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2nd Satellite Symposium & Workshop on Molecular Pathology

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DEAR READER

This Supplement to the **BIOMEDICAL PAPERS, Volume 150** (Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub), is devoted to the 33rd Congress of the Czech Society of Pathologists and the 2nd Satelite Symposium & Workshop on Molecular Pathology held at the Regional Centre Olomouc & Faculty of Medicine, Palacký University Olomouc, May 4–6, 2006.

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I hope that the content of this Supplement will not only represent a basic brochure useful to all participants of the Conference but also provide a lot of valuable new information on recent results of leading laboratories working in all the fields of pathology.

Vilím Šimánek
Co-Editor-In-Chief

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2nd Satellite Symposium & Workshop on Molecular Pathology

Olomouc, Czech Republic, May 4–6, 2006

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THE 100TH ANNIVERSARY OF THE HOSPITAL DEPARTMENT OF PATHOLOGY AND THE 60TH ANNIVERSARY OF THE INSTITUTE OF PATHOLOGY OF THE MEDICAL FACULTY, PALACKÝ UNIVERSITY IN OLOMOUC

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At this time, several important anniversaries are commemorated in relation to the Institute of Pathology of the Medical Faculty and the Department of Pathology of the Faculty Hospital in Olomouc. Firstly, it is 110 years since the completion of a New District Hospital in Nová Ulice in Olomouc in **1896.** Second, it is 100 years **since** the foundation of the Pathology Department and finally, it is the 60th anniversary of the Institute of Pathology of the Medical Faculty following the restitution of Palacký University in Olomouc on 21st February **1946**.

The above-mentioned **100th anniversary of the Pathology Department** is of special importance for the current institute. From the outset, the department undertook not only autopsy and diagnostic-histological work but also a wide range of other activities, particularly bacteriology, clinical chemistry, experimental work (animal tests mainly for infectology purposes), as well as scientific work including cooper0ation with clinicians. During its long existence, the department was influenced by the work of generations of its leader and hundreds of employees of various levels and specialization. Gradually, their efforts led to the modern Institute of Pathology with the high standard of activities, research and teaching, an important part of the present-day Palacký University Medical Faculty and Faculty Hospital in Olomouc.

The **University Institute of Pathology** founded 60 years ago as part of the re-established University in Olomouc continued its more than 200-year-old **tradition of teaching** anatomy and later pathology at the old Olomouc University based at the Philosophical Faculty since 1778 and historical health care facilities – the **State Hospital** created by fusion of the old church hospitals (Hospitales Sanctu Spiriti) in 1785 and the **School of Medicine and Surgery** (1787–1875). Prominent anatomists and pathologists of that time include the state hospital prosector **G. Wezel** (1786–1788), **J. John** (1807–1853; a founder of the anatomical cabinet – "Theatrum anatomicum"), **R. Heschel** (1854–1855; an assistant to professor K. Rokitanský at University of Vienna; author of a pathology manual called "Compendium Pathologico-Anatomicum") and **Arthur Willigk** (1855–1881), originally an assistant-pathologist in Prague (1850–1855), later a professor of the School of Medicine and Surgery (1869) and a founder of an extensive collection of pathological-anatomical preparations "Museum pathologicum".

For a long time, the **School of Medicine and Surgery** (1787–1875) was a parallel part of the university-type **Lyceum** with leading personalities such as Professor **A. Willigk**, **Andreas Jeitteles-Justus Frey** – collections of pathological-anatomical preparations, **Filip Hartman** (1806–1811) – a manual of pathology used at that time in the Austro-Hungarian Monarchy, and **J. Mošner** – a manual of obstetrics.

It was at the University of Olomouc that the founder of classical genetics **J. G. Mendel** began his studies. (His work on experimental genetics was published in 1865 in the Brno Natural Science Society Journal).

The **University in Olomouc** is among the oldest in Central Europe and the second oldest, after Prague's Charles University in our country. Originally founded as a **Jesuit**

college by Bishop Vilém Prusinovský between 1566 and 1573, it was **turned into a university** with a papal seminary **Collegium Nordicum** in 1573. The foundation charter of Pope Gregory XIII and Emperor Maximilian II ("Great confirmation privilege") gave the university the right to confer degrees and the same position and rights as those of other European universities in Germany, France, Italy and Spain. The following stages of its history were characterized by periods of both prosperity and decline, interrupted teaching caused by the plague epidemic until 1715, and repeated abolition – the anti-Habsburg uprising of the Czech estates and the Jesuit exodus from Olomouc (1618–1620), the Thirty Year's War (1618-1648) and the 7-year Swedish military occupation of Olomouc. In 1782 the institution was reopened but only as a lower 3-year **"Lyceum"**. Soon after 1848 the **School of Medicine and Surgery** was established (named "K. und K. Medizin-Chirurgische Anstalt").

In 1827, the Austrian Emperor, Francis I promoted the "Lyceum" to a university ("**Caesaro-Regia Universitas Franciscea Olomucensis"**). However, after the 1848-1849 revolution was suppressed, the emperor's court was moved from Vienna to the Olomouc fort and the period of Bach's absolutism began. The university was **abolished** once again in 1860 and its **rector's historical insignia** were first transferred to Brno and later to the University in Innsbruck, Austria. Only the theological faculty remained in Olomouc until 1939 when all Czech high schools were closed during the German military occupation.

The health care facilities also underwent very complicated developments. Between 1785 and 1787, municipal hospitals were joined into one **state-administered hospital** with departments of surgery, internal medicine and obstetrics, and an orphanage. Since 1862, the hospital was administered by the country of Moravia as the **Moravian General Country Institutes** in Olomouc with a capacity of 184 beds and 20 children's beds. The hospital was located in the place of the present-day university buildings in Křížkovský street.

The new boom of the hospital facility dates back to 1892 (after the town fortifications were torn down in 1886) when the Moravian assembly decided to build a **new hospital** (1892–1896) in the new Olomouc area of Nová Ulice – Tabulový vrch. The **New Moravian General Country Institutes** were the basis of the later **Faculty Hospital**. Ten years later, in 1906, the **pathology department** was established, first headed by **Dr F. Berka** (1906–1920), later a professor at the Institute of Forensic Medicine of Masaryk University in Brno. Shortly after, between 1920 and 1922, the department was led by **Dr V. Neumann**, later a professor and the head at the Institute of Pathology of Masaryk University in Brno, and then by **Professor Dr J. Kabelík** (1922–1939). During World War II the head was **Dr J. Jung.**

After the university was renewed in 1946 and **named "Palacký University" –** according to a well-known Czech historian, politician and cultural worker František Palacký (1798–1876) – the Medical Faculty was founded. The Institute of Pathology was headed by **Professor Dr. F. Pavlica** (until 1948), **Doc. Dr K. Kučera** and **Prim. Dr Krajčiřík** (1948-1949), **Doc. Dr Č. Dvořáček** (1949–1958) – the establishment of the **Catheder of Pathology** of the Medical Faculty of Palacký University. In 1958–1960 the acting head was **Dr R. Koďousek.**

In 1960 the Institute of Pathology was moved to a new medical faculty building (the "Theoretical Institutes") and headed by **Doc. Dr V. Valach** (1960–1974). In 1964 the **Research Laboratory of Cyto- and Histochemistry** was established (headed by Doc. Dr R. Koďousek, 1964–1974), closely cooperating with the Czechoslovak Society of Cyto- and Histochemistry, a part of the Czechoslovak Academy of Sciences.

Between 1974 and 1994, when the institute was led by **Professor Dr R. Koďousek**, it was organized into specialized divisions including an integrated biopsy service (according to a Swedish model). Simultaneously, efforts to introduce systemic classification and computerization of biopsy findings were initiated (Dr P. Vácha). The Section of Clinical Pathology and Cytology was established in 1976 as part of the Society of Czechoslovak Pathologists. Diagnostic and scientific research activities of the institute were supported by the division of electron microscopy (Professor Dr J. Dušek), cytology diagnostics, hepatopathology (Dr V. Jezdinská), neuropathology (Dr Z. Skatula, Dr O. Rozhold), dermatopathology (Dr O. Černý) and perinatal pathology (Dr L. Židová). The health care service section of the department was led by Prim. Dr K. Vojáček. The stomatopathological section for dentistry students was directed by Prof. Dr P. Jansa.

After return from a stay at McGill University in Canada, **Professor Dr J. Dušek** was the head of the Institute of Pathology for the period 1994–2000. He was also a prominent Palacký University representative (a vice-rector for scientific and research work) and education innovator (introduction of the "PBL" system – problem-based learning – and pathology lessons taught in English to foreign medical students).

Following Professor J. Dušek's tragic death in 2000, the institute has been directed by **Professor Dr Z. Kolář**, an organizer of the modernization and reconstruction of the laboratories, scientific researcher and founder of the **Research Laboratory of Molecular Pathology**.

After Professor Z. Kolář became the Dean of the Medical Faculty of the Palacký University in 2004, the leading post in the institute has been held by **Doc. Dr M. Tichý** who continues to safeguard the progressive trends of the Institute, both in the health care divisions as well as in the education of medical students. He is also a co-organizer of this national congress.

Of former personalities we should especially acknowledge the work of **Doc. Dr Č. Dvořáček** (1949–1958), a founder of the **university-type institute of pathology**, organizer of a network of many pathology institutes in the North Moravia region and representative of the so called school of North-Moravian pathologists. During **Doc. Dr V. Valach's era** the diagnostic health care service reached its heights with more than 20 thousand biopsies and nearly 2,500 autopsies a year. This state remained until

pathology institutes all over North Moravia were taken over by experienced pathologists, mainly trained at the Institute of Pathology in Olomouc.

Many outstanding professionals and several Palacký University dignitaries began their careers at the Institute of Pathology, including 13 leading pathologists in regional pathological departments, 6 heads of university institutes and several specialists working abroad (Great Britain, USA, Canada).

We would also like to briefly mention the **current situation** at the institute. It comprises several divisions. The **diagnostic health care services** (Prim Dr L. Kučerová) include autopsies, routine and peroperative biopsies and cytology diagnostics and a **special reference laboratory for oncological diagnostics** (special orientation to breast cancer). Important scientific and research activities are also seen in the recently established **research laboratory for molecular pathology**. We should also mention other areas such as neuropathology (earlier work on prion diseases), nephropathology, hepatopathology (liver biopsies), oncohematology, malignant lymphomas and immunohistochemistry.

The teaching programmes for medical students include the study of pathology along general medicine, dentistry and bachelor science degree lines. A programme in English for foreign students is also in existenced. The institute has also been accredited for **postgraduate doctoral studies**.

The **scientific and research activities** have traditionally been of high a methodological level in all divisions. In the past, these were supported by the Research laboratory of cyto- and histochemistry (Professor Dr R. Koďousek), electron microscopy laboratory (Professor Dr J. Dušek), and later – especially in routine diagnostic work – by the immunohistological laboratory (Professor Dr J. Mačák). At present, in addition to histochemistry, methods of molecular pathology and genetics are used in the Research laboratory of molecular pathology (Professor Dr Z. Kolář) in cooperation with some research institutions of the university.

For its scientific, research and publishing activities, the Institute has gradually become one of the well-recognized university centres in our country. The institute members have published a number of original papers in the field of applied clinical-pathological research which have received favourable international response. An example includes research on the introduction of macro-enzymatic histochemistry in necroptic pathology of ischemic conditions (succino-/malico-dehydrogenase macroreactions in acute encephalomalatias, early heart lesions in coronary insufficiency and early phases of myocardial infarction). Other research has focused on the etio-pathogenesis of Whipple's disease including demonstration of bacterial agent in enterobiopsies and in cases of Whipple's encephalopathy. Publication of original research on human **pulmonary Adiaspiromycosis** defining a new nosological entity in medicine attracted unusual attention. Another important research was that concerning experimentally induced demyelinization of the brain and spinal cord using copper-binding metal chelating agents (Dr J. Můr and Dr M. Záruba). From more recent communications, an original observation proving positivity of aspartate-protease Cathepsin D in the case of cytoinvasive stages of Coccidia (possible spreading factor in coccidiosis) is worth mentioning. Extensive materials were gathered in cooperation with other institutions including those abroad, which enabled publication of a monograph on medically significant mycotic diseases. A wide range of papers on molecular pathobiology have also been published in a number of international journals. These include special issues of immunohistochemical diagnostics, interpretation and prognostic factor evaluation.

The q**ualifications** of the institute personnel include 2 doctor of sciences dissertations, 4 professors of pathology, 6 assistant professors and several PhD academic titles.

The effort to maintain the high qualification level can be demonstrated by the acquired accreditation for postgraduate doctoral studies and scientific doctoral dissertations.

L**ooking back** at the time since the pathology department of the hospital in Olomouc and the medical faculty institute of pathology were established, we can see the progressive trend in development towards the present-day modern institute with excellent facilities located in the building of the Theoretical Institutes of the Medical Faculty. The institute of pathology has become a significant part of the Medical Faculty of Palacký University integrating at the present time seven faculties with more than 18,000 students.

In the previous years, the Institute of Pathology was developed thanks to many staff members, heads, scientific and research workers and teachers who deserve appreciation for their work. This is an opportunity to remind ourselves of a classic quotation characterizing an individual's contribution to common efforts: "Suum cuique decus rependit posteritas".

Heinz HÖFLER, MD

Heinz Höfler is Professor of Pathology of the Technical University Munich (Germany) and Head of the Department of Pathology at the GSF (Munich-Neuherberg). He was trained in Graz (Austria) at the University Hospital, in Basel (Switzerland), London (UK) and spent two years sabbatical at Tufts University in Boston. 1989 he was appointed by the Technical University Munich to head the Institute of Pathology. He was President of the Society of Histochemistry (1991–1993), President of the German Society of Pathology (1999–2000). Dean of the Faculty of Medicine, Technical University Munich (1997–1999) and is currently Chairman of the Scientific Council of the GSF. He is member of several societies and currently Editor in Chief of Virchow's Archive. In addition to his administrative and diagnostic activities Professor Höfler has published over 430 full scientific peer reviewed papers and several books/chapters (14). His main interest in pathology is GI and molecular pathology. He is currently acting chairman of the Working Group of Molecular Pathology of the ESP.

PREDICTION OF RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN CARCINOMAS OF THE UPPER GASTROINTESTINAL TRACT

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Multimodal treatment protocols are increasingly employed to improve the survival of patients with locally advanced adenocarcinomas of the upper gastrointestinal tract. However, only 30–40 %y of patients respond to 5-FU and cisplatin based neoadjuvant chemotherapy. The goal of our studies is identification of reliable genetic markers, on the genomic DNA-level, mRNA or protein level, that can predict the response of upper gastrointestinal carcinomas prior to neoadjuvant chemotherapy.

