (Re), as classified by parameters R0, R1 or R2, was not reported for instance in 6 % of breast, 12 % of rectal, 18 % of bronchogenic, 38 % of pancreatic and 50 % of hepatocellular carcinomas. Microscopic (R1) or macroscopic (R2) residual tumor will certainly dictate a postoperative therapeutic strategy different from R0.

C) Clinical stage (St) of malignant disease was not reported in 7 % of malignant melanomas, 10 % of breast, 15 % of colorectal, 16 % of uterine cervix, 18 % of prostate, 18 % of renal, 30 % of bronchogenic and 48 % of pancreatic carcinomas, and 39 % of soft tissue sarcomas. In this array of cancer diagnoses the unknown stages reflect both difficult and defective diagnostic efforts. However, an appropriate therapeutic strategy cannot be determined without knowing the stage of disease or estimate of treatment efficacy.

D) Investigation of tumor grade (InG) in terms of degree of malignancy or aggressiveness in each individual case involves several aspects from standard histological grading criteria in various tumor types to new panels for gene expression grading indices. The approach and results in each treatment center/hospital have become more dependent on pathological expertise and the use of newer molecular methods. For instance, it has been suggested according to gene expression arrays that breast cancers are classified as basal cell-like, HER2-like, luminal A and luminal B. However, these subtypes also correspond to different phenotypes as detected by ER, HER2 and p53 immunohistochemistry. On the other hand standard histological grading, despite its impact on prognosis and indication for adjuvant therapy, was missing for 23 % of breast, 18 % of colorectal, 24 % of uterine cervix and 16 % of bladder early stage carcinomas. In soft tissue sarcomas, where the clinical stage is determined by grading, the histological grade was not reported in 42 % cases.

In conclusion, it needs to be stressed that new molecular markers and predictors can be effectively utilized only if the standard classification criteria of malignant tumors are not missing or ignored.

Marián Hajdúch, Associate Professor, M.D., Ph.D.

CURICULUM VITAE

Marián Hajdúch graduated from the Faculty of Medicine of Palacký University in Olomouc in 1997. He obtained his Ph.D. in pediatrics in 2003 and received an Associate Professorship in oncology in 2006. He is an active researcher and head of the Laboratory of Experimental Medicine, Department of Paediatrics, Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc since 1997 and clinical fellow at the Department of Oncology, Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc since 2003. His research portfolio involves the development of novel anticancer therapies and drugs, regulation of the cell cycle in tumor cells, the role of cyclin-dependent kinase inhibitors, molecular, cellular and clinical aspects of multidrug resistance in human cancer and predictive and prognostic oncology. He is a member of a number of Czech and international scientific societies as well as a member of editorial boards or editor of scientific journals. His bibliography includes 125 original papers and review articles, 11 books/chapters in books and 17 patents.

National experience with the implementation of predictive medicine in oncology: Example of Her-2/neu (c-erbB-2) gene.

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Determination of Her-2/neu status is crucial for the effective indication of trastuzumab (Herceptin®) treatment. In the Czech Republic, Her-2/neu status is evaluated by FISH and immunohistochemistry at six Reference Centres (Laboratories of Predictive Medicine). However, some samples cannot be evaluated by FISH due to DNA degradation and those tumors were evaluated by quantitative real-time PCR for Her-2/neu gene. Moreover, due to polysomy of chromosome 17 (CH17) at least 5–7 % of patients are not indicated for trastuzumab treatment as they do not fulfill the criteria of an Her-2/neu: CH17 ratio > 2.2. The efficacy of trastuzumab in polysomic patients has not yet been confirmed. In the Czech Republic, such patients are indicated for trastuzumab treatment only when they are immunohistochemically positive (3+).

More than 2818 breast cancer samples were evaluated in our institution over a period of seven years. Overall, 148 (5.25 %) cases failed to be concluded by FISH. Absence of cancer cells and/or DNA degradation in the tumor biopsy were the major causes of the failure. For such cases, quantitative real-time PCR comparing the Her-2/neu gene status to reference genes dck, gcs1 and epn2 was established. Among 148 cases which failed using FISH technique, we have successfully investigated 77 patient samples by qRT-PCR achieving unambiguous results in 78 % (60/77).

In 13.8 % (368/2670) cases, polysomy of C17 was detected by centromeric probe (CEP17). Using locus specific probe mapping of 17p11.2 region, we found that 57 % (212/368) of such a" polysomic cases" contain only 2 copies of CH17. We found in some instances, the hybridization of centromeric probe was not specific enough and the probe also hybridized to centromeres of other chromosomes or the cells showed complex cytogenetic rearrangements which misrepresent the number of CH17.

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Bohuslav Melichar, Professor, M.D., Ph.D.

Bohuslav Melichar is currently Head of the Department of Oncology of the Faculty of Medicine and Dentistry of Palacký University and University Hospital in Olomouc. He graduated from Charles University Medical School in Hradec Králové in 1989 and obtained a Ph.D. in immunology in 1999. From 2000 he was affiliated as a medical oncologist to the Department of Oncology and Radiotherapy, Charles University Medical School and University Hospital in Hradec Králové. He was appointed Associate Professor in 2001 and Professor in 2006. Bohuslav Melichar has published more than 120 articles with more than 450 citations (SCI).

CURICULUM VITAE

Targeted therapy from the point of view of medical oncology

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In the last few years, targeted therapy has become an integral part of the armamentarium of medical oncology. Targeted agents have been introduced into the treatment of the most common malignant tumors, including breast, colorectal and non-small cell lung carcinoma. The advent of targeted therapy has also changed the course of tumors that were hitherto resistant to any systemic agents, e.g. renal cell carcinoma and gastrointestinal stromal tumors. The term targeted therapy implies a precise knowledge of the target but with some targeted agents the expanding knowledge on tumor biology has so far failed to translate into defining a predictive factor. On the other hand, evidence is emerging that the host immune response may play a crucial role in determining the response to some targeted agents, specifically monoclonal antibodies. Thus, algorithms for the prediction of response to targeted treatment may include not only molecular targets expressed by the tumor cell but also parameters determining the host immune response.

CURICULUM VITAE

Martin Puhr, M.Sc.

Upon completion of his master´s degree in biology at the Karl F. University of Graz, Martin Puhr joined the Department of Urology of Innsbruck Medical University, where he is currently finishing his Ph.D. study in Molecular Cell Biology and Oncology under the supervision of Professor Zoran Culig. During his studies he also worked in the laboratory of professor Nevalainen at the Kimmel Cancer Center of the Thomas Jefferson University in Philadelphia. He has already been honoured by several awards, either as a co-author or as the first author of articles or presentations (EAU award for the best paper published in 2007 in the field of urology, SBUR travel award for 7th World Basic Urological Research Congress in 2007, ARTP award for the best research presented at the ESUR meeting in 2008 and Alken Price for the best paper in the field of urology in 2008). He is interested in various aspects of prostate cancer development and progression with major emphasis on the role of the suppressor of cytokine signalling-3 (SOCS-3) in the survival of prostate cancer cells.

Down-regulation of suppressor of cytokine signaling-3 causes prostate cancer cell death through activation of the intrinsic apoptosis pathway

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Suppressor of cytokine signaling (SOCS) -3 acts as a negative feedback regulator of the Janus kinase/signal transducers and activators of transcription factors signaling pathway and plays an important role in the development and progression of various cancers. In order to better understand the role of SOCS-3 in prostate cancer, SOCS-3 expression was down-regulated in DU-145, LNCaP-IL-6+, and PC3 cells by consecutive SOCS-3 siRNA transfections. SOCS-3 mRNA and protein expression as measured by qRT-PCR and Western blot, respectively, were decreased approximately 70–80 % compared to controls. We observed a significant decrease in cell proliferation and viability in all SOCS-3-positive cell lines but not in the parental LNCaP cell line, which is SOCS-3-negative. In this study, we show that down-regulation of SOCS-3 leads to an increased cell death in prostate cancer cell lines. We found a remarkable increase in the activation of the pro-apoptotic caspases 3 and 9. A significant up-regulation of cPARP and inhibition of Bcl-2 expression was observed in all SOCS-3-positive cell lines. Overexpression of Bcl-2 could rescue cells with decreased SOCS-3 levels from going into apoptosis. Tissue microarray data prove that SOCS-3 is highly expressed in castration-refractory tumor samples. In conclusion, we show that SOCS-3 is an important protein in the survival machinery in prostate cancer and is overexpressed in castration-resistant tumors. SOCS-3 knock down results in an increase in cell death via activation of the intrinsic apoptosis pathway.

CURICULUM VITAE

Asher Castiel, M.Sc.

Asher Castiel graduated from the Life Science Faculty of Tel-Aviv University in 2005. His master´s degree focused on retroviruses and lentiviruses. He is currently finishing his Ph.D. study in the field of cancer research, regulation of the cell cycle and mitosis at the Faculty of Medicine of Tel-Aviv University and Sheba Medical Centre. Asher Castiel has published important research articles in the above mentioned fields.

The SIL (STIL) gene in mitosis and cancer

Asher Castiel
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The SIL gene (SCL Interrupting Locus) was cloned from the most common chromosomal rearrangement in T cell acute lymphoblastic leukemia (T-ALL). In this rearrangement, SIL promoter assumes control of a downstream gene, SCL. The resulting aberrant expression of SCL leads to the development of leukemia. The SIL gene encodes a large (150kDa) cytosolic protein, with almost no known functional motifs or cellular role. SIL mRNA expression is higher in rapidly proliferating cells and tissues, and decreases rapidly during terminal differentiation. The SIL protein reaches peak levels in mitosis during which it is phosphorylated and then degraded on transition to G1. SIL importance to cell growth and survival is supported by the phenotype of mouse and zebra fish embryos lacking a functional SIL protein. In both species, the loss of SIL is embryonically lethal and is associated with marked apoptosis of the developing nervous system. The phenotype of SIL-/- embryos, together with recent published data, suggests that SIL is required for the Sonic Hedgehog (Shh) pathway, a critical pathway for normal development and tumorgenesis. Mutations in the Shh pathway are associated with developmental defects and neoplasia, and over activation of this pathway can frequently be found in cancer. Additional data published recently show that SIL is mutated in the Primary Microcephaly Syndrome, together with other mutations in centrosomal genes, suggesting that SIL is a centrosomal protein.

We have previously shown that SIL is over expressed in multiple types of cancers and its expression correlates with the expression of mitotic checkpoint genes. To understand the role of SIL in cancer, we have constructed an inducible RNAi system targeting SIL in colon cancer cell line. Knockdown of SIL blocks mitotic entry and causes apoptosis of these colon cancer cells ***in vitro*** and ***in vivo***. The SIL knockdown related death phenotype was also observed in a variety of cancer cells representing the most common types of human cancer (cervical, lung, breast, prostate, gliomas and renal cancer) using anti-SIL siRNA oligonucleotides. This lethal phenotype can be rescued by the murine SIL, showing specificity of the siRNA effect. SIL is not necessary, however, for survival of normal proliferating cells as there are mouse embryonic stem cells and fibroblasts that are negative for the SIL gene.

SIL seems to be a novel regulator of mitotic entry, and thus crucial for the survival of cancer cells. Since cancer cells are extremely sensitive to anti-mitotic drugs, much effort is being invested in the development of drugs targeting mitotic regulators. Thus, targeting SIL (by either our shRNA or specific small inhibitors) may be a novel anti-cancer therapy. Since SIL may be a part of a novel mitotic and survival pathway, the combination of anti-SIL therapy with exiting mitotic drugs may improve anti-cancer treatment efficacy.

CURICULUM VITAE

Petra Knížetová, M.Sc., Ph.D.

Petra Knížetová is currently working as a postdoctoral fellow in the laboratory of Professor Jiří Bártek at the Institute of Cancer Biology of Danish Cancer Society in Copenhagen and also in the Laboratory of Genome Integrity of the Faculty of Medicine and Dentistry of Palacký University in Olomouc. Under the supervision of Dr. Alice Hlobilková and Professor Jiří Bártek she successfully defended her Ph.D. degree from Palacký University in Olomouc in 2008. The main focus of her Ph.D. was study of the molecular carcinogenesis of astroglial brain tumours and VEGF signalling. Petra was awarded by the Dean of the Faculty of Medicine and Dentistry for the best student research article published in 2007 and Sigma Young Scientist Award 2007. In 2008 she was awarded a Lundbeck Foundation fellowship. The focus of her research is cell cycle control and DNA damage response in brain tumours and tumour-derived cancer stem cells.

DNA damage response activation in human brain tumors

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Despite aggressive therapy comprising surgical resection, radiotherapy and chemotherapy, the prognosis for patients with glioblastoma multiforme (GBM) remains extremely poor. GBM is among the most malignant brain tumors with extensive areas of hypoxia, necrosis and robust angiogenesis. Hypoxia leads to increased oxidative stress, where the physiological function of reactive oxygen species (ROS) involves positive regulation of tumor angiogenesis. Formation of ROS leads to DNA damage and subsequent activation of DNA damage response (DDR) signalling pathways. Our preliminary data show DDR is massively activated in human tumors of astroglial origin, particularly in lower-grade astrocytomas (grade II). p53 mutated tumors (or areas within astrocytomas) are always accompanied by activated DDR but not ***vice versa***, which is consistent with the idea that DDR activation is a relatively early event creating an environment selective to the outgrowth of tumor cells with mutant p53. Indirect immunofloures-cence staining of a variety of GBM cell lines shows extensive γH2AX phosphorylation (Ser139) and foci formation, indicating high levels of spontaneous DNA damage, compared to normal human astrocytes (NHA). Cultivation of GBM cell lines at lower oxygen levels (3 %), together with data collected after ***in vitro*** induction of replicative stress allowed us to hypothesize that the high level of spontaneous DNA damage in astroglial tumors and cell lines is a consequence of the simultaneous effect of oxidative and replicative stress. Together, these observations point to oxidative and replicative stress as an enhancer of spontaneous DNA damage in GBM model cell lines ***in vitro***, giving them selective advantage in the expansion of highly aggressive clones.

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CURICULUM VITAE

Ondřej Slabý, M.Sc., Ph.D.

Ondřej Slabý gained the M.Sc. degree in biochemistry in 2005 and the Ph.D. degree in oncology in 2008, both at the Masaryk University in Brno. He is currently working at the Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno and University Cell Immunotherapy Center, Masaryk University, Brno. He is interested in diagnostic and predictive oncology, significance of microRNAs in cancer biology, microRNAs arrays, DNA microarrays, pharmacogenomics, nutrigenomics, biostatistics, and in colorectal cancer, breast cancer, kidney cancer and glioblastoma research. He is a member of European Association for Cancer Research, Czech Society of Oncology, Czech Society for Analytical Cytology, Czech Society for Biochemistry and Molecular Biology (member of FEBS) and member of Genetic Society of Gregor Mendel.