In esophageal carcinomas, a higher gene expression of methylentetrahydrofolate reductase (MTHFR), an enzyme involved in folate metabolism, is more frequently found in responsive patients. In addition high gene expression of caldesmon and of the two drug carrier proteins, MRP1 and MDR1 is associated with response to therapy. By performing a genomwide profiling at the protein level in a small group of patients, new potential markers were identified, which have to be validated in ongoing studies.

In gastric carcinomas, mutations of the *p53* gene revealed no association with treatment response or survival but tumours with a high rate of loss of heterozygosity determined by microsatellite analysis showed a better response to a cisplatin-based chemotherapy. Analysis of expression of 5FU- (*TS, DPD, TP*) and cisplatin- (*ERCC1, ERCC4, GADD45A, KU80*) related genes, demonstrated an association of *DPD* expression with response and survival. Consideration of the combined TP and GADD45 gene expression, provided the most obvious association with therapy response in this tumor type.

Our studies point to promising markers with potential use in chemotherapy response prediction of adenocarcinomas of the upper gastrointestinal tract but prospective studies for validation are necessary.

INTRODUCTION AND OBJECTIVE

Multimodal treatment protocols are increasingly employed to improve survival in patients with locally advanced adenocarcinomas of the esophagus and stomach. Neoadjuvant chemotherapeutic treatment, mainly based on cisplatin and 5FU, has been used since 1989 in several clinical trials and recently a statistically significant improvement in: progression-free and overall survival in operable gastric and lower esophageal cancer has been demonstrated in a large randomized, controlled phase III trial (MAGIC trial)[1]. However, only 30–40 % of patients respond to therapy and the majority of patients undergo several month of toxic, expensive therapy without survival benefit. In particular, in the case of esophageal carcinomas, it has been shown that patients with non-responding tumors seem to have an even worse prognosis than patients treated by surgery alone, which may be related to therapy-induced side effects, selection of chemotherapy-resistant, more aggressive tumor cells and delay in surgery[2]. Thus, identification of reliable genetic markers that can predict response is in high demand.

Several molecular markers have been investigated as potential response predictors. Thymidylate synthase as the target enzyme for 5-FU has been widely studied for 5FU containing regimens in gastrointestinal cancer but the re-

sults are inconsistent[3–5]. Dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) are two other important regulatory enzymes involved in the degradation of 5FU and low levels of DPD have been shown to be associated with response in gastric carcinoma[5,6], whereas conflicting results have been reported for TP.

The other major component used for the treatment of carcinomas of the upper gastrointestinal tract is cisplatin, which supposedly directly damages DNA. A significant association of the gene expression of the nucleotide excision enzyme *ERCC1* which is involved in DNA repair, with response to neoadjuvant chemotherapy has been reported[4].

Other markers such as glutathione S-transferase vascular endothelial growth factor and apoptosis-related genes such as bcl-2, bax and p53 have mostly been studied by immunohistochemistry but the results have been inconclusive. The result is that no markers have been found to be clinically relevant to date[3,7].

Thus the goal of our studies was to identify effective molecular markers for response prediction in patients with esophageal and gastric carcinomas treated by neoadjuvant chemotherapy. We used different strategies based on the one hand, on targeted approaches to characterize pretherapeutic biopsies for tumor specific molecular alterations in genomic DNA and mRNA-level. We also analysed constitutional genetic factors, e.g DNA-polymorphisms in therapy related genes. On the other hand, we performed a genomwide profiling on the protein level to identify new marker proteins.

RESULTS

Characterization of pretherapeutic biopsies of esophageal carcinomas

Analysis of m-RNA expression of therapy-related genes

In this study, paraffin-embedded, formalin-fixed endoscopic esophageal tumor biopsies of 38 patients with locally advanced esophageal adenocarcinomas (Barrett's adenocarcinoma) were included. All patients underwent two cycles of cisplatin and fluorouracil (5FU) therapy with or without additional paclitaxel followed by abdominothoracic oesophagectomy. RNA expression levels of 5-FU-metabolism associated genes Thymidylate Synthase, Thymidine Phosphorylase, Dihydropyrimidine Dehydrogenase, Methylenetetrahydrofolate Reductase, MAP7, ELF3, as well as of platinum and taxane related genes Caldesmon, ERCC1, ERCC4, HER2-neu, GADD45 and multidrug resistance gene MRP1 were determined using real-time RT-PCR. Expression levels were correlated with response to chemotherapy histopathologically assessed in surgically resected specimens.

The results demonstrated that the responding patients showed significantly higher pretherapeutic expression levels of MTHFR ($p = 0.012$), Caldesmon ($p = 0.016$), MRP1 ($p = 0.007$). In addition, patients with high pretherapeutic MTHFR and MRP1 levels had survival benefit after surgery ($p = 0.013$ and $p = 0.015$, respectively)[8]. Additionally, investigation of intratumor heterogeneity of gene expression of relevant genes (MTHFR, Caldesmon, Her2-neu, ERCC4, MRP1) – verified in 9 untreated Barrett's adenocarcinomas by examination of 5 distinct tumor areas – revealed no significant heterogeneity in gene expression indicating that expression profiles obtained from biopsy material may yield a representative genetic expression profile of total tumor tissue[8].

Thus, in conclusion, the results indicate that determination of mRNA levels of a few genes may be useful for predicting the success of neoadjuvant chemotherapy in individual cancer patients with locally advanced Barrett's adenocarcinoma.

Differential quantitative ProteoTope analysis of fresh frozen biopsies

A comprehensive protein profiling approach, using the ProteoSys platform, has been performed to date for a small group of patients. Quantitative and qualitative protein expression analysis was performed using 2D ProteoTope techniques after radioactive labelling of the protein extract with I-125 and I-131. The results so far point to an interesting group of proteins which may be associated with response. Validation of specific proteins by immunhistochemical analysis in a high number of cases is now part of ongoing studies.

Characterization of pretherapeutic biopsies of gastric carcinomas

Microsatellite analysis and p53 mutation analysis

We evaluated microsatellite instability (MSI) and loss of heterozygosity (LOH) in 53 pretherapeutic gastric carcinoma biopsies using 11 microsatellite markers. The entire coding region of the p53 gene (exons 2 -11) was analyzed for mutations by DHPLC (denaturing high pressure liquid chromatography) and sequencing. P53 protein expression was evaluated by immunohistochemistry. Patients were treated with a cisplatin-based, neoadjuvant chemo-therapy regimen. Therapy response was evaluated by CT-scan, endoscopy and endoluminal ultrasound[9,10].

We identified p53 mutations in 19 of the 53 (36 %) analyzed tumors. No significant association with response or survival was found for p53 mutation or for p53 protein expression. Microsatellite instability (either MSI-H or MSI-L), showed no correlation with response. With respect to LOH, LOH at chromosome 17p13 showed a significant association with therapy response ($p = 0.022$), but this did not reach statistical significance in terms of patient survival. The global LOH rate, expressed as fractional allelic loss (FAL) was assessed and tumors were classified into tumors with a high (> 0.5), a medium (> 0.25–0.5) and a low (0–0.25) FAL-value. A statistically significant association of FAL with therapy resoponse was found ($p = 0.003$), with a high FAL being related to therapy response.

Thus, a high level of chromosomal instability (high FAL-value) defines a subset of patients who are more likely to benefit from cisplatin-based neoadjuvant chemotherapy. p53 mutation status is not significantly associ-

ated with therapy response and is not a useful marker for response prediction[9, 10].

Methylation analysis

We investigated the methylation profile of six genes, which are frequently methylated in gastric cancer (14-3-3σ, E-cadherine, HPP1, Lysyl oxidase, MGMT and p16) for an association with response and survival in a set of 61 neoadjuvant treated gastric cancer patients by bisulfite/methylation specific PCR using the TaqMan system. 46% of the patients showed tumor specific methylation signals in four or more genes. There was no significant correlation of response with global methylation status or with any of the genes alone. Patients with a low methylation status showed a tendency to respond to therapy and patients with no or only one methylated gene demonstrated a statistically significant better survival ($p = 0.027$). This interesting finding raises the question if the use of inhibitors of DNA methylation and/or histone deacetylase inhibitors would represent a therapeutic alternative for gastric cancer patients demonstrating a high methylation status in their tumors[11].

Analysis of m-RNA expression of therapy-related genes

For gastric carcinomas we performed gene expression analysis, focussing on genes related to the effects of 5FU or cisplatin. Pretherapeutic, formalin-fixed and paraffin-embedded biopsies of 61 patients, who received a 5-FU and cisplatin based chemotherapy were included. The expression of the 5-FU related genes *TS*, *DPD* and *TP* and of the cisplatin related genes *ERCC1*, *ERCC4*, *KU80* and *GADD45A* were analyzed by quantitative real-time PCR. The expression levels of single genes and of various combinations were tested for an association with response and overall survival[5]. High DPD levels were more frequently found in nonresponding patients and were associated with worse survival. *GADD45A* and *TP* levels demonstrated weak associations with response, but *GADD45A* expression correlated with survival. There was no association with response for TS expression, but tumors with a high TS-level were associated with worse survival. The combination of *GADD45A* and *TP* revealed the strongest predictive impact. High expression values of *TP* and/or *GADD45A* were exclusively found in nonresponding patients ($p = 0.002$) and were associated with a significantly poorer survival ($p = 0.04$).

Thus in conclusion, the combined gene expression levels of *TP* and *GADD45A* represent a new parameter for predicting clinical outcome after neoadjuvant chemotherapy in gastric cancer. The association of *DPD* expression with response and survival underlines the predominant role of *DPD* in predicting 5-FU sensitivity. The association of *TS* expression levels with survival, but not with response, suggests the importance of this gene for tumor progression[5].

OUTLOOK

Although some of our studies point to promising markers with potential use in chemotherapy response prediction for adenocarcinomas of the upper gastrointestinal tract, prospective studies for validation are necessary before they can be used in clinical practice. As chemotherapy response is considered highly complex, depending on tumor-specific characteristics as well as on the constitutional genetic makeup of the individual patient, integrative approaches for response prediction may be necessary. In addition the incorporation of early response evalution by positron emission tomography (PET) for the therapeutic decision together with molecular markers, might result in superior sensitivity and specificity for successful application of individual therapy-strategies in patients with upper gastrointestinal malignancies.

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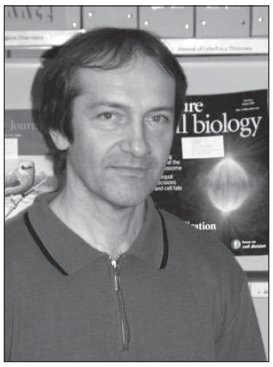

Jiri BARTEK

Czech born in 1953, Jiri Bartek studied general medicine in Olomouc, the Czech Republic. He was awarded an M.D. degree in Olomouc in 1979, and a Ph.D. degree in cell biology by the Institute of Molecular Genetics, Czech Academy of Sciences in Prague in 1983. Important milestones in J. Bartek's scientific career include group leadership position at the Oncology Institute in Brno, Head of Department at the Institute of Hematology in Prague, (both in the Czech Republic), and his stays as a visiting scientist at the Imperial Cancer Research Fund Laboratories (with Joyce Taylor-Papadimitriou and David Lane) in London, UK (1983/84, 1988-1990), and with Werner Franke at the German Cancer Research Center in Heidelberg (in 1987 and 1990).

In 1992, J. Bartek was appointed to a senior scientist position at the Danish Cancer Society in Copenhagen, Denmark, where he later (in 1997) became the Head of the Department of Cell Cycle and Cancer, and where he currently still works. J. Bartek studies molecular mechanisms that regulate mammalian cell division cycle and the ways these mechanisms are employed to preserve genome integrity. His scientific achievements in this field include: 1) Insights into the role of p53 as a tumour suppressor and effector of DNA damage signalling; 2) Elucidating the role of the Retinoblastoma tumour suppressor pathway in mammalian cell cycle progression and the ways these mechanisms (1 & 2) are subverted in cancer; 3) Identifying several checkpoint pathways activated in response to DNA damage, such as the Chk1/Chk2- Cdc25A, Chk1-Tlk and Chk1-Rad51 cascades; 4) The roles of ubiquitin ligases and protein degradation in cell cycle control and DNA damage checkpoints; and 5) Identification of the DNA damage response machinery as a critical anti-cancer barrier in early human tumorigenesis.

Jiri Bartek is a member of EMBO, Professor at the Universities in Aarhus and Copenhagen, and since 2005 also Deputy Director of the new Centre for Genotoxic Stress Research in Copenhagen. His current research interests, pursued jointly with Jiri Lukas, focus on the molecular basis of DNA damage response, its links with cell cycle machinery, and the involvement of these mechanisms in human cancer.

MOLECULAR PATHOLOGY OF DNA DAMAGE CHECKPOINTS AND CANCER

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The lecture will provide a brief outline of our most recent work on mechanisms of cell cycle control and DNA damage responses in mammalian cells, with emphasis on the following specific issues and their potential implications for the fields of molecular pathology, mechanisms of oncogenesis and cancer therapy. First, the emerging biological differences between the ATR-Chk1-regulated and ATM-Chk2-regulated pathways in unperturbed cell cycles and in response to genotoxic insults will be discussed. This part will include presentation of our results on the functional assessment of DNA damage signaling and effector pathways directly in human biopsy material, as well as data on new roles and substrates of Chk1 kinase and consideration of Chk1 kinase inhibition as a potential strategy to sensitize cancer cells to DNA damaging treatment modalities. Second, our data supporting a concept of the DNA damage response machinery as an inducible barrier against progression of early stages of human tumours in vivo, and in response to various oncogenes in cell culture models, will be presented. Here, emphasis will be mainly on unpublished results on distinct patterns of DNA damage response activation seen in diverse types of human malignancies, discussion of the potential cellular and molecular basis of such differences, as well as the role of genetic and epigenetic defects in the DNA damage response machinery in the predisposition to familial cancer. The lecture will finish by demonstrations of the most recent techniques that allow us to analyze these signaling and checkpoint mechanisms in real time, directly in live human cells.

Yrjö COLLAN, M.D., Dr.Med.Sci., FRCPath.

Yrjö Collan, from 1989 professor of pathology at the University of Turku, Finland, studied medicine at the University of Helsinky, and presented his published thesis 1972. After training in pathology he served as laboratory supervisor at the Institute of Occupational Health, Helsinky, Finland and was appointed professor of Pathology at the University of Kuopio, Finland in 1980. There he developed quantitative pathology in particular and became a leader in the development of the field. He served as president of the International Society for Diagnostic Quantitative Pathology for four years. 1988 he moved to the University of Turku. The years have been active in pathology service, and in pathology research. Visits to research laboratories of the University of Maryland in Baltimore (1974-1975) and AFIP in Washington D.C. (1988-1989) greatly expanded his understanding of research and diagnostics in pathology. Dr. Collan is one of those who has fully lived through the fast, comprehensive, and diversified changes and development of research since the late 1960s. As a keen follower o scientific development he has acquired good knowledge in many laboratory methods including electron microscopy, various aspects of quantitative pathology, and most recently molecular pathology. In the 1980 he served as a contract professor at the University of Ancona, Italy, with the mission to teach and study quantitative pathology. He is active in daily diagnostics and research, and has an international research group associated with his work in research. Dr. Collan is a fellow of the Royal College of Pathologists. Recently he has paid much attention to the potential of the findings of molecular biology and the human genome project in developing a modern diagnostic histo- and cytopathology laboratory. It seems that pathology diagnostics cannot solely be based on molecular medicine, but that the methodologies, which have helped us to create the present practises and classifications, will be important for generations. It is also important to realize that pathology is much influenced by the development of computer methods, as is medicine in general.