Significance of microRNAs in predictive oncology: experimental and clinical observations in colorectal cancer, breast cancer and glioblastoma

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MicroRNAs (miRNAs) are short (18–25 nucleotides in length) noncoding RNA molecules that post-transcriptionally regulate gene expression. Bioinformatic tools predict that miRNAs are able to regulate approximately one-third of mammalian genes, including a significant number of oncogenes, tumor suppressor genes and genes associated with the invasion, dissemination and chemoresistance of tumors. In our colorectal cancer study, we examined by real-time PCR expression levels of miR-21, miR-31, miR-143, miR-145 and let-7a-1 in bioptic samples of 35 colorectal cancer (CRC) patients including 5 cases of IUCC Stage I, 13 of Stage II, 8 of Stage III, 9 of Stage IV. For 6 cases of CRC samples adjacent non-tumor tissue was also analyzed. The expression levels of all tested miRNAs significantly differ in tumor and normal mucosa, miR-21 ($p = 0.0001$) and miR-31 ($p = 0.0006$) were up-regulated and miR-143 ($p = 0.013$) and miR-145 ($p = 0.018$) were down-regulated in tumors. miR-21 was also correlated with CRC stage. High expression of miR-21 was associated with lymph node positivity ($p = 0.025$), development of distant metastases ($p = 0.009$) and also with poor survival (long-rank $p = 0.043$) in CRC patients. Tumors with down-regulated miR-143 and miR-145 were larger and more frequent (not significantly) in proximal CRC. In the case of glioblastoma, we examined the expression levels of miR-21, miR-221, miR-222, miR-181a-c, miR-125b and miR-128a in 22 primary glioblastomas and six specimens of adult brain tissue by the Real-Time PCR method. In addition, we examined the methylation status of MGMT (O^6-methylguanine–DNA methyltransferase) promoter by methylation-specific RT-PCR, as this has previously been shown to be a predictive marker in glioblastomas. MGMT status and microRNA expression levels were tested for any association with the patient's response to concomitant chemoradiotherapy with temozolomide (RT/TMZ). MGMT methylation status did not correlate with age, gender, performance status, expression level of any microRNA analyzed and, most importantly, with response to RT/TMZ. Patients who responded to RT/TMZ tended to have lower expression levels of microRNA-181 family members than those with progressive disease. MiR-181b and miR-181c were significantly down-regulated in patients with a response to treatment ($p = 0.016$; $p = 0.047$, respectively) in comparison to patients with progressive disease. The "basal-like" mammary carcinomas occur in 15 % of breast cancer with higher frequency in patients with altered BRCA1. Typical invasive "basal-like" carcinoma is characterized by triple negative phenotype (ER, PR and HER2 negative) and higher frequency of mutations in tumor suppressor p53. It seems probable that alterations in the apoptotic p53-signaling pathways are one of the causal events in the pathogenesis of this molecular subtype of breast cancer. In our study of the "basal-like" mammary carcinoma we focused on the significance of miR-34a-c family known to be under direct transcriptional control of p53. The results of this study will be part of our presentation.

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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.

Alterations in cancer-causing lipids induced by Epstein-Barr virus in lymphoma

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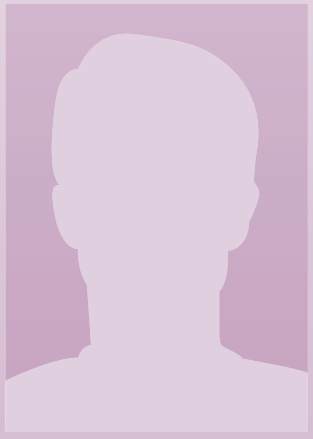
Oncogenic viruses are implicated in the pathogenesis of a number of different types of cancer. For example, the Epstein-Barr virus (EBV) is associated with the development of several types of lymphoma which include Hodgkin's lymphoma. When attempting to define the contribution of this oncogenic virus to the development of lymphoma, we have identified important cellular events induced by infection which not only provide a better understanding of the mechanism of cancer development in general, but which also suggest that alternative therapies that target these pathways could reverse the malignant character of these tumours. Of particular interest is the observation that EBV induces hitherto unrecognised effects on cellular physiology, which include the activation of lipid signalling. This presentation will reveal how EBV modifies the generation of the small bioactive lipids, sphingosine-1-phosphate and lysophosphatidic acid, how they contribute to lymphoma development, and how they might be targeted therapeutically. It will also consider how the regulation of these lipids may be controlled by cellular and viral microRNAs.

Petr Zdílna, M.Sc.

Petr Zdílna is currently in the position of the Guarantee for the field of Accreditation of medical laboratories and Lead Assessor for testing laboratories in the chemical and medical field of the Czech Accreditation Institute. He gained his master degree in Polymer Chemistry at the Faculty of Chemical Technology of the University of Pardubice in 1997. In 2006, he worked as a Quality Manager at the Czech Society for Quality. He is also an active member in the European co-operation for Accreditation in the field of medical laboratories and Trained Assessor of the World Anti-doping Agency. He has four years extensive experience in the fields of conformity assessment and accreditation for medical and testing laboratories. He organised courses on standards both in the Czech Republic and Croatia: ISO 15189:2007, ISO/IEC 17025:2005, ISO/IEC 19011:2002.

CURICULUM VITAE



Accreditation of medical laboratories – contribution to a quality medical care for a patient

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Accreditation is a procedure by which an accreditation body gives formal recognition that a laboratory is competent to carry out specific tasks, according to a specific standard. In the case of medical laboratories it is a standard ČSN EN ISO 15189:2007, Medical laboratories – Particular requirements for quality and competence.

Medical laboratory services are essential for patient care and therefore have to be available to meet the needs of all patients and the clinical personnel responsible for the care of those patients. Such services include arrangements for requisition, patient preparation, patient identification, collection of samples, transportation, storage, processing and examination of clinical samples, together with subsequent validation, interpretation, reporting and advice, in addition to the considerations of safety and ethics in medical laboratory work.

All accredited laboratories are characterised by professional competence, and, independent and impartial approaches to providing medical laboratory services, functional and implemented quality management system, including internal and external quality control of examination results.

Huntington's disease and postural stability impairment

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Huntington disease (HD), a hereditary neurodegenerative disease, is characterized by a triad of motor, cognitive, and psychiatric symptoms. Chorea is the major motor sign of HD. Choreic movements are continuously present and cannot be voluntarily suppressed by the patient. Posture is designed to maintain support against the force of gravity. The well-balanced activity of agonist and antagonist muscle groups stabilizes the given position of the body and its parts. Two information sources monitor the changes in body position: the head and the feet. The feet's pressure receptors monitor changes in pressure distribution. The head has specialized vestibular and visual systems. Posture maintenance therefore presents an outstanding example of the somatosensory and motor system integration. During our preliminary study the HD patients' postural stability will be measured usinga NeuroCom Balance machine. NeuroCom International is a specialised computerized tool for the assessment and rehabilitation of patients with balance and mobility disorders. Appropriate protocols were chosen from a broad spectrum. We emphasised visual and proprioceptive cues in the testing. The results are expected to show the pattern and abnormality of the postural balance in HD patients´ control according to the role of the basal ganglia in different aspects of postural control. The data could be used to prevent the fall of HD patient.

Estrogen receptor beta signalling is potentiated by coactivators p300 and CBP in PC3 prostate cancer cells

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Introduction: It has been demonstrated that increased expressions of p300 and CBP coactivators in prostate cancer contribute to androgen independent proliferation. The aim of this project was to investigate the relevance of the coactivators for estrogen receptor beta (ER beta) – mediated transcription in prostate cancer.

Materials and methods: Luciferase assays were performed in PC3 cells after transfection with ERE reporter and vectors affecting the expression of p300/CBP (expression vectors for both p300 and CBP, siRNAs against both p300 and CBP). Luciferase activity was assessed after stimulation of ER beta by genistein alone and in combination with ER antagonists ICI 182 780 or ICI 164 384.

Results: Stimulation of ER beta with 1000 nM genistein was further potentiated by acquired p300 or CBP expression in PC3 cells. The stimulation was abolished by 100 nM ICI 182 780 or 1000 nM ICI 164 384. Anti-estrogens exerted no agonistic effects on ER beta in the presence of either p300 or CBP. Knockdown of p300 and CBP by specific siRNAs abolished genistein stimulation of ER beta. The impact of p300/CBP coactivators on proliferation and apoptosis of PC3 cells after genistein treatment is currently being investigated. Other prostate cancer cell lines (DU145, LNCaP, LNCaP-abl, C4–2) were similarly tested. However, the luciferase signals were very low, probably due to low levels of the ER beta receptor.

Discussion: Coactivators p300 and CBP, which are both increased in advanced prostate cancers, potentiate signalling via ER beta. This might be important in tailored treatment with ER beta ligands.

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Detection of phenotypic changes in malignant melanoma. Immunohistochemical study of c-Myc, PDGF, bFGF and nestin expression in benign and malignant cutaneous melanocytic lesions

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Malignant melanomas are highly aggressive tumours characterised by a rapidly increasing incidence and unpredictable prognosis. Tumour progression is typified by aberrant proliferation of malignant cells associated with alterations to cell growth and cell regulation. We analyzed expression of growth factors PDGF, bFGF, c-Myc oncogene and nestin belonging to a newly identified intermediate filament associated with highly malignant neuroectodermal tumours in primary malignant skin melanomas as well as benign nevi. Formalin-fixed and

paraffin-embedded tissue sections of 11 nodular melanomas (NM), 12 superficial melanomas (SSM), 9 dysplastic and 10 benign nevi were examined by indirect immunohistochemistry using EnVision visualisation system. In groups of superficial and nodular melanomas an increased expressions of c-Myc and PDGF in comparison to benign and dysplastic nevi were observed. The results for nestin expression were interesting: the protein was slighly increased in SSM but strongly so in a group of NM. A higher FGF positivity of FGF was detected in NM compared to SSM and dysplastic nevi. This protein was also present in benign nevi and this could be caused by the gradual maturation of cells. We conclude that melanoma progression is characterized by aberrant angiogenesis associated with alteration of growth factors PDGF, bFGF, c-Myc oncogene and nestin protein. Nestin therefore may be a useful marker of malignant progression and grade of tumour differentiation.

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Triple negative breast cancer – cytogenetic change assessment of EGFR (HER1) and TOP2A genes with clinical and histopathological data analysis

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Introduction: Triple negative breast cancer (TNBC) – negative for hormonal receptors (HR) and HER2 receptor (HER2/neu, c-erbB2) – is a subgroup of 10–17 % of all breast cancer cases. Treatment approaches targeted at HR and HER2 are not eligible for these patients because of receptor negativity. The treatment in these cases remains surgery, radiation and chemotherapy. The results however could be improved with the support of biological treatment. Our study focuses on cytogenetic markers for TNBC which could be used as targets for treatment.

Materials and methods: A total of 59 patients diagnosed with nonmetastatic TNBC between 1998–2005 were studied. Histologically their tumors were invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), medulary carcinoma (MC) and ductal carcinoma in situ (DCIS). Formalin-fixed paraffin-embedded peroperative tissue samples were prepared for fluorescent ***in situ*** hybridization. We evaluated genes encoding for epithelial growth factor receptor (HER1, EGFR) and topoisomerase 2α (TOP2A) in relation to chromosomes 7 and 17 (CH7, CH17), respectively. Amplification (A) cutoff used was EGFR/CH7≥1.5, polysomy (P) cutoff used was CH7≥2.5. The data were analyzed using standard statistical methods.

Results: Median age was 57 years at diagnosis. We found EGFR amplification in 20.7 % (12/58) cases, high (EGFR/CH7≥2.5) amplification was found in 3 patients (25 %). CH7 polysomy occurred in 22.4 % (13/58) cases. Cytogenetic changes involving EGFR amplification and CH7 polysomy were found in 43.1 % (25/58) patients. One case (1.7 %; 1/58) displayed rare EGFR deletion together with TOP2A amplification. Only tumors with nonamplified EGFR carried TOP2A amplifications. These were present in 6 patients (12.8 %; 6/47), high TOP2A amplification (TOP2A/CH17 ≥ 2.5) in 1 patient (16.7 %). TOP2A deletion was identified in 3.4 % (2/59) of all cases. TOP2A/CH17 ratio showed a trend toward asociation with higher HER2 gene copy number (p=0.0883). There was a significant correlation between EGFR amplification and higher grade (G) and negative axillary lymph nodes (p=0.0179, resp. p=0.0086). EGFR polysomic patients tended to be older (p=0.0741). There was a significant relation between MC and young age at diagnosis (p= 0.0069). Lower expression of bcl-2 correlated with higher EGFR gene copy number (p= 0.0194).

Conclusion: Higher G was found in EGFR amplified tissues. In contrast, relation to axillary node involvement revealed an association between N0 and EGFR amplification. Increased EGFR gene copies might therefore enhance tumor proliferation without supporting metastasis. Survival analyses showed no significant differences in patient subgroups. To confirm the outcome in the CMF-treated group and to assess more precisely the impact of radiation we need to increase the patient sample. TOP2A amplifications were found in low frequency in EGFR nonamplified cases. TOP2A is a marker predicting probability of better outcome after anthracycline based treatment. Based on EGFR evaluation we identified patients who might be expected to benefit from EGFR targeted therapy.

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HPV16 – transformed cells influence the phenotype of normal keratinocytes to stem cell-like character

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Epidermal stem cells play a key role in epidermal self-renewal and healing processes. Hair follicles or the epidermal basal layer are the assumed sites for epidermal stem cells. As well as stem cells in normal tissue, cancer stem cells also require a special

microenvironment to support their survival and growth. This microenvironment is represented by the tumor stroma which can also be formed by the epithelial-mesenchymal transition from tumor epithelial cells.

In an attempt to create a system capable of testing some aspects of the mesenchymal cell-keratinocyte interactions, we studied the effects of the fibroblastoid murine TC-1 cells on the phenotype of normal human interfollicular and hair follicle keratinocytes. TC-1 cells were prepared by the introduction of human papillomavirus type 16 (HPV16) genes E6 and E7 to murine embryonic lung epithelial cells. We used them as a model of stromal cells formed by the epithelial-mesenchymal transition of cells from HPV-induced squamous cell carcinoma. 3T3 murine embryonic fibroblasts, routinely used for keratinocyte cultivation, were used as control cells. Both 3T3 and TC1 cells were co-cultured with normal human follicular and intefollicular keratinocytes. To characterize the phenotype of co-cultured keratinocytes, we detected a panel of keratins, nucleostemin, vimentin. When compared with 3T3 fibroblasts, TC-1 cells strongly influenced the size of keratinocytes, the shape of their colonies and their expression profile. In co-cultured keratinocytes they induced expression of keratins 8 and 19 as well as vimentin.