THE CHANGING WORLD OF THE PATHOLOGIST

Yrjö Collan

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The pathologist used to be considered as a helper of the clinician. The clinician's main job was to find the best management and treatment for the patient. The pathologist worked in his laboratory, or in the autopsy rooms, and served the clinician with diagnoses, on the basis of which clinical decisions could be made, and explanations given to relatives. Today, this basic arrangement is changing. One of the reasons is the dramatic diversification of medicine. New knowledge is generated from many sources. Especially important in this general context is the expansion of therapy options, and the development of new drugs which often need evaluation from the pathologist so that they can be used optimally. This is important not only from the medical point of view, but is also important economically, as the newest drugs are often the most expensive ones. For the efficient use of new information and for the election of the right treatment options clinical therapy selection meetings are common, and the pathologists have an increasingly active role in these. The pathologists of today should feel that they, too, are representatives of patients. Only through this means can clinical meetings fully benefit from the results of the pathology department.

To understand the present situation and future developments we have to look back. The generation which studied medicine in the 1960's is starting to retire. Since those days medical knowledge base has expanded dramatically, and not only through the development within medicine, but also through the development in biology, and especially through the newest field of biology, **molecular biology**. What knowledge in pathology and genetic pathology were medical students taught then in the 1960's? A look at the textbook of pathology by William Boyd[1] can provide an answer.

In 1952 the Watson – Crick model of DNA structure was published, and this information was already in the pathology textbook, and clearly the model has influenced the thinking in pathology. The textbook also described the knowledge base of genetics quite well and referred especially to the works of Mendel and Morgan. The concept of aneuploidy was mentioned in contrast to euploid number of chromosomes or amount of DNA. The importance of mutations was also mentioned. Four basic types of inheritance were listed (autosomal dominant, autosomal recessive, modified autosomal, sex-linked inheritance). Chromosomal abnormalities were known to exist and Turner's syndrome (44 X0) and Klinifelter's syndrome (44XXY) were listed. The carcinogens were listed quite thoroughly (chemicals, radiation, viruses, hormones, environment & occupation, co-carcinogens) but a general unifying theory was lacking. In fact those were the days when there was a conceptual fight between chemical car-

cinogenesis and viral carcinogenesis: nobody really dared to say which was the most important in human cancer. In line with the lack of unified theory of carcinogenesis there was no talk of oncogenes or tumor suppressor genes, which emerged through the development of molecular biology. Many hereditary diseases were mentioned and the concept of inborn error of metabolism was clearly presented. The clinical genetics was shaping itself those days, and the still continuing work of McKusick (OMIM, www.ncbi.nlm.nih.gov) was in its early phases. Numerous genetic diseases were listed (haemophilia, sickle cell anemia, Gaucher's disease, Nieman-Pick disease, Wilson's disease, glycogenoses, phenylketonuria, renal tubular defects, cystinuria, gout, and many hereditary skeletal defects, neuromuscular diseases, skin diseases, eye diseases, mental and neurological diseases) were mentioned. Even hereditary types of colorectal carcinoma were mentioned. It was more or less clear from the pages of the textbook that there was only a limited help available to patients suffering from most of these diseases.

If one looks at more specific textbooks on genetics published in the 1960's, one cannot help admiring the achievements which were already there before the time of molecular medicine. Molecular genetics was very advanced, especially in virology. Its medical relevance only was much less than today.

The difference between Boyd's textbook and that edited by Underwood[2] in 2004 is obvious. In addition to the more impressive type of printing work and extensive editing, the new information on molecular biology (or in this context, molecular pathology) clearly shapes the text. The genomic theory of carcinogenesis best outlined by the groups of Varmus and Weinberg is in substance presented in the text. This textbook stresses the difference between genetic and environmental causes of diseases, and the influence of the interplay of these causes in the actual situation of the patient. The structure of genes is clearly described, and concepts earlier unknown, like reverse transcription, explained and put in context of pathology. The importance of gene polymorphisms in disease is outlined. Numerous genomic changes not listed in the 1960's are outlined. The landmarks of the development of molecular biology are similarly outlined. Growth factors and intra- and extracellular mediators are listed and described in connection with inflammation, immunopathology and carcinogenesis. The thinking associated with molecular therapy is introduced. Apoptosis and its regulators are studied. The molecular changes associated with genetic disease are explained and shown in a few examples (like cystic fibrosis). The complex systems of mediators and adhesion molecules of immunity are outlined. Oncogenetic mechanisms are explained in many conditions on the basis of available information from molecular pathology. In line with the extended knowledge the textbook serves for becoming physicians, the pathologists are today served with classifications which include morphological and genomic information. This approach is now in WHO tumor atlases[3], which stress the importance of molecular pathology and genomic information in the work of the pathologist.

It is now absolutely clear, that molecular pathology is indispensable to the training of that pathologists to meet the challenges of the future. However, we have to understand that it is not only **molecular pathology** which we have to understand. We also need the understanding of traditional methodology. This much stresses the fact that we have to see the pathology discipline as a discipline in which thorough and continuous education is vitally important. Time, space, opportunities, and money should be given for pathologists to participate in continuous training.

It is a great mistake to think that development is not taking place in other fields than molecular pathology. We have to be knowledgeable about these developments too.

Light microscopy is the most important and basic method in histopathology. In tumor diagnosis light microscopy gives the basic background knowledge for diagnosis. What is normal and what is normal in relation to age is often more important than the exact knowledge of the classification of finding. Classification after the first examination under light microscope can be and probably should always be studied from books. The use of updated books is especially important in pathology.

A couple of years ago nobody could imagine that light microscopy could change in other respects than in ergonomy or improved optics. However, through digital imaging, and the methods of virtual microscopy we are today in a situation in which the traditional microscopy is no longer necessary. That means that the pathologist does not necessarily need a microscope in front of him As a basic solution to virtual microscopy the department has a microscope which scans all the sections produced by the histopathology laboratory to make digital images. Scanning is done on high power so that the number of pixels presenting the whole slide can be enormous. Such presentation, however, will allow evaluation of the image at low power on the computer screen, and allow zooming into areas which look interesting. In fact this is microscopy done on the computer screen, without a microscope in the hands of the pathologist, and called virtual microscopy. Because in this arrangement the slide is transformed into an image presented by electrical signals, the image can be transferred long distances through the internet. The pathologist of the future does not need to do his work at the microscope, and he can be consulted when abroad, and he can even do his work – in theory – while being on holiday. There is evidence that the pathology community is not yet completely ready for this change, but it is clear that virtual pathology is rapidly coming, especially in consultation over long distances, medical education, and pathology training. It is clear that the training pathologists should learn about virtual microscopy.

Electron microscopy has become an important research method. Clinical applications, however, are today more limited than the most optimistic predictions once

suggested. This is only partly associated with the limits of the method itself. The method is more laborious than immunohistochemistry and needs more intense participation by the pathologist than immunohistochemistry. However, many pathologists still feel that at least in problem cases electron microscopy of tumors is helpful. However, there are areas, in which electron microscopy has been incorporated in the diagnostic protocol, and as part of the protocol serves the diagnostics well. The best example is kidney biopsy diagnosis, in association of which the diagnostic process benefits in about 70 % of biopsies[4], either by showing positive diagnostic association, or by excluding disease entities. So, for covering diagnostic process electron microscopy in kidney biopsy diagnosis is essential, yet, it is much less utilized in tumor diagnosis. It is clear, that electron microscopy should be part of the training among training pathologists.

Enzyme histochemistry, which is used for locating specific enzymes in tissues through the reactions they themselves activate, has today limited value in the diagnostic process, but not totally forgotten, as shown by the tartrate resistant acid phosphatase in the diagnosis of hairy cell leukaemia[5]. However, **histochemistry associated with traditional stains** is diagnostically more important. Congo stain, of course, is an example, as it detects the various types of amyloid proteins. It is clear that the chemistry theory associated with staining, and the principles of histochemistry should be part of pathologist's training.

Quantitative pathology including DNA cytometry has become more popular than it used to be. Mitotic counts (e.g. in producing reliable histological grade in breast cancer) are important, and often have dramatic prognostic value[6], but also morphometric methods are valuable. DNA cytometry has much potential, and should be practiced more than it is practiced today. Much basic research with this method will be necessary in grouping tumors which potientially can be treated with newly developed drugs. Quantitative pathology training among pathologists is clearly insufficient. Much theoretical and practical diagnostic knowledge can be gained through courses on quantitative pathology.

Immunohistochemistry and molecular pathology clearly overlap. One can detect the expression of certain genes by detecting the presence of the transcribed messenger RNA (mRNA) by in situ hybridisation or other means. However, immunohistochemistry for detecting the corresponding translated protein is much more widely practiced. This area has developed fast and is still developing in the histopathology laboratory. The diagnostic value of immunohistochemistry is immense, to the degree that immunohistochemistry changes the classifications, and changes the interpretations of classifications in our everyday work. It is quite normal that the histopathology laboratory of a university hospital uses more than 100 antibodies for detecting a corresponding number of antigens in association with the daily diagnostic work. It is here that the diagnostic emphasis is today, especially in association with tumor classification. Pathologists should be trained thoroughly in immunohistochemistry, both in theory, laboratory practice, and interpretation. The methods of quantitative pathology in the interpretation can be especially fruitful.

It is difficult to predict how the science of pathology will develop. Some points are clear, however. Immunohistochemistry will still increase in importance for a long time. Molecular pathology is gaining importance daily. Traditional laboratory methods will stay for a long time, because morphology is so important, especially in tumor diagnosis. Quantitative methods will be introduced in all fields of pathology because they improve the accuracy of the diagnostic process. Virtual microscopy will become more commonly practiced than it is practiced today. Electron microscopy will stay at least in kidney biopsy diagnosis. DNA cytometry may give help in tumor diagnosis, but its role will be much dependent on whether this method is also studied in association with drug studies. But one aspect is certain: If any field of medicine, pathology will be the field of a life long study. The medical community should realise this, and we, as pathologists should keep an open mind towards scientific development.

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Gábor CSERNI, M.D., Ph.D., D.Sc.

Gabor Cserni was born in Kecskemét (Hungary) in 1966. He finished his university studies in 1990 in Albert Szent-Györgyi University of Medicine, Szeged, Hungary with *summa cum laude.* The theme of his Ph.D. thesis was "Methods of assessing axillary lymph nodes in breast cancer and their significance" (1999). In 2005 he obtained a D.Sc. (Doctor of the Hungarian Academy of Sciences) for "Evaluation of sentinel lymph nodes in breast cancer". Since 2005 he has been Head of Department of Pathology, Bács-Kiskun County Teaching Hospital. He is a member of the Society of Hungarian Pathologists, Hungarian Division of the International Academy of Pathology, European Society of Pathology, European Working Group for Breast Screening and Pathology International Sentinel Node Society (founding member). Moreover, Dr. Cserni has been a member of the Editorial Board of Journal of Clinical Pathology since 2000. He has published 109 publications in peer reviewed journals and some additional comments and letters and 76 abstracts in biomedical journals or their supplements.

NODAL STAGING OF BREAST CANCER VIA SENTINEL NODE BIOPSY

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Sentinel lymph node biopsy (SLNB) has gained a lot of interest in the staging of solid malignancies. In connection with breast cancer, it was first reported in the early nineties[1-3], and was claimed to represent a tool for a more reliable staging[4].

There have been many controversies concerning the optimal histological assessment of the SLNs. Some of the early reports documented a standard histological work-up, whereas others used enhanced pathology[5]. In routine practice, the SLNs are usually subjected to more detailed evaluation than other lymph nodes[6], and this may raise the question of whether or not the upstaging rate of SLNB derives simply from differences in methods used for the SLNs and the non-SLNs. A few studies have compared the yield of enhanced histopathology for both SLNs and non-SLNs, and have shown that the SLNs are indeed the most likely sites of regional nodal metastases[7-11]. By using an enhanced histopathology consisting of a combination of multilevel assessment and immunohistochemistry for epithelial markers, the upstaging rates vary between 9 and 47 % (Ref.[12]), and this rather large range is principally explained by differences in methods.

Molecular methods of nodal staging have been studied by several groups. Their use should result in ultrastaging of breast carcinomas, as it seems that both RT-PCR assays and flow cytometry are more sensitive than microscopic methods for metastasis detection[12]. Despite the fact that this conference is about novel techniques and concentrates on molecular pathology, the participants should not be misled in this respect. Molecular nodal staging is sensitive but we cannot neglect microscopic checking at present. Most series I know about have found at least a few cases that were positive by microscopy and negative by the molecular assay used[11] – these should obviously by classified as node-positive. On the other hand, those cases that have only molecular marker positivity and lack histological evidence for nodal involvement should be classified as node-negative for staging and treatment purposes[13-16].

Based on the weakest level of evidence, experts' opinion and review of available data[11], what constitutes standard evaluation of SLNs is a method that can identify all macrometastases and preferably all micrometastases too[16, 17]. Cytokeratin immunohistochemistry may be a part of the analysis, especially for lobular carcinomas[18], but molecular nodal analysis still represents an area of research despite the fact that positivity coincides with markers of poor prognosis[19].

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John DARLING, Professor B.Sc. (Kent), M.Sc. (Reading), PhD (London).

Originally trained as a microbiologist at the University of Kent in Canterbury and as a virologist at the University of Reading, in 1978 John joined the Institute of Neurology at the University of London now part of University College, London. He undertook a PhD in the Gough-Cooper Department of Neurological Surgery within the Institute on the chemosensitivity of human high grade astrocytomas. As a lecturer and subsequently Senior Lecturer he undertook research on the experimental therapeutics, immunology and molecular pathogenesis of malignant brain tumours. A programme of collaborative work still continues with UCL where John is an Honorary Senior Fellow. In 2001 John was appointed Professor of Biomedical Science at the University of Wolverhampton and since 2003 has been Director of the Research Institute in Healthcare Science (Associate Dean) at the University. RIHS is a cross-school venture. With Professor David GT Thomas John was the co-founder of the British Gliomas Group that subsequently became the British Neuro-oncology Group and has been known as the British Neuro-oncology Society since 2004. John has served as secretary of the BNOG and now serves as Vice President (President Elect) of the British Neuro-oncology Society. John was also one of the founder members of the European Association of Neuro-oncology. He is on the Scientific and Medical Advisory Board of the Samantha Dickson Research Trust and Brain Tumour U.K. which are major funders of neuro-oncology research in the UK.