In conclusion, TC-1 cells exhibited marked biological activity and they influenced the behaviour of the normal human follicular and intefollicular keratinocytes equally. This indicates that stromal cells play an important role in tumour progression and spreading.

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Extending Her-2/neu assessment using a larger spectrum of cytogenetic probes and monoclonal antibodies

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Breast carcinoma is a major cause of cancer mortality in women. In an estimated 25 to 30 % of breast tumors there are amplifications/overexpression of the Her-2/neu (c-erbB-2) gene and these changes are markers of poor prognosis. For these patients selective and genetically targeted treatments are currently being designed using the humanized monoclonal antibody, transtuzumab (Herceptin). To date, immunohistochemistry (IHC) is the most used method for Her-2/neu assessment and IHC positive cases are verified by fluorescence in situ hybridization (FISH). It is necessary to optimise the quality of treatment for Herceptin to be effective. To compare the results using different agents, we carried out sample treatment using several polyclonal antibodies designed by the company Exbio in conjunction with our laboratory. The results were compared with the immunohistochemical findings for antibodies commonly used in Her-2/neu diagnostics (HercepTect; DakoCytomation). In the same way we proceeded with the FISH assay using the probe, made by labeling plasmid vs. commercial probe (PathVysion Vysis). The three newly designed monoclonal antibodies produced by the Exbio company showed the same results as the HercepTest, immunohistochemistry detection system of Dako Cytomation. The sample of 524 patients was assessed by both plasmid and commercial probes. A high correspondence was found in the assessment of amplification of the Her-2/neu gene (divergence in 2.6 % of samples; $p<0.001$). Based on comparison of different probes and antibodies it is concluded that for Her-2/neu treatment, it is possible to use diverse detection systems but it is necessary to confirm their reliability with commercially verified systems.

Acknowledgement: The project was supported by grants *MSM6198959216, LC07017* and grants of International Grant Agency of Ministry of Health care in *CR NR/9076* and Ministry of industry and Commerce Department *MPO 56110041*. Special thanks go to all cooperating departments, and health insurance companies.

Testing and control of the postural stability of the human body

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Background: Posture and stability control is a process of maintaining the balance and position of the body and its parts in a constantly changing environment. The purpose of our study is to determine which morphological indicators influence postural stability and its control. Methods: We used NeuroCom Balance-12 protocols, anthropometric measurements – 32 values, an anamnestic questionnaire and 41 individuals took part in it. For this project we analyzed three chosen protocols: Sensory Organization Test-Equilibrium, Adaptation Test and Rhythmic Weight Shift.

Results: Very interesting statistically significant coherences were found during analysis of test Rhythmic Weight Shift, Sensory Organization Test-Equilibrium on level $p<0.01$, $p<0.05$.

Conclusions: This study shows us a direction and we may consider it a convenient base for more specific continuation in this problematic area.

Minimal residual disease as a new prognostic factor in pancreatic carcinoma patients

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Minimal residual disease (MRD) in pancreatic carcinoma patients implies the presence of circulating tumor cells (CTCs) in patients who have undergone curative surgery but who remain symptom-free The CTCs can be the precursor of micrometastasis and relapse. The presence of MRD can be identified in patients with poor prognosis who have minimal benefit from surgery.

The human telomerase (hTERT) can be a potential marker for MRD detection in pancreatic carcinoma patients. Circulating/disseminated tumor cells in the peripheral blood and bone marrow were detected using real-time RT-PCR for hTERT. The aim of this study was validation of hTERT as a diagnostic and prognostic marker for MRD detection in pancreatic carcinoma patients.

A total of 60 patients were included in the study from 2007 to 2008. All patients underwent surgery for pancreatic carcinoma. The expression of hTERT, EGFR1 (receptor for epidermal growth factor 1) and CEA (carcinoembryonic antigen) was determined in samples of peripheral and portal blood, bone marrow, peritoneal lavage and tumor tissue. We also tested the hTERT expression in 52 blood samples of healthy blood donors.

We foundthat hTERT is not a useful marker for MRD detection in pancreatic carcinoma patients because of its low tumor expression and high peripheral blood and bone marrow expression in the control group. This is probably due to the high hTERT expression in circulating blood stem and embryonic cells. We will present the relationship between hTERT expression and other clinical and pathological parameters of the disease.

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Monitoring of metallothionein level in cancer tissue cultures exposed to cadmium

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Introduction: The association of heavy metal exposure and the risk of cancer is known. Arsenic, cadmium, chromium and nickel have been formally classified as carcinogens. Heavy metals are thought to promote cancer by a number of common mechanisms, e.g. formation of free radicals, influence of cell control via alteringgene regulation etc. Heavy metals are also strong co-carcinogens, promoting synergistic effects in the presence of other carcinogenic agents. Metallothioneins (MT) are low-molecular mass proteins capable of binding to heavy metals. They are involved in transporting and/or detoxifying metal ions. It is also known that MTs contribute to the development of resistance to heavy metal-based cytostatics. Apart from heavy metal homeostasis and detoxification, their regulatory functions in many cell processes, e.g. apoptosis inhibition, transcription and enzyme regulation are known. It is assumed that heavy metal-induced metallothioneins serve as a host-derived factor in malignant diseases and closely relate to metastasis.

Aims: the aim of this research was to determine metallothionein levels in human fibroblast cell cultures (derived from an individual with cancer) exposed to cadmium.

Materials and methods: BR-175 (tumour) and BR-142 (control) human fibroblast cell cultures were exposed to CdNO3 in concentrations 0, 1, 5 and 10 μM. In intervals of 0, 1, 3, 6, 12 and 24 hours the cells were harvested. MT level was determined by using Western blotting, Dot-Immunobinding Assay (DIA) and Differential-Pulse Voltammetry-Brdicka reaction (DPV).

Results: MT levels determined in studied cell lines were within the range from 180 to 48.900 μg/mg of the soluble proteins. We observed a different behaviour of tumour cells in response to heavy metal exposure compared to controls. In control cells (0 μM Cd2+) MT levels were 2–3 mg/g of soluble proteins and did not change over time compared to treated cells, in which we observed a marked enhancement of MT level even after one hour long treatment. Compared to controls (BR 142) the MT level was three-times greater in tumour cells (BR 175) at the beginning of the experiment. The increase in MT content in BR 142 cells exhibited a linear trend over time, while for BR 175 cells MT induction was faster, but with prolonged exposure, MT content in tumour cell was below 75 % compared to control cells in relation to Cd concentration.