MOLECULAR PATHOLOGY OF BRAIN TUMOURS – HOW WILL MOLECULAR AND CELL BIOLOGY CONTRIBUTE TO IMPROVED OUTCOMES IN PATIENTS WITH MALIGNANT BRAIN TUMOURS?

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Although in comparison to breast, lung and colon cancer, the brain is a relatively uncommon site for the development of cancer, the brain is the tenth most common site for the development of cancer in men and about the twelfth in women. This translates to about 6,000 individuals in the UK developing a primary malignant brain tumour every year. Cancer of the brain develops in two distinct age groups, although the types of tumour that develop in these two age groups differ markedly. There is a peak of incidence in the first decade of life, and brain tumours rank with leukaemia as a leading cause of cancer death in children. These tumours tend to be indolent low-grade astrocytomas or highly malignant primitive neuroectodermal tumours like medulloblastoma. However, the vast majority of brain tumours occur with increasing frequency in the sixth, seventh and eight decade of life and they are the second fastest growing cause of cancer death among those over 65. These tumours tend to be malignant astrocytomas particularly the most malignant variety, glioblastoma multiforme (GBM). Unlike lung cancer or malignant melanoma there is no strong evidence of an environmental carcinogen associated with the development of these tumours and no change in behaviour reduces risk.

The commonest malignant brain tumours in adults are derived from the supporting glial cells in the brain (Rampling et al., 2004). These tumours, known collectively as glioma, include tumours derived from astrocytes, astrocytomas; from oligodendrocytes, oligodendroglioma; and the ependymal cells that line the cerebral ventricles, ependymoma. In practice, the vast majority of primary brain tumours are derived from astrocytes. These tumours display a wide variation in malignant potential, ranging from low grade astrocytomas that, although they display in adults a marked propensity for malignant progression, have median survivals measured in decades to highly malignant tumours like glioblastoma multiforme (GBM) where the median survival is of the order of 9–12 months.

The histological features that are characteristic of GBM are marked nuclear atypia and mitotic activity, areas of necrosis bordered by areas of glial cells displaying characteristic pseudopallisading and significant evidence of endothelial proliferation. A further hallmark is that there are always both within different areas of the same tumour and between different GBMs marked heterogeneity in histological and cytological appearance. Although it is clear that GBMs are monoclonal tumours the cellular heterogeneity, presumably a result of significant genetic instability, suggests that there is likely to be considerable complexity in terms of therapeutic response between different parts of the tumour. Perhaps as a consequence of this cellular heterogeneity there is often considerable variation in the survival of patients with these malignant

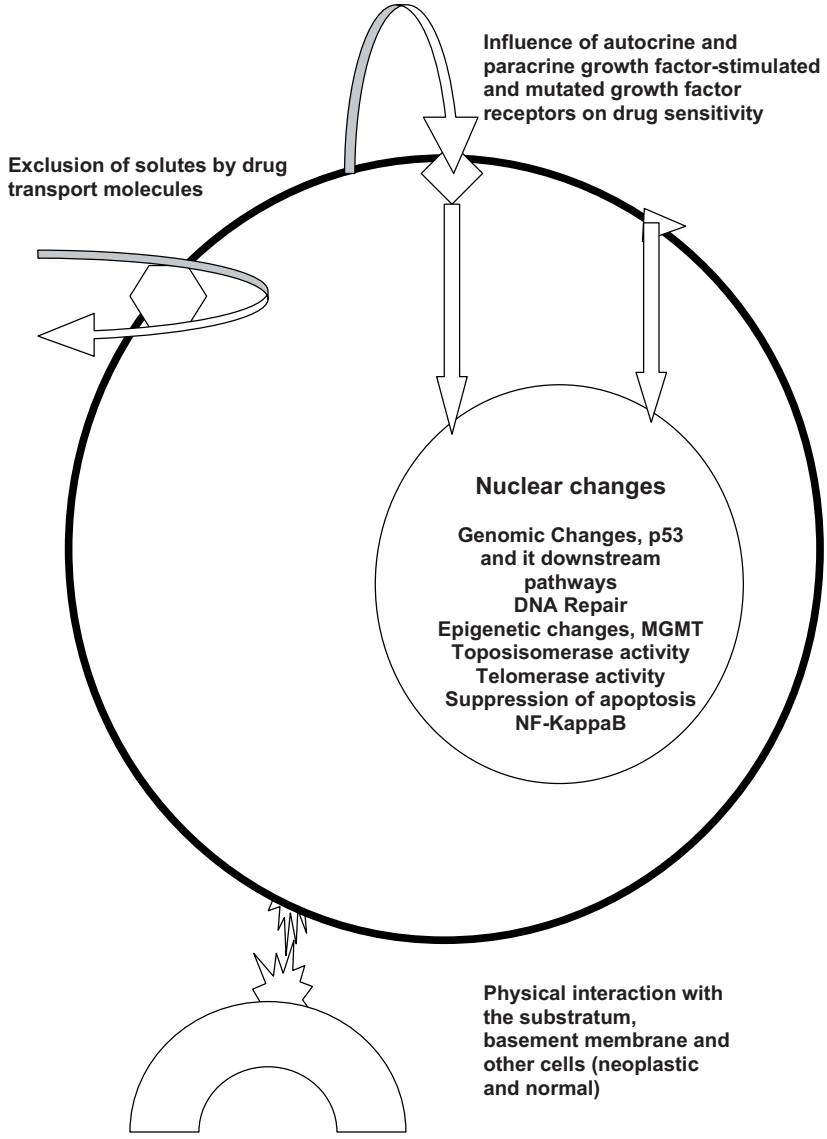

Fig. 1

tumours. Whilst it is true that the median survival may be of the order of 9–12 months, many clinical trials have shown that there is a proportion of patients (perhaps of the order of 10–15 %) who derive disproportionate benefit from the radiotherapy and adjuvant chemotherapy that they receive. It is from this group of patients that now a significant minority of patients with GBM survive 2 to 3 years from diagnosis. Although clinical features like age and performance status are powerful prognostic factors in patients with GBM, it seems reasonable to assume that biological features of the tumour also influence survival. There certainly seem to be two major types of GBM in adults that have different patterns of genetic changes. The most common variety is primary GBM that appears to arise de novo with signs or symptoms extending only a few months or weeks and no evidence of a pre-existing tumour. Typically these tumours do not have p53 mutations but often express a mutated form of the epidermal growth factor receptor (EGFR). On the other hand, there are those cases of GBM that arise as a result of malignant transformation of a low-grade astrocytoma. These secondary GBMs do not typically express altered forms of the EGFR but commonly do have p53 mutations. However, once the tumour has progressed to GBM, there is no systematic evidence of a difference in survival between these two groups of GBM patients. One feature that is common to the two groups of GBM is the presence of abnormalities on chromosome 10 that seem to be obligatory for the development of the GBM phenotype. However, we have been able to show that GBM in children and young adults (<25 years old) does not conform to this scheme. In a series of 10 young patients with GBM we have been able to identify a wide variety of genomic imbalances. The most common recurrent copy number aberrations were loss of 16p (54 % of cases), 17p (38 %), 19p (38 %), and 22 (38 %) and gain on 2q (38 %), 12q (38 %), 13 (38 %), 4q (31 %), 5q (31 %), and 8q (31 %). Seven regions of high copy number amplification were observed at 8q21-22, 7q22-23, and 1p21-22, 2q22, 12q13-pter, 12q15-21, and 13q11-14. In other words, the common genetic changes

in adult GBMs are not features of GBM in children and young adults and vice versa. Of course the aim of studies like this is actually to produce data that is of clinical relevance in individual cases. For example, can we identify molecular signatures that are predictive of long-term survival or response to particular cytotoxic drugs? There are some tantalising data that suggests that certain genomic changes are associated with long-term survival in patients with GBM. Burton and colleagues (2001a,b) have reported that losses of chromosome 9p and 10 and gains of 19q were associated with poor survival and that 19q loss was restricted to long-term survival (i.e. those GBM patients who survived longer than 3 years from diagnosis). Interestingly, p53 mutation and aberrant expression of p53 protein was also associated with longer survival (Schmidt et al., 2002). This suggests that p53 disruption might be associated with long-term survival. We have analysed the relationship between p53 mutation and chemosensitivity in short-term cultures derived from GBM biopsy samples. In this system, there is a marked relationship between p53 mutation and sensitivity in vitro to two drugs used clinically to treat patients with GBM, CCNU and vincristine (Ashmore et al., 2006). A recent study has indicated that using expression microarrays, clinically meaningful subgroups can be identified (Liang et al., 2005). However, a drawback with many of these studies is that the number of patients included in these studies is small and the exact therapies individuals received is unclear. This precludes a definitive analysis of the relationship between molecular changes and outcome in individual cases.

A clearer indication of the clinical utility of using molecular genetics to detect sub-groups of brain tumour patients that are likely to respond to particular cytotoxic drugs comes from patients with oligodendroglioma. These are rare tumours and most are well differentiated with little evidence of proliferative activity. However about a third of patients present with a anaplastic variant which displays aggressive features like increased mitotic activity, nuclear pleomorphism and vascular proliferation. Over the last fifteen years it had become clear that about 70 % of these malignant oligodendroglioma responds to combination chemotherapy with procarbazine, CCNU and vincristine (the PCV protocol) and consequently have a considerably more favourable prognosis than patients with malignant astrocytoma. The apparent chemosensitivity of anaplastic oligodendroglioma appears to be associated with loss of sequences on chromosome 1p and 19q. It is unclear which genes are responsible for this. Are there specific genes that are lost in these tumours that produce chemosensitivity or does this represent the identification of two types of tumour, one sensitive and one not chemosensitive but with the same histological appearance?

The relative ease with which samples of GBM produce short-term cell lines composed of replicating neoplastic glial-like cells and eventually established cell lines provides a powerful model system for investigating the relationship between molecular features and therapeutic sensitivity. The relationship between p53 mutation and sensitivity to cytotoxic drugs has been mentioned above. We have developed a panel of sixteen short-term cell lines derived from surgical biopsies and have been characterising the phenotype and genotype of these cultures in relation to response to cytotoxic drugs. A striking feature is the very complexity of the drug resistance mechanisms that can be identified in these cultures. In addition to the p53 status of these cell lines we have investigated the expression of O^6-methylguanine DNA methyltransferase (MGMT) together with the methylation status of its promoter, cytogenetic profile, expression of drug transport molecules, "stem cell" content and the NF-KappaB status. It is clear that multiple drug resistance mechanisms are present in a single cell (Figure 1). This very complexity in a comparatively simple situation in vitro is no doubt responsible for the difficulties there have been in correlating molecular changes and response to therapy in clinical studies. Nevertheless, such in vitro experiments will provide the background for properly designed clinical studies to be developed in the future.

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RE-EXPRESSION OF INTERMEDIATE FILAMENT NESTIN IN ADULT TISSUES

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Key words: Nestin/Rat/Human/Embryo/Adult intact tissues/Tumours/Myocardium infarction

We examined immunohistochemical expression of intermediate filament protein nestin in paraffin-embedded sections of rat and human tissues. During prenatal development, nestin expression was mainly confined to nervous, muscular and cardiovascular systems with no major inter-species differences in its cellular distribution. In intact adult tissues, nestin expression was down-regulated and the protein was detected only in the immature cells that were generated to balance tissue homeostasis. We concluded that in these systems, nestin could be considered a reliable developmental marker that allows us to distinguish undifferentiated from mature cells. Finally we examined whether IF nestin was re-expressed in adult tissues affected by disorders associated with cell growth such as neoplasia and regeneration. We focused on tumours derived from nervous tissue and samples tissue affected by myocardial infarction. High and consistent levels of nestin expression were observed in high grade astrocytomas, malignant melanoblastomas and capillary haemangiomas. In these tumours, nestin detection may help us in precise classification of tumours and might be also used as a predictive factor. In all specimens of myocardial infarction, intermediate filament nestin was re-expressed in cardiomyocytes and capillaries providing evidence that nestin could be a novel and invaluable marker in studies of the pathogenesis of heart diseases.

INTRODUCTION

The class VI intermediate filament protein nestin was named according to its abundant expression in neuroepithelial stem cells (nestin is an acronym for neuroepithelial stem cell protein)[1]. However nestin was also found to be expressed in other cells of neural origin[2,3,4]. Outside the nervous tissue, high levels of nestin were detected in the developing skeletal and cardiac muscle[1,2,5,6,7]. Another type of cell that expresses nestin is the immature endothelial cell[8]. Expression of nestin in nervous tissue is controlled by the second intron of the nestin gene whereas its expression in muscle precursor- and endothelium-specific regulators was identified in the first intron of the gene[9,10].

In most cases, the nestin gene is expressed in undifferentiated cells and is down-regulated in postmitotic differentiated cells, being replaced with another type of intermediate filament[6,11]. As a result, nestin can be consid-

ered a remarkable developmental marker that is expressed in early phases of ontogenesis. In lost cells adult tissues, nestin appears in immature cells that are produced to replenish or in dedifferentiated cells produced during tumorigenesis, e.g. in brain tumours[4,12]. Thus temporary nestin expression is induced under situations that correspond to recapitulation of developmental phases.

In this study, we examined immunohistochemical expression of nestin in archival material of rat and human tissues. The specimens were taken from embryonic as well as intact adult tissues and their examination showed no inter-species differences in protein localization. To judge whether nestin immunodetection could be exploited as a useful marker in human histopathological diagnosis we focused on disorders affecting the central nervous and cardiovascular systems because both systems are associated with nestin expression during neurogenesis, cardiogenesis and angiogenesis. Our results confirmed the usefulness of nestin immunodetection in the diagnostics of astrocytomas, capillary haemangiomas, melanoblastomas and in examination of early changes that occur in the myocardium after heart attack.

MATERIAL AND METHODS

For examination of both rat and human tissues we used archived samples fixed in 10% formalin and embedded in paraffin. Pseudoperoxidase activity in deparaffinized sections was suppressed in solution of 3% H_2O_2 in methanol. Revitalisation of epitopes was performed by pretreatment in the microwave oven. Following thorough washing in PBS containing 5% Triton X-100 (Sigma), sections were incubated with a primary antibody at 4°C overnight. The rat tissues were incubated with primary monoclonal antibody Rat-401 (DSHB, Iowa). Nestin in the human tissue was identified with monoclonal antibody 10C2 (Chemicon, UK). Following washing with PBS, sites where primary antibody bound to nestin epitopes were visualized with a goat anti-mouse biotinylated antibody, streptavidin conjugated to horseradish peroxidase and diaminobenzidine (Sigma). Sections were counterstained with the methyl green or haematoxylin. To avoid false positivity, parallel sections were processed according to the same protocol but primary antibodies were omitted. To receive intense signal, some detections were not developed in diaminobenzidine but were twice amplified with biotinylated tyramine and visualized with streptavidin conjugated to fluorochrome Cy3 (Jackson ImmunoResearch Laboratories). Nuclei were then counterstained with DAPI and sections were mounted in polyvinylalcohol/glycerol with DABCO; for light microscopy the sections were mounted in DPX. Sections were examined in Olympus BX51 microscope equipped with epifluorescence and DP-70 camera.

RESULTS

We first concentrated on studying nestin expression in developing rat tissues and then evaluated whether the corresponding developing human tissues expressed also this protein. In both rat and human embryonic tissues, nestin was mainly localized in corresponding cells of the nervous, cardiovascular and muscular systems. In the CNS, nestin was found in neural stem cells, radial glial cells and developing astroglial cells. As astroglial cells differentiated, nestin protein was redistributed to their endfeet processes that formed superficial and perivascular limiting membranes. Nestin was also expressed by cerebral capillaries that were ensheathed by apposed perivascular astrocytic endfeet. Examination of non-neural rat and human tissues revealed that nestin was expressed in endothelial cells of capillaries and larger blood vessels that grew in the developing organs. Moreover, we observed nestin also in extraembryonic blood vessels inside the rat and human placenta. The third typical structures expressing high levels of nestin were represented by muscular tissues. The smooth muscle (in developing intestines or arteries) showed just low levels of nestin. On the contrary, myoblasts and myotubes of the skeletal muscle contained high levels and during the skeletal muscle differentiation, nestin was gradually replaced by desmin. In the myocardium, cardiac muscle cells in both atria and ventricles showed intense immunoreactivity for nestin during early stages of development, which is lost after maturation in cardiomyocytes.

Nestin expression in adult intact tissues was relatively rare. In the forebrain, nestin was observed in the subependymal zone and even more abundantly in the rostral migratory stream of rats. In the brain, the protein was also detected in tanycytes (especially around the third cerebral ventricle) and astrocytes (mainly fibrillary astrocytes and endfeet of those participating in formation of perivascular and superficial limiting membranes). Cerebral blood vessels contained nestin-positive endothelial cells sporadically. The same was true for other tissues including muscular tissues. The only exception was represented by those tissues, which grew in response to cyclic hormonal changes like the corpus luteum or endometrium. Such tissues contained lots of nestin-immunoreactive blood vessels of different calibres. Adult intact muscular tissues were devoid of nestin.

Situation changed during pathological processes. In astrocytomas, nestin immunoreactivity appeared in some undifferentiated neoplastic cells, reactive astrocytes and in neocapillaries. High grade astrocytomas revealed higher histoscore than low grade astrocytomas. Nestin was also observed in radial-glia like elements in the medulloblastomas. From other tumours, nestin was found in medulloepitheliomas and two-thirds of supratentorial primitive neuroectodermal tumours. In haemangioblastomas, immunoreactivity for nestin was observed in fenestrated capillaries and some types of stromal cells. High levels of nestin were observed in melanoblastoma, where all tumour cells expressed this protein. Moreover, blood ves-

sels represented mainly by capillaries, found within the tumour or at its periphery also showed high immunoreactivity. High levels of nestin were also typical for capillary haemangiomas where immunopositivity was found in endothelial cells and pericytes. On the contrary, cavernous haemangiomas were nestin-negative.

Nestin elevation was also detected in adult tissues suffering from non-tumourigenic diseases like ischaemic heart disease. In patients who survived for few days after acute heart attack, nestin reappeared in their myocardium. Immunoreactivity was confined to blood vessels and also to cardiac muscle cells in the vicinity to necrotic areas. Depending on time interval from the onset of myocardial infarction, nestin immunoreactivity appeared in the entire sarcoplasm of cardiomyocytes or was reduced to subsarcolemic areas or to intercalated discs.

DISCUSSION

Intermediate filament protein nestin confers the cell cytoskeleton remarkable flexibility. Nestin itself cannot form stable filament network but it co-polymerizes with type III intermediate filaments, e.g. vimentin[13]. Such heteropolymers are less stable than vimentin oligomers, which is essential for promotion of disassembly and restructuring of the vimentin intermediate filament network during mitotic division and migration of progenitor cells[14]. As a result nestin expression is typical for developing cells and for that reason it is heavily expressed in developing neural, muscle or endothelial cells. As these cells differentiate nestin disappears being replaced by another and permanent type of intermediate filament[6,11]. From this point of view, nestin can be considered as a remarkable developmental marker: it is expressed in early phases of development associated with cell proliferation and it is lost after cell maturation.

In adult organs, tissue homeostasis keeps the cells at appropriate amounts balancing well cell death and cell renewal to preserve tissue maintenance. Here again, production of new cells in nervous, cardiovascular and muscular systems has to be associated with nestin expression to provide the cell cytoskeleton properties necessary for a proper development in desired phenotypes. Therefore nestin expression in areas of active adult neurogenesis like the subependymal zone or rostral migratory stream can not be surprising[15]. Similarly growing tissues like the uterine endometrium[10], corpus luteum[16] or tumours[4,12,17] are characteristic with nestin immunoreactivity. Nestin is confined to newly formed capillaries that enter the growing tissue or may be expressed in newly formed cells e.g. in case of tumours of neural origin. Nestin immunohistochemistry can be used for the grading of astroglial tumours and its expression has significant value in astrocytomas and malignant melanomas as an auxiliary indicator of dedifferentiation and progression[18].

The most surprising is nestin expression in the myocardium. Although the protein is expressed during early phase of development, its expression in cardiac muscle cells in embryonic mice is limited to a short interval of two days[7]. After embryonic day 11 to adulthood, the authors were not able to detect nestin in the mouse myocardium. Since nestin expression was observed in the same cell types and under same physiological situations in mouse and human tissues, we can expect that nestin expression in the human heart is also confined to embryonic heart and it is presence disappears in intact adult heart tissue. Our preliminary results confirm this hypothesis. Observation of nestin$^+$ cardiomyocytes and endothelial cells in samples of infarcted myocardium might correspond to the tissue repair. Recently identified stem cells in the myocardium confirm the potential of cardiac muscle tissue for its regeneration[19]. If new cardiac muscle cells are generated in response to ischaemic injury, newly produced cardiomyocytes have to be nestin$^+$. Moreover, changes in nestin distribution in cardiac muscle cells are closely similar to those observed in developing skeletal muscle where immature myoblasts and myotubes are first immunostained for nestin homogenously but as the skeletal fibres mature, nestin gradually disappears and its immunoreactivity is concentrated to area of motor end-plate[20].

To summarize our results, we confirm that intermediate filament nestin is primarily expressed in developing nervous and muscular tissues and endothelial cells both in rodent and human tissues. As the cells differentiate, nestin levels decrease being substituted by permanent intermediate filaments proteins. However, nestin expression reappears in adult nervous and muscular tissues as well as in blood vessels when new cells are generated in the course of maintenance of tissue homeostasis or tumourigenesis. In the former case, few cells in intact tissues are generated in response to physiological stimuli to replace dead cells. Production of cells is higher in specialized sites of neurogenesis such as the subependymal zone. Increase in nestin$^+$ cells can also be associated with increase in cell production during tissue reparation. In the latter case, nestin reappears in tumours arising from cells that express this protein in early phases of their ontogenesis. Nestin expression is characteristic for undifferentiated phenotypes which may be used as a predictive factor in histopathological evaluation of some tumours. Nestin expression in the infarcted myocardium may be associated with generation of new cardiac muscle and endothelial cells or may correspond to changes in the cytoskeleton of cardiomyocytes that adapt to new conditions. Further investigation is necessary to reveal a precise role of nestin in diseased cardiac muscle cells.

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The major focus of the Murray group is to provide a better understanding of the molecular events leading to the development of Hodgkin's lymphoma (HL) and especially of the role of the Epstein-Barr virus in this process. Although the tumour cells of HL have rearranged Ig genes, they do not express a functional B cell receptor (BCR), either because of crippling mutations in their somatically mutated Ig genes or by the loss of transcription factors important for Ig-transcription. Thus, a fundamental pathogenic event in the development of HL is the ability of HRS cells to escape the apoptosis that would be the normal fate of GC B cells lacking functional BCR expression. We have recently shown that HRS cells are intrinsically resistant to Fas-induced cell death and that they express the Fas inhibitory protein, c-FLIP (Dutton A *et al.*, *Proceedings National Academy Sciences USA* 2004). We have observed that down-regulation of c-FLIP, through the use of specific small interfering (si) RNAs, leads to HL cell death. Furthermore, we have also demonstrated that HRS cells express Fas ligand (FasL) and that treatment of HL cells with both c-FLIP- and FasL-specific siRNAs in combination restores cell viability. These results provide a mechanism whereby HRS cells appear to be protected from autonomous FasL-mediated cell death. Work is ongoing to determine whether FasL expressing HRS cells can evade immunosurveillance through a counterattack mechanism involving Fas-induced death of HRS-specific CTLs. Other work has identified the epigenetic silencing of key tumour suppressor genes with pro-apoptotic functions in HL cells (e.g. Murray *et al*., *Oncogene* 2003) which might be an important step allowing BCR-negative cells to survive in the GC. Clearly, EBV might provide important survival signals in GC B cells which might facilitate the escape of BCR-negative progenitors- we are currently investigating the likely nature of these potential signals. More recent work is focused on a detailed analysis of the HL phenotype *in vivo* and how EBV infection contributes to this. Thus, we have recently shown that EBV confers growth and survival promoting effects upon HL cells via the transcriptional alteration of key target genes, including autotaxin (Baumforth *et al*., *Blood* 2005).

CONTRIBUTION OF THE EPSTEIN-BARR VIRUS TO THE HISTOLOGICAL PHENOTYPE OF HODGKIN'S LYMPHOMA

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The Epstein-Barr virus (EBV) is a ubiquitous herpes virus which can establish a persistent but usually harmless infection in the human host following colonisation of the memory B cell pool. However, EBV can also contribute to the development of Burkitt's lymphoma (BL), post-transplant lymphoma, and classical Hodgkin's lymphoma (HL), where it is detected in approximately half of all cases. HL is characterised by malignant Hodgkin/Reed-Sternberg (HRS) cells surrounded by an abundance of non-malignant 'reactive' cells. The detection of crippling immunoglobulin (Ig) gene mutations in a proportion of HL indicates that HRS cells derive from pre-apoptotic germinal center (GC) B cells that would normally have undergone apoptosis. Despite their B cell origin, HRS cells not only lack expression of the B cell receptor (BCR) but also show a unique pattern of downregulation of B cell- and lymphoid-associated genes and surface markers.

EBV can rescue BCR-negative human tonsillar GC cells from apoptosis indicating that viral genes may interfere with the GC reaction. Using transgenic mice models, the EBV-encoded latent membrane protein 2A (LMP2A) has been shown to enable the survival of BCR-deficient B cells by providing a surrogate BCR signal. The other EBV latent membrane protein, LMP1, mimics a constitutively active CD40 receptor and can promote the survival of CD40-deficient transgenic murine B cells. Furthermore, LMP1 is a potent oncogene as it transforms rodent fibroblasts and is essential for B cell immortalization

Both LMP1 and LMP2A are expressed in EBV-positive HL, suggesting an important role for these viral proteins in the pathogenesis of this tumor. LMP2A expression in mouse B cells and in a non-Hodgkin's lymphoma cell line leads to changes in gene expression resembling those seen in HRS cells, including downregulation of B cell lineage-specific genes. However, the contribution of LMP1 to the HRS cell transcriptional signature has yet to be defined. Since LMP1 can redirect the transcriptional programme of B cells, by activating a range of signalling pathways (e.g.

NFκB, Jak/STAT) that are also known to be deregulated in HL, it seems plausible it might also contribute to the HRS phenotype.

We have undertaken gene expression profiling following EBV infection or gene transfection of transformed cell lines and of normal human GC B cells. This revealed transcriptional changes consistent with those observed in cultivated and primary HRS cells including the characteristic loss of B cell identity. These data not only implicate EBV as an important mediator of the HRS-cell phenotype, they also suggest that EBV infection of GC B cells modulates changes in gene expression that may play an important role in the early stages of the development of HL.

Paul N. NELSON, Dr., Associate Professor

Paul N Nelson is Reader/Associate Professor in Biomedical Sciences (Immunology) and a core member of the Research Institute in Healthcare Science at the University of Wolverhampton, U.K. His research group focuses on viral immunology, in particular the detection and role of human endogenous retroviruses (HERVs) in autoimmunity and cancer and the development and application of antibodies as probes and diagnostic/prognostic reagents in immunoassay systems. The immunology team consists of three senior lecturers, two research assistants and ten PhD students plus additional visiting fellows. The reputation of the group has gained much acclaim from many publications, invitations to conferences and book chapters plus media presentations. More importantly, Dr. Nelson has worked closely with Professor Zdenek Kolar (University of Palacky) on the ERASMUS exchange link for several years that has brought much benefit to the training and research experience of postgraduate students. At the heart of his team's ethos is the encouragement of all staff to reach their potential, to try novel ideas and expand the scientific knowledge of how viruses affect the immune system.

After gaining his degree in immunology and physiology at the University of London, Dr. Nelson was fortunate to work at the prestigious Royal Postgraduate Medical School, London, under Professor Mitchison and Dr. Ann Rees. The research focused on TB and provided an insight and a real interest into the world of microbes and disease. He was subsequently awarded a CASE PhD studentship in immunology and forensic science under Professor Roy Jefferis at the Medical School, University of Birmingham. It was at Birmingham that skills in antibody technology for clinical benefit were established. Indeed, one monoclonal antibody A57H (pan-IgG) was marketed and others (anti-allotypic reagents) have proved useful to this day for probing epitopes on immunoglobulin G. Much of Dr. Nelson's postdoctoral experience was gained at St. George's Hospital Medical School, London. Training in pathology was initially provided by Professor Tim Chambers whose interest was in bone diseases. Subsequently a major opportunity arose to work with Professor Frank Hay, Dr. Claire Sharrock and Professor Andrew Lever. Much is owed to these individuals for opening the way for research into RNA viruses and their role in autoimmune diseases. To this day, Dr. Nelson still works with Professor Hay and Professor Andrew Lever on HERVs, rheumatoid factors and, epitope mapping. His experience in virology was then expanded under the guidance of Dr. Jim Booth. Here Dr. Nelson led the team that provided a unique polymerase chain reaction test for cytomegalovirus in bone marrow transplant (BMP) patients. This test is still used routinely at St. George's Hospital on all BMT patients and has no doubt saved many hundreds of lives. This probably serves as one of the best examples of 'bench to bed' medical research projects. Following a stint in cardiac immunology and an appreciation of other viruses, an opportunity arose at the University of Wolverhampton to establish an immunology research group. The position was an act of faith and the risk was considerable since the university at that time had no immunology research or facilities to run postgraduate research programmes. Fortunately a like-minded colleague in the name of Dr. Paul Murray was already interested in DNA viruses, immunology and oncology. Right from the start an excellent working relationship began (and continues to this day) with many papers and research grants on HERVs, EBV and the link with the Czech Republic. From this beginning, new laboratories within RIHS were established with a thriving research community. Current projects include the development of monoclonal, polyclonal and phage antibodies to a number of HERVs including those implicated in breast cancer (Dr. Jan Martin) and brain tumours (Professor John Darling). In addition, various projects are investigating serological responses to HERVs in autoimmune diseases such as rheumatoid arthritis and system lupus erythematosus. In addition to research, Dr. Nelson is co-editor of the 3rd edition of the 'biology of disease' and will edit a book on HERVs in 2007. Fundamentally, he is also a teacher and trusts that his students all benefit in terms of knowledge from many years experience and his enthusiasm for immunology. To relax, Dr. Nelson enjoys skiing with his family and he flies aircraft.