Conclusions: The increase in MT synthesis and different kinetics of MT induction after cadmium exposure in tumour cells in comparison to controls, found by analytical methods used, indicates a possible role of MT in carcinogenesis and metastasis

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Collagen volume fraction, CTGF and TGF-beta expression in patients with end-stage heart failure and atrial fibrillation

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Atrial fibrillation is a common disorder and its relation to myocardial fibrosis is currently under investigation. The aim of our study was to evaluate the level of fibrosis manifested as collagen volume fraction (CVF), together with the immunohistochemical detection of transforming growth factor-beta (TGF-beta) and connective tissue growth factor (CTGF) in patients suffering from end stage heart failure with or without atrial fibrillation. TGF-beta and CTGF are involved in fibrotic processes in various organs and their role in myocardial fibrosis has been proposed, as well. The study was performed in two groups of patients – one group with atrial fibrillation and one group with sinus rhythm. In both of these groups were patients indicated for heart transplantation, who were diagnosed with ischemic heart disease or cardiomyopathy. The atrial and the ventricular samples were fixed with paraformaldehyde and embedded into paraffin. Histochemical detection of collagen for quantification of collagen volume fraction (CVF) was performed using Sirius red staining. Immunohistochemical detection of TGF-beta and CTGF was performed using three-step immunoperoxidase reaction. Image analysis software was used for the quantitation of CVF and immunohistochemical reaction product. When the CVF was quantified in the right atrial myocardium and in the left ventricular myocardium, there was nosignificant difference in CVF between the atrial fibrillation and the sinus rhythm group. CTGF was detected in all samples obtained from both groups of patients. It was constantly expressed in cardiomyocytes. TGF-beta was also detected in all samples. However, its expression in atria was not as widespread as that of CTGF. TGF-beta was found mainly in the endothelial lining of atria and in the capillary endothelium. Cardiomyocytes expressed TGF-beta in some of the samples; mainly in regions adjacent to endocardium. TGF-beta was more abundant in ventricles than in atria. In summary, we found that in patients with end stage heart failure there was no difference in CVF between groups of patients with atrial fibrillation and with sinus rhythm. In addition, we detected both CTGF and TGF-beta in atria and ventricles of patients from these two groups.

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Biological activity of stromal fibroblasts in basal cell carcinoma

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Introduction: The role of stem cells in cancer development and spreading has been established. As well asnormal tissue stem cells, cancer stem cells also require a special microenvironment supporting their growth. Stromal fibroblasts a.k.a. cancer-associated fibroblasts are important components of the tumor stroma. The mutual interaction between cancer cells and surrounding stromal fibroblasts is mediated through direct cell-cell contacts and paracrine signals. Basal cell carcinoma is the most common form of cancer of all with about a million new cases estimated in the U.S. each year. This kind of tumor can be locally aggressive, damaging the surrounding skin and sometimes invading bone and cartilage. However the rate of metastases is low.

Aim: This study reports on marker profiling changes of normal keratinocytes under the influence of mesenchymal cells derived from basal cell carcinoma in vitro, and especially on the induction of stem-cell-like features in affected epithelia. Material and methods: Weperformed a genomic search for up/down-regulated genes for growth factors in stromal fibroblasts. We compared the phenotype of human tumors with an in vitro model where growth and phenotypic pattern of normal human keratinocytes were monitored under the influence of various types of fibroblasts including those prepared from stroma of basal cell carcinomas.

Results: We visualized a panel of keratins, endogenous lectins and binding sites for endogenous lectins, and markers of stem cells and growth factors which were upregulated. The observed epithelial cells in coculture subsequently expressed tumor markers as well as some putative stem cell markers.

Conclusion: Epithelial-mesenchymal interactions are of crucial importance in the course of malignant tumors, where stroma influence the behavior of the transformed epithelial cells. This biological activity can be attributed to the produc-

tion of soluble paracrine factors and can be blocked by proper monoclonal antibodies. This observation can be of a great future therapeutical importance.

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Polysomy of chromosome 17 in breast cancer patients and its impact to diagnosis and treatment

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Amplification and/or overexpression of the Her-2/neu gene has been reported in approximately 20 % of patients with breast cancer. These changes are associated with poor prognosis and higher tumor aggressiveness. Humanized monoclonal antibody trastuzumab (Herceptin, Genentech; anti-p185Her2) was developed for treatment of breast cancer patients with ratio Her-2/neu: chromosome 17 (CH17) copy number ≥ 2.2 and/or immunohistochemically positive 3+. Roughly 5–7 % of breast cancer patients are not indicated for trastuzumab therapy because they do not match Her-2/neu: CH17 criteria due to CH17 polysomy. The efficacy of trastuzumab in polysomic cases has not yet been confirmed. Apart from Her-2/neu, the most frequently altered genes in breast cancer are TOP2A (topoisomerase 2α), C-MYC and CCND1 (cyclin D1). We focused on the status of genes C-MYC and CCND1 and corresponding chromosomes 8 and 11 for two reasons:: amplification of C-MYC gene has been described as a positive predictor for 5-fluorouracil therapy in colon cancer (for breast cancer the comparable study has not been published yet) and amplification of CCND1 gene is considered to be a negative predictive marker for tamoxifen therapy in patients with early stagebreast cancer and positive hormonal receptors. These numerical changes can be prognostically important as well as useful in predictingof tailored therapy.

For the pilot study, we chose 280 patients: 112 (40 %) with confirmed chromosome 17 polysomy (CH17 copy number ≥ 2.5) and 168 (60 %) with diploid status. Amplification of C-MYC, resp. CCND1 was determined in 48.2 % (54/112), resp. 47.3 % (53/112) cases with CH17 polysomy vs. 10.7 % (18/168), resp. 23.2 % (39/168) without CH17 polysomy. Extra copies of chromosome 8 and 11 ≥ 2.5) were found in 34.8 % (39/112) and 23.2 % (26/112) cases with CH17 polysomy vs. 2.4 % (4/168) and 4.2 % (7/168) without CH17 polysomy.

Our data demonstrate more frequent genetic alterations in CH17 polysomic tumors. Their clinical relevance is being analyzed.

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Induction of G_1-phase cell cycle arrest and apoptosis in human breast and prostate cancer cells by natural brassinosteroids

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The study of plant-derived compounds with effect at the molecular level has become an important approach in the selection of new agents with antitumour activity in humans. Brassinosteroids (BRs), polyhydroxylated sterol derivatives with close structural similarity to animal and insect steroid hormones are plant growth regulators representing a group of newly-discovered agents with relatively wide-ranging effects in plants. Based on the structural motifs, one putative explanation for their strongly cytotoxic effect is their binding to steroid receptors. In this study, we characterized the effect of natural BRs (28-homocastasterone and 24-epibrassinolide) on cell growth and apoptosis in human hormone-sensitive and hormone-insensitive breast and prostate carcinoma cells. The aim was to identify the processes associated with apoptosis induction and hormone-independent status in these cancer cells. The agents inhibited cell growth in all cell lines and resulted in alterations in the cell cycle progression and levels of cell cycle related proteins. Using flow cytometry, we found that BRs can disturb cell cycling in breast and prostate cancer cells. The results showed that treatment with either 28-homocastasterone or 24-epibrassinolide induced blocks in the G_1 phase of the cell cycle in the MCF-7, MDA-MB-468 and LNCaP cell lines, associated with decreased expression of cyclin D_1 and pRb phosphorylation and induction of cyclin kinase inhibitors $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. In hormone-dependent cells, BR treatment led to induction of apoptosis and resulted in alterations of localization and expression of the steroid hormone receptors (ER-α, ER-β, AR). Based on our data, the effect of BRs can be compared to the effect of antagonists to steroid hormone receptors. Our results suggest that the tested BRs are

promising leads in the development of a new generation of potential anticancer drugs.

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N-cadherin as a potential predictor of metastases to central nervous system in NSCLC

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We have been screening genes encoding transmembrane/secretory proteins that are up-regulated in lung cancers and their brain metastasis, with cDNA microarrays and tumor cells purified by laser-capture microdissection. To verify the predictive value of these gene products from the point of view of brain metastases, we have been performing tumor tissue microarray analysis of clinical lung cancer materials.