HUMAN ENDOGENOUS RETROVIRUS HERV-K10 IMPLICATED IN RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS: POTENTIAL PATHOLOGICAL TRIGGERS?

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Human endogenous retroviruses (HERVs) are a group of integrated RNA viruses within our human genome. Whilst many are regarded as defective, a number possess the potential to generate retroviral products. Indeed HERVs such as those belonging to the HERV-K family produce retroviral particles in the teratocarcinoma cell line GH and the breast cancer cell line T47D. It has been argued that some retroelements may be beneficial to the

human host, perhaps conferring a selective advantage, whereas others may be harmful. Furthermore certain HERVs might be involved in the pathogenesis of autoimmune diseases. The precise mechanisms in diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) may include molecular mimicry and superantigen motifs that evoke and augment unwarranted immune responses. In the RA joint, tissue destruction is evident over time with recruitment of lymphoid and other cells plus the presence of rheumatoid factor that exhibits increased affinity and change in isotype; evidence of an antigen-driven immune response. The precise trigger of course, remains unknown although certain HERVs have been implicated. In a previous study we found evidence for increased expression of HERV-K10 mRNA in patients with RA. Here we have extended this work by investigating the serological expression to HERV-K10 in patients with RA, SLE, osteoarthritis, normals and other inflammatory disease groups. The study utilised a novel peptide ELISA immunoassay using segments of HERV-K10 identified through bioinformatic analysis. In particular, biotinylation of peptides was necessary for serological discrimination between patients. Overall a significant difference ($p<0.05$) was found for RA patients in terms of antibody activity to HERV-K10. There was also an increased level of antibodies to HERV-K10 in patients with renal lupus although this was below the level of significance. It is possible that HERV-K10 could act as a trigger in RA/SLE through regions of similarity to host proteins. In this case, the immune response to HERV-K10 could lead to collateral damage and pathogenesis of disease.

Miroslav STRNAD, prof. Ing., DSc.

Professor Miroslav Strnad born in 1958 has been Head of the Laboratory of Growth Regulators since 1996. He is interested in cytokinine and fytohormones research and he is actively involved in the development of inhibitors of cyclin dependent kinases (olomoucin). He is author of 324 original articles (botany, biochemistry, physiology, analytical and organic chemistry), 12 chapters and reviews in textbooks, author of 3 books and 7 international patents. His citation index according SCI is more 1500 citations. He obtained the Award of European Fytochemical Society in 1998 for the analyses and identification of aminopurine fytohormones. Professor Strnad has also been an honorary member of Olomouc City since 1999.

PURINE INHIBITORS OF CYCLIN-DEPENDENT KINASES AS NEW GENERATION OF ANTICANCER DRUGS

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Our research focused on the primary mechanism of action of plant hormones cytokinins (N^6-substituted adenine derivatives; 6-benzylaminopurine – BAP as representative) in cell division cycle has showed that natural plant cytokinins are rather non-specific inhibitors of various protein kinases[1]. Surprisingly, among aromatic cytokinin derivatives, we have discovered a compound, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, named "olomoucine" (OC, Fig. 1), which specifically inhibits some cyclin-dependent kinases (CDKs) at micromolar concentrations [1]. The total lack of the inhibitory effect of olomoucine on major kinases, such as cAMP- and cGMP-dependent kinases, protein kinase C, and others, suggests that OC might be a useful tool for cell cycle regulation studies. The design and inhibitory activity of OC was further improved by modifications at positions 2, 6, and 9, i.e., the positions that control binding to CDK1. This led to discovery of novel specific CDK inhibitor named roscovitine, etc. (Fig. 1)[2, 3], which displays enhanced inhibitory activity toward CDK1, a higher selectivity toward

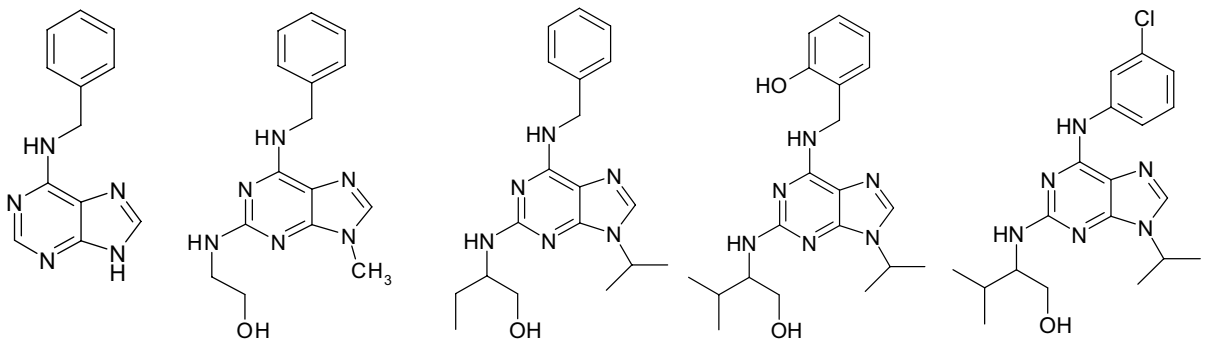

Fig. 1. Chemical structures of 6-benzylaminopurine (BAP), olomoucine, roscovitine, olomoucine II, and purvalanol A (from left to right).

some CDKs, an increased antimitotic activity at the G1/S and G2/M points of the cell cycle, and stronger and more selective antitumour effects. Finally, we have tested different CDK inhibito rs on several tumour cell lines (CTLL-2, rhabdomyosarcoma cell lines, etc.). The average concentration that causes 50% growth inhibition range between 0.1–100 μM. The compounds are also effective *in vivo* and one is already in clinical trials (roscovitine ⇒ Seliciclib®, Cyclacel Ltd, U.K.). We have developed several new generations of CDK inhibitors derived from the trisubstituted purines[4–14]. The purine analogues bohemine and roscovitine were also used to study the role on CDKs in cell cycle progression and microtubule organisation in *Vicia faba* root tip cells[15]. Both drugs inhibited the activity of immunopurified *Vicia faba* and alfalfa CDC2-kinase. An observed transient arrest at the G1/S and G2/M regulatory points indicated that inhibition of the CDC2-kinase had an effect on both transitions. In contrast to the regular bipolar spindle in untreated cells, in drug treated metaphase cells abnormally short and dense kinetochore microtubule fibres were observed. The chromosomes were not aligned on the metaphase plate but were arranged in a circle, with kinetochores pointing inwards and chromosome arms pointing outwards. γ-Tubulin, which plays a role in microtubule nucleation, also localised to the centre on the monopolar spindle. These compounds also induce apoptosis in different cells *in vitro* and *in vivo*. We also recently discovered that compounds known as anticytokinins are rather specific CDK inhibitors[16]. The results of recent development will be presented during the meeting.

ACKNOWLEDGEMENT

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ABSTRACTS

A1 MICROVESSEL DENSITY IS ELEVATED IN BONE MARROW OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS BUT IS NOT ASSOCIATED WITH PATTERN OF INFILTRATION

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Key words: Angiogenesis/Bone marrow/fVIII (von Willebrand)/Microvessel density/CLL (chronic lymphocytic leukemia)

BACKGROUND

Angiogenesis is considered a potential prognostic factor in chronic lymphocytic leukemia (CLL). Elevated levels of various angiogenic factors have been identified in plasma and serum of CLL patients. However, it is still controversial whether neovascularisation in CLL bone marrow (expressed as microvessel density – MVD) is elevated, in part because methods of angiogenesis assessment are not directly comparable between studies published so far due to different antibodies used for immunohistochemical identification of vessels and different methods of calculating MVD. Moreover, there are insufficient data on the association of bone marrow angiogenesis with type of marrow infiltration (focal/nodal, interstitial, diffuse) which have prognostic impact on the clinical course.

AIM

To assess MVD in marrow biopsies from CLL patients and a control group and to compare MVD according to infiltration type,

METHODS

We analyzed 22 CLL bone marrow biopsy specimens. The control group consisted of 11 biopsies from individuals with no evidence of bone marrow malignancy. Neovascularisation was assessed using immunohistochemical staining of endothelial cells with anti fVIII Ag (von Willebrand-related antigen, factor VIII) monoclonal antibody. Microvessel density was assessed in digitalized pictures from Lucia-M image analysis software and calculated using the hot spot method, i.e. identification of three loci with highest accumulation of microvessels under low (100x) magnification and counting microvessels in three high-power fields (x400 magnification) per hot spot. MVD was expressed as number of microvessels per high-power field and per square micrometer.

RESULTS

Twenty-two CLL samples and 11 control specimens were technically evaluable. Type of infiltration was focal/nodular in 6, interstitial in 5 and diffuse in 11 samples. MVD was significantly elevated in the CLL group compared to controls ($p < 0.0001$, Mann-Whitney U test). On the other hand, we found no significant difference between CLL subgroups.

CONCLUSION

Our study shows that microvessel density in CLL is highly statistically significantly elevated over controls. However, we found no significant difference between focal vs. interstitial vs. diffuse type. In conclusion, this study adds further support to the importance of angiogenesis in CLL biology. Further studies assessing the association of MVD with other prognostic factors (e.g. IgVH mutation status, genetic aberrations) are necessary.

ACKNOWLEDGEMENT

Supported by grant No. NR/8373-3 and research project MZO 00179906 from Ministry of Health of the Czech Republic.

A2 MICROARRAY ANALYSIS OF GENE EXPRESSION IN MICRODISSECTED MAMMARY NORMAL AND TUMOR CELLS

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Key words: Wnt pathway/Cell adhesion/Ductal and lobularcarcinoma/Breast cancer/Microdissection/ Microarray

AIM

Breast cancer is a complex genetic disease characterized by the accumulation of multiple molecular alterations. The resulting clinical heterogeneity makes current diagnostic and therapeutic strategies unsuited to individual patient needs. Breast cancer is extensively studied by many methods, including microarrays. The aim of this study was to contribute to the understanding of breast cancer by the microarray analysis of microdissected normal and cancer cells of both ductal and lobular type.

PATIENTS AND METHODS

We examined surgical specimens obtained by mastectomy from 10 postmenopausal breast cancer patients. Of these 5 were invasive ductal, and 5 were lobular carcinomas. All tumors were negative for c-erbB-2 by immunohistochemistry. Tumor tissue and normal mammary gland tissues were snap-frozen in liquid nitrogen. 7–8 μm frozen sections were stained by hematoxylin in RNase-free conditions. Normal lobular and ductal cells as well as infiltrating ductal and lobular tumor cells were microdissected from cryosections using Veritas™ Laser Capture Microdissection System. Fifty nanograms of total RNA were amplified by combined PCR and in vitro transcription. RNA was labelled during in vitro transcription and analysed on Affymetrix Human Genome U133 Plus 2.0 Arrays. Gene expression analysis was performed by Affymetrix Gene Chip Operating Software, dChip software and Internet databases.

RESULTS

The genes overexpressed by tumor tissue were involved primarily in cell-matrix interactions, nucleic acid, zinc ion, ATP and protein binding (FN1, MMP2, CSPG2, POSTN, ASPN, BGN, MXRA5, AEBP1, LRRC15, SULF2). The expression of several genes was decreased in tumor tissue: structural constituents of cytoskeleton (keratins 5, 14, 15, 17, 23), genes with transcription factor and regulator activity (ELF5, ID4, BHLHB3, TRIM29, FOSB), with protein serine/threonine and thyrosine kinase activity (ANXA1), with cytokine and growth factor activity (PTN, CX3CL1), genes involved in calcium regulation pathway (SFN, CAB39L, EGFR, DST, MGP), Wnt signaling pathway (SFRP1), cell differentiation and apoptosis (CLU, GPM6B, NDRG2), purine metabolism (AK5), cell adhesion (CAV2), also oxytocin receptor gene (OXTR).

Genes, differently expressed in lobular carcinoma and normal lobular cells, are involved in cell adhesion (E-cadherin), electron (TSTA3, LOC203427) and protein (VTI1B) transport, N-Glycan biosynthesis (DDOST), calcium ion binding (DEF6), ERBB2 signaling (IL6R), negative regulation of transcription (COBRA1), zinc ion (ARFGAP1), GTP and DNA binding (SPG3A), ubiquitin activity (USP53). Genes, differently expressed in ductal carcinoma and normal ductal cells, are involved in zinc ion and DNA binding (PCGF4, EGR1, RAB18), cell cycle and cell-cell adhesion (DLG5), apoptosis (SULF1), Wnt signaling pathway (TCF7L2), protein and heparin binding (TXNL2, CD59, LAMC2), transcription (ELF3).

CONCLUSION

One of the major differences between breast cancer and normal breast epithelium lies in the expression of genes involved in Wnt/Ca^{2+} signaling pathway. This pathway is important for both cell-cell and cell-matrix interactions and affects invasion and metastasis of cancer cells. Wnt signaling molecules may represent potential targets for cancer therapy.

ACKNOWLEDGEMENT

The work was supported by grants NR 7844-3 and MSM 6198959216.

A3 QUANTITATIVE ANALYSIS OF CYCLIN D1 EXPRESSION BY REAL-TIME RT-PCR IN BONE MARROW AND TISSUE SPECIMENS OF MANTLE CELL LYMPHOMA AND OTHER NON-HODGKIN'S LYMPHOMAS

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Key words: Mantle cell lymphoma/Non-Hodgkin lymphoma/Cyclin D1expression/ Quantitative real time PCR

AIM

Overexpression of cyclin D1 as a result of the chromosomal translocation t(11;14) is a highly specific marker for the diagnosis of mantle cell lymphoma (MCL). The aim of this study was to design a useful tool to quantify cyclin D1 transcript in fresh, frozen, formalin-fixed, paraffin-embedded tissue sections and bone marrow specimens.

A second aim was to develop a precise and reliable approach for distinguishing MCL from other non-Hodgkin's lymphomas (B-NHL).

MATERIAL/METHODS

We studied 107 primary tumours from patients diagnosed with a variety of B-NHL. These included 60 patients with MCL and 47 cases of other B-NHL, of which 26 had mucosa associated lymphoid tissue (MALT) lymphoma. In 36 specimens of MCL and 15 specimens of MALT lymphoma only formalin-fixed, paraffin-embedded tissue was available. In all remaining patients frozen tissue was analysed while lymph node tissue of five patients with a reactive lymphadenopathy was used as a control. In addition, we examined 100 bone marrow (BM) specimens. These consisted of 27 BMs with no MCL infiltration, 26 BMs with MCL infiltration, 25 BMs with no infiltration by other B-NHL, 19 BMs with infiltration by B-NHL other than MCL and 3 BMs from healthy individuals.

We used real-time quantitative reverse transcription-polymerase chain reaction (RQ RT-PCR) to quantify levels of cyclin D1 mRNA. The assay involved RT-PCR with product detection using TaqMan technology. RQ RT-PCR is a specific, sensitive and effective method of molecular analysis which permits monitoring the level of analyzed molecules.

RESULTS

The range of cyclin D1 expression in patients with MCLs exceeded the range found in other lymphomas and reactive lymph nodes by a considerable margin. Overall, the overexpression of cyclin D1 was detected in 58 of 60 MCLs. Increased levels of cyclin D1 mRNA was observed in several extranodal tissues of MALT lymphoma. This phenomenon can be explained by an admixture of non-neoplastic epithelial cells present in the specimens because epithelial tissues are known to express cyclin D1. All other lymphoproliferative diseases and reactive lymph nodes were found to have low or no cyclin D1 expression and were easily distinguishable from cyclin D1 overexpressing MCLs. There were no differences when expression levels in frozen tissues and formalin-fixed, paraffin-embedded material were compared. Measurement of cyclin D1 mRNA in BM specimens allowed us to differentiate BM from MCL infiltration and all other types of BM samples. All differences were statistically significant.

CONCLUSION

We were able to distinguish MCL from other B-NHL using this rapid, simple, and highly reproducible real-time PCR assay which is applicable to both routine archival material and bone marrow aspirates. Overall, the difference in cyclin D1 expression between MCLs and extranodal MALT lymphomas was statistically significant but the levels of expression overlapped to some extent. We therefore recommend correlating the level of cyclin D1 mRNA with morphological, immunohistochemical and cytogenetical profiles in cases in which only extranodal tissues are available.

ACKNOWLEDGEMENT

This work is supported by the Research project MZO 00064203/6704 and the Internal grant of faculty hospital in Motol 9756.

A4 PATHOLOGICAL FINDINGS OF UTERINE LEIOMYOMAS AND ADENOMYOMAS FOLLOWING UTERINE ARTERY EMBOLIZATION.

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Key words: Leiomyoma/Adenomyoma/Embolization/ Uterus

AIM

To assess histopathological findings in surgical specimens following uterine artery embolization.

MATERIAL AND METHODS

Between 2000 and 2005, 91 women were treated by uterine artery embolization because of symptomatic leiomyomas and prospectively followed at our institution. Twenty subsequently underwent surgery. One patient underwent four surgical procedures. Embolic material used was tris acryl gelatine microspheres (TGMS) in 15 patients (18 surgical specimens), polyvinyl alcohol particles (PVA) in three cases and a combination of both PVA and TGMS in another two cases. Twenty-three post-UAE specimens from 20 patients were evaluated. The material was routinely processed and sections from formalin-fixed paraffin-embedded tissue block were stained by hematoxylin-eosin. In addition selected sections in each case with

identifiable particles were stained with Elastica van Gieson and examined immunohistochemically with antibody directed against CD31 antigen

RESULTS

Histologically, of the 23 examined specimens, 20 revealed leiomyoma and 3 adenomyoma. Particles used for embolization were found in all but 3 specimens. Necrosis was present in 15/20 leiomyoma specimens. In 12 specimens it was hyaline necrosis, in 1, coagulative necrosis and in another 2 cases it was a combination of hyaline and coagulative suppurative necrosis. The 3 adenomyomas remained unaltered.

CONCLUSION

The morphologic changes we found in post-UAE specimens were comparable regardless whether TGMS or PVA was used. We should be aware of the morphological changes associated with arteficial embolization of various types of particles to avoid the misdiagnosis leiomyosarcoma especially in cases with coagulative necrosis, which can be accompanied by nuclear atypias and increased mitotic activity. In our study we failed to find any relation between extent of the regressive changes of leiomyomas and their size reduction. It is possible that other factors related to the host response to necrotic leiomyoma tissue play a role in the tumor size reduction.

A5 ULTRASTRUCTURAL MORPHOMETRY OF THE INTERNAL LIMITING MEMBRANE (ILM) IN THE LUCIA IMAGE ANALYSIS SYSTEM

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Key words: Internal limiting membrane/Ultrastructural morphometry/Diabetic macular edema/Idiopathic macular hole

BACKGROUND

The Vitreoretinal interface and the Internal Limiting Membrane (ILM) of the retina is a recent subject of concentrated interest in the pathogenesis and surgical treatment of diabetic macular edema (DME) and idiopathic macular hole (IMH) based on removal of the posterior hyaloid and ILM during microsurgery for. Complete release of traction forces and inhibition of reproliferation of fibrous astrocytes seem to be prudent in eyes with tractional maculopathy. Removal of the ILM leads to expedited resolution of DME, closure of the IMH and improvement in visual acuity.

Morphological identification of the peeled material as ILM and subsequent morphometry evaluation provides useful information about the role of ILM in the pathogenesis of these diseases.

MATERIAL AND METHODS

Ultrastructure of the peeled ILM from patients that underwent vitreoretinal surgery was analyzed using following protocol:

1) 2.5% glutaraldehyde fixed surgically peeled ILM was embedded into arteficial resin (Durcupan-Epon)
2) Semithin sections were stained with toluidine blue and ILM identified
3) Ultrathin sections were contrasted with uranyl acetate and lead citrate
4) ILM was photographed with the electron microscope YEOL 1200 EX under the standard enlargement 5000x with a 1 μm scale.
5) In the LUCIA G5 (Laboratory Universal Computer Image Analysis, Laboratory Imaging, Prague) the digitalized images were loaded, superimposed with a square grid of 500 px (= 3.25 μm). Any hit of the grid on a membrane was a place for the transversal ILM thickness measurement. The ILM of any patient with sufficient length of the membrane provided was measured on 10 photographs providing thus 40-50 dimensions for subsequent arithmetic mean + SD evaluation
6) MS Excel predefined table served the final arithmetic mean + SD evaluation.

RESULTS

In 18 from 20 patients the material supplied for evaluation was identified as ILM and measured. Mostly a substantial thickening of the ILM was found, especially in patients with DME (12 patients). It reached the thickness 1.68 ± 0.43 μm to 4.69 ± 0.30 μm (a norm being 0.5 μm).

Some cellular elements (mostly resembling macrophage) were observed on the vitreous side of the peeled ILM in both groups. The thickness of the peeled ILM in the DME group and the IMH group were significantly increased.

CONCLUSION

We conclude that ILM thickening and cell presence on the vitreous surface might contribute to the pathogenesis of the DME and IMH.

Ultrastructural morphometry of the ILM represents a tool for an objective vitreoretinal interface surgery evaluation and with the qualitative evaluation and patients' clinical status monitoring it is the basis for further study of the pathogenetic mechanisms in the traction maculopathy diseases.

A6 A RELATION OF THE P27 EXPRESSION TO OCCURRENCE OF GENETIC ABNORMITIES AT NOT GENERALIZED PROSTATE CARCINOMA.

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Key words: Chromosomal abnormalities/Carcinoma of prostate/p27

Published studies on prostate carcinoma report a strong connection between the c-myc gene amplification and immunohistochemical overexpression of the Myc protein. It is assumed that excessive Myc protein expression causes degradation of protein p27 resulting in activation of the pathway of cyclin E/cyclin-dependent kinase 2 and cell proliferation.

Since it is known that numerous chromosomal and genetic abnormalities are connected to prostate carcinoma progression, the aim of the study was to show whether any relation exists between protein p27 expression and various genetic abnormities. Given that in prostate carcinoma, chromosome 8 is most usually affected, deletions 8p22 and amplifications of zone 8q24 being the most frequent chromosomal changes, these were the most appropriate ones for evaluation.

Our findings are in complete accord with the literature. Degradation of protein p27 represented by expression in 0-25 % prevailed in a group of prostate cancers with a finding of chromosomal changes. In carcinomas with amplification of the c-myc gene, the finding was a significantly reduced expression of protein p27 in 89.47 %.

Table 1. Expression p27, Gleason score, Deletions 8p22, Amplifications 8q24, Polysomia of 8 chromosome

p27 % positiv nuclei	Gleason score (group)	Deletions 8p22	Amplifications 8q24	Polysomia of 8 chromozom
15	2	no	yes (AI)	no
5	2	yes	no	no
0	2	yes	no	no
0	2	yes	no	no
25	3	yes	yes	yes
25	3	yes	yes	yes
5	2	yes	no	no
0	3	yes	yes	yes
25	2	yes	yes	yes
5	2	no	yes	yes
0	3	no	no	no
25	2	no	no	no
5	3	yes	yes	yes
50	3	yes	yes	yes
5	2	no	yes	yes
0	2	no	yes (AI)	no
0	2	no	no	no
25	2	yes	no	no
5	2	no	no	no
25	3	yes	yes	yes
0	2	yes	yes	yes
5	2	no	no	no
5	2	yes	no	no
5	2	yes	no	no
25	2	yes	no	no
5	2	no	no	no
0	1	yes	yes	no
0	1	no	no	no
25	3	yes	yes	yes
5	1	no	yes	yes
25	1	no	no	no
25	2	yes	yes	no
75	1	yes	yes	yes
0	2	no	yes	yes
0	2	yes	yes	yes

A7 PROSAPOSIN DEFICIENCY – A RECENTLY RECOGNIZED COMPLEX LYSOSOMAL DISORDER

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Key words: Prosaposin deficiency/Lysosomal lipid storage/ Neuropathology

AIM

Definition of the molecular basis of a fatal infantile most probably underdiagnosed complex neurovisceral lysosomal disorder and description of its phenotype at all essential levels.

MATERIAL AND METHODS

Three postmortem studied cases, cultured fibroblasts, white blood cells and urine studied histologically, confocal microscopy, electron microscopy, enzyme and lipid biochemistry, lipid loading tests and molecular genetics.

RESULTS

The above disorder is defined by the absence of prosaposin (pSap) a multifunctional protein with trophic, mainly neurotrophic functions which is converted in lysosomes to four peptides (Saps), activating a set of six lysosomal sphingolipid hydrolases which are profoundly deficient even in wild type state. Absence of prosaposin is caused by mutation in its gene leading to either nuclear nonsense mediated decay (frame shifts) or interference with translation. At the lysosomal level the disorder is manifested in nonneuronal cells by absence of SAPs leading to deficiency of about six sphingolipid hydrolases which leads in turn to extensive multiple lipid storage encompassing ceramide, galacto- and glucocerebroside, globotriaosylceramide, lactosylceramide, sulphatide. Neuropathologic study of three cases of prosaposin (pSap) deficiency, ages at death 27, 108 and 119 days, carried out in standard autopsy tissues revealed neurolysosomal pathology different from that in nonneuronal cells. The lysosomes in the central and peripheral neurons were distended by pleiomorphic nonlipid aggregates which lacked specific staining and autofluorescence. Lipid storage was borderline on day 27 and in low abundance at later stages. Neurolysosomal storage was associated with massive ubiquitination that was absent in nonneuronal storage cells. Confocal microscopy and cross-correlation function analysis revealed positive correlation with late endosomal/lysosomal markers. We hypothesise that the neuropathology reflects disregulated influx of membrane components (either in bulk or as individual molecules) into neurolysosomes. Cortical neurons appear to be uniquely vulnerable to pSap deficiency. Whereas on the day 27 they populated the cortex, on days 108 and 119 they were replaced by dense populations of both glial phagocytes and astrocytes. We suggest that massive neuronal destruction reflects a cortical crisis precipitated by the lack of pSap. The results of our study may extend knowledge of the neurotrophic function of pSap that should be considered essential for the survival and maintenance of human neurons.

CONCLUSION

The disorder is most probably underdiagnosed. To the date six cases, has been diagnosed in five families. Simple diagnostic tests are recommended.

ACKNOWLEDGEMENT

The studies were supported by the project MSM 0021620806.

A8 SUBCLINICAL COURSE OF ADULT VISCERAL NIEMANN-PICK TYPE C1 DISEASE. A RARE CASE DIAGNOSED POSTMORTEM BY MOLECULAR GENETICS

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Key words: Niemann-Pick disease type C1 adult visceral type/Lysosomal storage

AIM

Diagnosis of an adult type of visceral lysosomal storage disorder in a 53 year old female patient who died of acute pulmonary embolism without significant prior health problems. Autopsy disclosed hepatosplenomegaly, lymphadenopathy with storage histiocytes originally interpreted as Gaucher cells.

MATERIAL AND METHODS

Formaldehyde-fixed paraffin-embedded tissues were available for analyses. We used standard histology and histochemistry together with DNA analysis of relevant genes using DNA extracted from the autopsy samples and from peripheral leukocytes of family members.

RESULTS

Samples of the liver, spleen and some lyphnodes were infiltrated by variously dense storage ceroid-rich vacuolized histiocytes. Initial suspicion of Niemann-Pick disease type B were excluded by showing normal ASM activity in obligate heterozygotes and by direct sequencing of the *SMPD1* gene which revealed no pathogenic sequence variations. This raised suspicion of the NPC disease. No pathogenic sequence variations were found in the *NPC2*

gene. Two novel sequence variations c.1997G>A (S666N) and c.2882A>G (N961S) were detected in *NPC1* gene not found in more than 300 control alleles. Final diagnosis: NPC1 disease.

CONCLUSION

The patient represents a third case of this extremely rare adult visceral variant of NPC1 disease (1,2) and it may be underdiagnosed.

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ACKNOWLEDGEMENT

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A9 THE EFFECT OF ANDROGEN RECEPTOR ANTAGONIST BICALUTAMIDE ON STATS EXPRESSION IN PROSTATIC CELL LINES.