Preliminary results: RNA from 28 primary NSCLC, 8 samples of normal lung that were taken from the same patients, seven independent brain metastasis and one specimen of normal brain (commercial RNA that is derived from a pool of normal brains) were hybridized to Affymetrix U95 Chips (containing 12625 probe sets). Of the 28 primary NSCLC cases 6 developed brain metastases and 7 extra-cranial metastases during a minimal follow-up of three years. Limited space precludes a detailed description of the analysis. The microarray results were confirmed by qRT-PCR of selected genes. ADAM8 and N-cadherin are according to these analysed genes associated with brain metastasis in NSCLC patients screened above. After verification on symplex from independent NSCLC patient files, collected both in Israel and the Czech Republic, we found a significant association between N-cadherin expression and brain metastasis (p= 0.008).

Conclusion: N-cadherin is a very strong predictor of brain metastasis in NSCLC patients.

Her-2/neu (c-erbB-2) gene evaluation in breast cancer; samples with ambiguous FISH results

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Amplification and/or overexpression of Her-2/neu indicates unfavourable prognosis in breast cancer patients. Genetically tailored therapy with trastuzumab (Herceptin®) shows great benefit in these individuals. Her-2/neu status is crucial for effective indication of trastuzumab treatment. In the Czech Republic, Her-2/neu status is evaluated by FISH and immunohistochemistry at six Reference Centres (Laboratories of Predictive Medicine). However, some samples cannot be evaluated by FISH due to DNA degradation and those tumors are evaluated by quantitative real-time PCR for Her-2/neu gene. Moreover, due to polysomy of chromosome 17 (CH17) at least 5–7 % of patients are not indicated for trastuzumab treatment as they do not fulfill the criteria of Her-2/neu: CH17 ratio >2.2. The efficacy of trastuzumab in polysomic patients has not yet been confirmed. In the Czech Republic, such patients are indicated for trastuzumab treatment only when they are immunohistochemically positive (3+).

More than 2818 breast cancer samples were evaluated in our institution over a period of seven years by fluorescence in situ hybridisation assay. Overall, 148 (5.25 %) cases failed to be concluded by FISH. Absence of cancer cells and/or DNA degradation in the tumor biopsy were the major causes of the failure. For such cases, quantitative real-time PCR comparing the Her-2/neu gene status to reference genes dck, gcs1 and epn2 was established. Among 148 cases which failed using FISH technique, we have successfully investigated 77 patient samples by qRT-PCR achieving unambiguous results in 78 % (60/77). In 13.8 % (368/2670) cases, polysomy of CH17 was detected by centromeric probe (CEP17). Using locus specific probe mapping of 17p11.2 region, we found that 57 % (212/368) of such a "polysomic cases" contain only 2 copies of CH17. We found in some instances, the hybridization of centromeric probe was not specific enough and the probe also hybridized to centromeres of other chromosomes or the cells showed

complex cytogenetic rearrangements which misrepresent the number of CH17.

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Histone deacetylase inhibitors affect androgen receptor activity through corepressors

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Background: Histone acetylases (HATs) modulate the activity of diverse proteins including the androgen receptor which is a member of the nuclear receptor family. Deacetylation is mediated by class I histone deacetylases (HDACs) which are primarily nuclear while class II HDACs are shuttled between the nucleus and cytoplasm. The histone deacetylase inhibitors (HDACI) sodium butyrate (NaB) and trichostatin A (TSA) have shown significant antiproliferative and apoptotic effects on various cancer cells, including prostate cancer. For this reason, they are potential therapeutic agents in the treatment of prostate cancer even if the mechanism of their action remains undefined. On the other hand, the transcriptional activity of the androgen receptor (AR) is also effected by coregulatory proteins known as coactivators and corepressors. The silencing mediator for retinoid and thyroid hormone receptors (SMRT) and the nuclear corepressor (N-Cor) are linked to HDAC3 and they interact with the AR in a transcription repression manner. Overall, new approaches to silence the AR, may have therapeutic importance in the treatment of prostate cancer: repressive pathways appear to be important regulators of cancerogenesis.

Experimental procedures: We treated androgen-dependent prostate cancer cell line (LNCaP) and androgen-independent prostate cancer cell lines (C4–2, DU145 and PC3) with sodium butyrate (NaB) and trichostatin A (TSA). The effects of NaB and TSA on AR, HDAC2 and HDAC3 (class I HDACs) were assessed in whole cell extracts of these cell lines by Western blot analyses. The AR immunoprecipitates were tested with anti-SMRT monoclonal antibody by immunoblotting. The chromatin extracts incubated with SMRT (SMRTe – 1542/H7) were used for chromatin immunoprecipitation assay (ChIP). Immunoprecipitated DNA was analysed by PCRs using primers specific for AR and prostate specific antigen (PSA). Analysis of siRNAs was performed by antibodies against HDAC3, N-Cor and SMRT.

Results and conclusions: NaB and TSA suppressed AR expression in LNCaP and C4–2 cell lines containing functional AR. Immunoprecipitation analysis revealed in C4–2 cells a higher formation of the AR – SMRT complex in samples affected by NaB compared to control samples. On the other hand, the NaB effect was not relevant in the LNCaP cell line. Further, we investigated the influence of co-repressors SMRT, N-Cor and HDAC3 on AR expression in LNCaP and PC3 cell lines affected by NaB. The results suggest that the co-repressor SMRT significantly contributes to the NaB-mediated AR suppression. In addition, all four cancer cell lines were tested for expression of HDAC2 and HDAC3. After NaB and TSA treatment we detected their decreased expression only in the androgen-independent DU145 cell line.

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Our experiences with molecular diagnosis of Ewing´s sarcoma in paraffin-embedded tissue

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Introduction: Ewing´s sarcoma is relatively uncommon tumor representing 6–8 percent of malignant bone tumors with variable morphology. No specific immunohistochemical marker of this tumor exists to date. Cytogenetically, Ewing´s sarcomas are characterized by a specific reciprocal chromosomal translocation t (11;22) (q24;q12). The presence of this chromosomal translocation has been detected in approximately 85 percent of cases. The translocation results in the fusion of EWS gene (Ewing sarcoma breakpoint region 1 gene) from chromosome 22 to FLI1 gene (Friend leukemia virus integration 1 gene) at 11q24 which is a member of ETS (v-ets erythroblastosis virus E26 oncogene homolog) family of transcription factors. Moreover, another chromosomal translocation t (11;22) (q22;q12) has been found in 10–15 percent of cases, which results in the expression of EWS-ERG fusion transcript. In 1 % or less cases t (7;22), t (17;22), and t (2;22) translocations and inv (22) have been described. The above mentioned secondary chromosomal aberration resulted in fusion between EWS gene and one of the ETS superfamily: Ets variant gene 1 (ETV1), Ets variant gene 4 (E1AF), fifth Ewing variant gene (FEV), and zinc finger sarcoma gene (ZSG), respectively.

Aim: In this study, we performed a comparison of two molecular diagnostic strategies, namely RT-PCR and FISH, in fresh, frozen and formalin-fixed paraffin-embedded tissues.

Results and discussion: We found that all PCR-negative Ewing´s sarcoma cases which we tested gave positive FISH results. We found that the storage time of paraffin block, even for as long as 10 years, did not affect the quality of the FISH results. We detected chromosomal aberrations in paraffin-embedded tissues stored as long as 31 years. However, the sensitivity of RT-PCR analysis in paraffin-embedded tissue could be increased if a smaller-sized amplicon (approximately

100 bp) was used for PCR because of degradation of RNAs in paraffin-embedded tissue.

Conclusion: We conclude that FISH is a more sensitive technique than RT-PCR for the diagnosis of Ewing´s sarcoma family in formalin-fixed paraffin-embedded tissue, although RT-PCR analysis provides additional information regarding fusion transcript subtype, which may play a role in prediction of prognosis. In conclusion, molecular pathology techniques, using reverse transcription-polymerase chain reaction (RT-PCR) and/or fluorescence in situ hybridization (FISH) are valuable diagnostic tools for the evaluation of undifferentiated small round-cell tumors like Ewing´s sarcoma. Finally, we assume that the diagnosis and prognosis of the Ewing´s sarcoma family can benefit from molecular testing with both techniques.

Molecular genetic investigations of breast carcinoma

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Aims: Breast cancer is a heterogeneous disease resulting from the acquisition of probably multiple static mutations which, in combination, define the characteristics of the tumour. Methods of molecular genetics have become an essential part of diagnostic and prognostic methods in oncology of solid tumours. Trastuzumab (Herceptin) is the cornerstone of molecular biology treatment of breast cancer women with HER2/NEU gene amplification. Accurate assessment of HER2/NEU status is therefore critical for identifying patients who may benefit from trastuzumab-based therapy. HER2/NEU gene amplification and overexpression were evaluated by IHC and FISH.

Methods: Breast carcinomas were investigated by imunohistochemistry (IHC), fluorescence in situ hybridisation (FISH) and comparative genomic hybridisation (CGH).

Results: IHC was performed with DAKO Cytomation HercepTest and FISH with PathVysion Probe Kit. A total of 213 (10,56%) of 2017 patients were positive by IHC. These positive IHC results (2+, 3+) were verified by FISH. HER2/NEU gene amplification was confirmed in 138 cases (70%). CGH was performed at 29 cases to screen breast tumors for copy number changes. We obtained successful results in 26 of 29 cases. 22 cases were positive for copy number changes, 4 cases were negative for copy number changes probably due to analysis of non tumour tissue. The most frequent DNA sequence copy number changes were gains of 1q (14 cases), 8q (13 cases) and loss of 1p (14 cases), 16q (12 cases).

Conclusions: Our results indicate that especially borderline results of IHC (2+) should be interpreted with caution using both IHC and FISH with standardised metodology. The prevalence of the most common copy number aberrations detected by CGH was roughly similar to that reported in the literature.

An investigation of the contribution of LMP1 to the differentiation of germinal centre B cells

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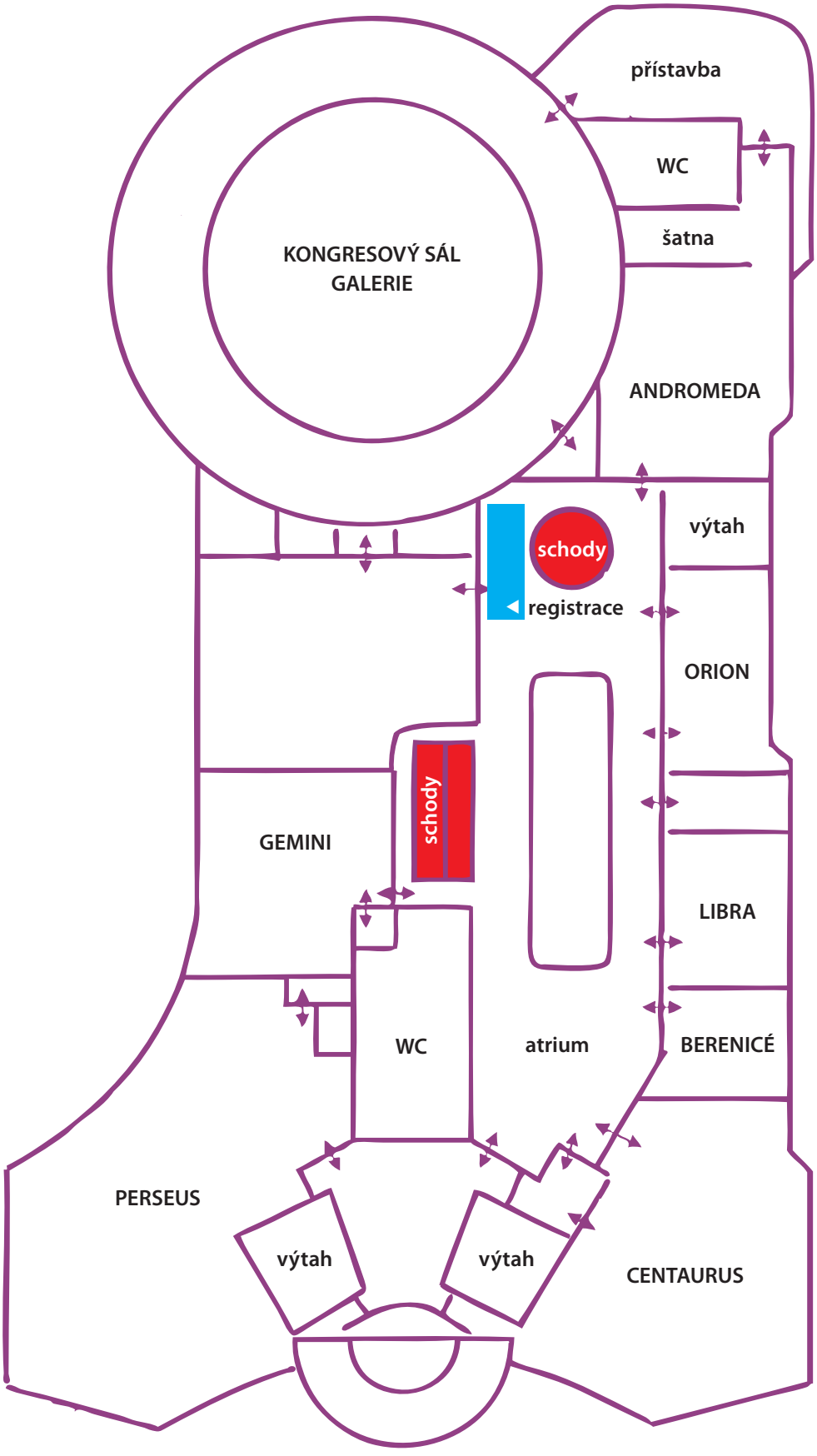
The latent membrane protein 1 (LMP1) of the Epstein Barr virus (EBV) is believed to be important for the transformation of germinal centre (GC) B cells which may contribute to the development of EBV associated lymphomas, such as Hodgkin´s lymphoma. When expressed in GC B cells, LMP1 induces transcriptional changes that show a striking overlap with those induced in the same cells by the B lymphocyte-induced maturation protein-1 (BLIMP1), a key transcription factor that is essential for plasma cell differentiation. For example, LMP1 and BLIMP1 coordinately regulate 230 genes, including the B cell differentiation-associated transcription factors, BCL6, PAX5 and IRF4. However, this mimicry is only partial, as unlike LMP1, BLIMP1 does not up-regulate the anti-apoptotic gene BCL2A1 or the chemokine CCL22. In addition, a proposed function of LMP1 is the up-regulation of the inhibitor of DNA binding 2 (ID2), which can inhibit PAX5 mediated maintenance of B cell identity. However, ID2 is not regulated by BLIMP1. Furthermore, the similarity between LMP1 and BLIMP1 targets is not a simple consequence of BLIMP1 up-regulation by LMP1. Additionally, LMP1 down-regulates BLIMP1 in GC B cells. Our data suggest that while LMP1-expressing cells are driven into the post-GC stages of B cell differentiation, they fail to induce BLIMP1 and so are prevented from completing plasma cell differentiation. Given that plasma cell differentiation is associated with induction of the virus replicative cycle, then the LMP1-mediated disruption of this process could facilitate viral persistence.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