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Key words: Prostate cancer/Androgen receptor/STATs/ Bicalutamid

BACKGROUND AND AIM

Proteins of the STAT family are transcription factors. Through binding to DNA specific sites and consequent regulation of gene transcription, these signaling proteins play an important role in many cell functions. Recent studies have demonstrated persistent activation of STATs and loss of their natural inhibitors SOCS and PIAS in various human neoplasias including prostate cancer. It has been found that prostate carcinoma cells lines expressing persistently activated STATs become dependent on their transcription factor and that experimental inhibition of STAT signaling in these cell lines lead to massive apoptosis. Therefore, experimental pharmacological or genetic modulation of their function offers a promising new approach in anticancer treatment. Moreover, to date, it is not clear whether STATs signaling is related to the hormone responsiveness of prostate carcinoma. The aim of our study was to evaluate the STATs expression profile in prostate cell lines (DU-145, PC-3, LNCaP and C4-2) and determine any changes after treatment by the androgen receptor antagonist, bicalutamide.

MATERIAL AND METHODS

The IC_{50} of bicalutamide was determined by the cell viability assay (MTT-test) and STAT1, phospo STAT1, STAT2, STAT3, phospo STAT3, STAT5 and phospo STAT6 expression was analysed by the Western blot technique.

RESULTS

Our results showed considerably lower expression of activated phospo STAT1, phospo STAT3 and phospo STAT6 in the hormone sensitive cell line LNCaP characterised by higher p27 expression, wild type p53 and less aggressive growth in contrast to the hormone negative cell lines DU-145 and PC-3. Treatment with IC_{50} of bicalutamide (80μm) led to reduced expression of STAT1, phospo STAT1, STAT 2, STAT3, phospo STAT3, STAT5 and phospo STAT6 expression in both hormone sensitive and in hormone negative cell lines.

CONCLUSION

These results clearly support the currently accepted theory that pharmacological reduction of STAT activity can lead to growth inhibition in prostatic cancer cell lines. We also demonstrated that STATs can be related to hormone sensitivity. The exact mechanism of this relationship however remains to be elucidated.

ACKNOWLEDGEMENT

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A10 STROMAL MICROENVIRONMENT IN MALIGNANT MELANOMA: AN IMMUNOHISTOCHEMICAL STUDY

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Key words: Stromal microenvironment/Malignant melanoma/Immunohistochemistry/Growth factors/Nestin

AIM

It is widely accepted that malignant transformation is caused by accumulation of genetic mutations of somatic cells which permit autonomous growth and the uncontrolled cell proliferation associated with loss of cell-cycle regulation. However, the behaviour of tumors is also influenced by its microenvironment. It has been discovered th0at tumor cells can regulate development of specific tumor promoting stroma and stromal cells reciprocically affect the differentiation of malignant cells. Changes in extracellular matrix composition, increased protease activity, cytokines and diffusible growth factor production, the presence of inflammatory cells, fibroblasts and increased angiogenesis are important factors determining tumor growth and invasion. The purpose of the present study was to evaluate the changes in the microenvironment surrounding malignant melanomas.

MATERIAL AND METHODS

Our study included superficial spreading (SSM) and nodular melanoma (NM) biopsies from 77 patients diagnosed at the Institute of Pathology between 2000–2005. As a control group, 43 benign pigment compound and intradermal nevi were examined. Immunohistochemical staining for VEGF-A, VEGF-C, PDEGF, bFGF, c-Myc and nestin were performed on formalin-fixed, paraffin-embedded tissue sections. The results were statistically analyzed by Chi-square test ($p < 0.05$).

RESULTS

Malignant lesions revealed enhanced expression of the proteins immunohistochemical staining exhibited mostly in fibrocytes, endothelial cells and some inflammatory cells, predominately macrophages and lymphocytes. Each protein was also detected in the neighbourhood of moles, but in lower levels. The differences between microenvironment immunoreactivity of these proteins in melanomas and benign nevi (VEGF C, $p < 0.0001$, PDEGF, $p < 0.0001$; bFGF $p < 0.0001$; c-myc, $p < 0.0003$; nestin, $p=0.0001$) were statistically significant except for VEGF-A.

CONCLUSIONS

Our study confirms that the investigated proteins have an impact on malignant stromal phenotype formation. Mild stromal changes were observed even in early staged melanomas but remarkable stimulation of stroma environment accompanied mainly advanced stages (Breslow III, IV, V). We conclude that stroma-tumor interaction can promote proliferation and dedifferetiation of malignant cells. These findings have important therapeutic implications.

ACKNOWLEDGEMENT

This work was supported by grant IGA MZ CR 1A8245-3 and MSM 6198959216.

A11 FIRST EXPERIENCES WITH IN VITRO CULTIVATION OF ASTROGLIAL CELLS FROM BIOPTIC SAMPLES OF BRAIN

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Key words: Astroglial cells/Cell culturel/Immunodetection

AIM

The aim of this work was to optimalise the cultivation of tumor and normal astroglial cells.

MATERIAL/METHODS

Tissue samples were taken from primary glioblastoma multiforme (grade IV) of a male aged 41 and from the

normal surrounding tissue from primary brain lymphoma of a female aged 61. Tissue samples were immediately homogenized and cultivated in medium DME F12 with 10% of foetal bovine serum and with addition of growth factors: epidermal growth factor (EGF), insulin, hydrocortisone and bovine pituitary extract (BPE). Cells derived from glioblastoma showed comparable level of growth in medium with and without growth factors. Cells isolated from nontumorous tissue needed growth factors for permanent cultivation . the first characterisation of isolated cells was achieved by immunocytochemistry and Western blot analysis using primary antibodies against: glial fibrillar acidic protein (GFAP), S 100 protein, vimentin, nestin, desmin, CD68 and PTEN. Antigens were visualised by fluorescent conjugates of secondary antibodies.

RESULTS

Cells isolated from glioblastoma showed higher growth than cells isolated from nontumorous tissue. Both types of cells were characterised by astroglial morphology with several long processes but tumorous cells were smaller and more elongated than nontumorous cells. After 60 days of cultivation, nontumorous cells grew very slowly almost stopped growing and changed morphology. Cells lost the ability to adhere and they detached from the surface. Nontumorous cells showed a limited lifetime probably determined by a limited number of cell divisions. Cell immortalization enables its cultivation. We aim to prepare stable transfection clones with gene for telomerase in hTERTpClneo plasmid. Both types of cells expressed S 100 protein (typical for cells of neural origin), and vimentin (marker of connective tissue and the precursor of GFAP in immature glial cells). The co-expression of vimentin and GFAP is described in mature astrocytes but never in ganglial cells. The absence of muscular marker – desmin and marker of phagocytic cells (oligodendrocytes in CNS) – CD68 exludes a muscular or oligodendroglial origin. The marker of stem cells – nestin was detected in nontumorous cells. In cells isolated from glioblastoma it was weakly positive, probably in connection with tumour dedifferentiation. GFAP is the main cytoskeletal protein of astrocytes in CNS and it was detected in the cytoplasm of tumor cells. No expression of GFAP in nontumorous cells was found. These results do not exlude less mature forms of astrocytes, where GFAP do not have to be presented. The expression of the tumor suppressor PTEN which is alterated in advanced stages of tumour progression was detected in both types of isolated cells.

CONCLUSION

Our results show the possibility of cultivating cells isolated from tumor and nontumor brain tissue. Establishment of stable nontumorous cell line is more difficult given the necessity of growth factors and immortalisation.

ACKNOWLEDGEMENT

This work was supported by grants: IGA MZ ČR NR/ 7828-3 and MSM 6198959216.

A12 POLYOMAVIRUS NEPHROPATHY: ANALYSIS OF INFLAMMATORY CELLS IN BIOPSY SAMPLES

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Key words: BK-virus nephropathy/Renal allograft biopsy/ Immunohistochemistry

INTRODUCTION

Polyomaviruses (BK and JC viruses named after the initials of the first patients with the disease) are ubiquitous infectious agents and around 90 % of adults in the general population have serological evidence of previous infection. Reactivation of BK virus infection can occur spontaneously or in individuals with a depressed immune response. BK-virus nephropathy (BKN) is also the most common viral disease in renal allografts. The disease outcome in this case is often unfavorable with functional deterioration and high risk of graft failure. Interstitial inflammatory cell infiltrates may be reactions to the viral infection or they may indicate acute rejection. Precise diagnostic interpretation of inflammatory changes in the setting of BKN in renal cortex is difficult, especially if the pathologist considers the “interstitial type of rejection” (I A or I B according to the Banff classification).

METHODS AND RESULTS

BKN was identified in 6 patients in 12 biopsies and 2 graft nephrectomy specimens of 1 115 biopsies between September 2000 and December 2003. The prevalence of BKN was 1.25 % in the graft biopsies. Definite virus identification was done by immunohistochemistry (monoclonal antibody α SV 40T, Oncogene, MA, USA; LSAB + kit, DakoCytomation, Denmark). Simultaneously CD 20, CD 45R0 and CD 68 positive cells in inflammatory infiltrate were detected by the immunoperoxidase double immunostaining method (α SV 40T, Oncogene, MA, USA; Histofine Polymer detection system, Nichirei, Japan; DAB, DakoCytomation, Denmark and α CD 20, α CD 45 R0, α CD 68 DakoCytomation Denmark, LSAB

+ kit, DakoCytomation, Denmark; AEC, DakoCytomation, Denmark).

The inflammatory infiltrate was composed mainly of a subpopulation of CD 45R0 positive cells. CD 20 positive B cells represented no more than 10 %. CD 68 cells were identified in the lumina of tubuli and scattered as a part of the inflammatory infiltrate.

CONCLUSION

At present, BKN is the most common viral disease affecting renal allografts with high risk of graft loss. Analysis of the inflammatory cells in our study showed that CD 45R0 positive cells represent the main subpopulation of lymphocytes without the morphological features of significant tubulitis. Macrophages and B cells were observed in low numbers as a part of the cooperating inflammatory cells. There is no simple and sensitive marker for recognizing inflammatory cells as due to rejection or infection. In the future pathologists will need to identify such a marker for indicating whether inflammatory cells are the result of alloantigen-dependent or – independent stimulation.

A13 INCREASED EXPRESSION AND SERUM LEVELS OF RESISTIN IN RELATION TO INFLAMMATION AND DISEASE ACTIVITY STATUS OF RHEUMATOID ARTHRITIS PATIENTS

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Key words: Adipocytokines/Resistin/Rheumatoid arthritis/ Osteoarthritis

AIM AND BACKGROUND

Resistin is an adipocytokine with proposed relation to insulin resistance, diabetes and obesity. Recently, its antiinflammatory properties have been suggested. The aims of the study were to evaluate the expression and localisation of resistin in synovial tissue samples from patients with rheumatoid arthritis (RA) and osteoarthritis (OA), to analyze serum and synovial fluid resistin levels and evaluate its association with inflammation and disease activity in RA patients.

MATERIAL AND METHODS

Synovial tissue was processed by routine paraffin technique and immunohistochemistry using a set of antibodies against resistin, CD20, CD3, CD138 and CD68. The results were confirmed with laser-confocal microscopy. Serum and synovial fluid samples were withdrawn simultaneously. The concentration of resistin was analyzed by commercial ELISA kits. Systemic inflammation was determined using high sensitive (hs) C-reactive protein measured by ELISA. The clinical activity of RA patients was assessed according the 28 joint count Disease Activity Score (DAS28).

RESULTS

Resistin was expressed in the lining, and particularly in contrast to OA samples, in the sublining layer of synovial tissue. It was localised predominantly to inflammatory cells such as plasma cells, B lymphocytes and macrophages. Serum and synovial fluid resistin levels were significantly higher in RA compared to OA patients (7.0 ± 3.0 vs. 4.4 ± 1.6 ng/ml, $p<0.001$ and 37.5 ± 13.4 vs. 1.6 ± 0.8 ng/ml, $p<0.001$, respectively). In RA patients, significantly higher resistin levels were observed in synovial fluid than in serum. In contrast to synovial fluid, serum resistin correlated with both CRP ($p<0.02$) and DAS28 ($p=0.05$) in RA patients. Resistin level did not correlate with leukocyte count in synovial fluid neither in RA ($r=0.17$, $p=0.49$) nor in OA patients ($r=0.17$, $p=0.49$).

CONCLUSION

Serum resistin may represent a new biomarker reflecting systemic inflammation and clinical disease activity in patients with RA. Moreover, increased expression of resistin in RA synovium suggests resistin plays a role in the pathogenesis of RA.

A14 ULTRASONOGRAPHICALLY CONTROLLED PROSTATE BIOPSY

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Key words: Carcinoma of prostate/Biopsy of prostate

OBJECTIVE

To evaluate the indications, techniques, strategies, complications and outcomes of the prostate biopsy performed in patients with suspected prostate cancer (PCa).

MATERIALS AND METHODS

Indications for prostate biopsy are either pathological findings on digital rectal examination (DRE) or more recently high levels of prostate specific antigen (PSA) or rapid PSA increase,PSA velocity (PSAV). Sextant biopsy was the standard method for a long time. Currently, according to the size of the prostate, a number of bioptic cores is taken (Vienna nomogram, multiplicated biopsy). Rebiopsy is indicated in cases of persistent suspicion of cancer after the first biopsy or unclear histological finding in the first biopsy. There are several clinically important and serious complications of prostate biopsy such as infection and haemorrhage. However, these occur in at a very low rate (< 1 %). Cancer detection is completely dependent on the indication criteria the prostate biopsy. Most authors report < 25 % cases.

RESULTS

As a result of the programme of early detection of PCa in the Olomouc region, we are registering a rising number of prostate biopsies at the Department of Urology, University Hospital in Olomouc. In 2003, there were approximately 350 biopsies, 720 in 2004 7, 1250 in 2005. We had less than 0.5 % complications. PCa detection was less than 25 %.

CONCLUSION

Prostate biopsy is a safe outpatient diagnostic method with minimal complications. From the viewpoint of a pathologist, the quality of the material acquired is crucial to determination of the histological type of tumor and Gleason score. These data have fundamental significance not only for prostate cancer diagnosis but also for predicting the course of the disease and treatment strategy.

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A15 STRUCTURAL ANALYSIS OF TISSUES AFFECTED BY CYTOCHROME C OXIDASE DEFICIENCY DUE TO MUTATIONS IN SCO2 GENE. CORRELATION OF STRUCTURAL, HISTOCHEMICAL, BIOCHEMICAL AND MOLECULAR FINDINGS

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Key words: Cytochrome c oxidase deficiency/SCO2 protein/Cardiomyopathy/Myopathy/Mitochondrial abnormalities

AIM

Cytochrome c oxidase (COX) deficiency represents a heterogenous group of disorders. In addition to the 13 structural subunits of this mitochondrial enzyme, there are numerous proteins encoded by nuclear DNA which are required for efficient COX assembly and maintenance. A structural and histochemical study was carried out in a series of 7 cases (from 5 families) with isolated cytochrome c oxidase deficiency caused by mutations in SCO2 gene. This research correlated phenotype at the clinical, histological, biochemical and histochemical levels with genotype.

MATERIAL AND METHODS

Paraffin-embedded, formaldehyde-fixed and frozen tissues from autopsy and less frequently from biopsy were studied using light microscopy, respiratory enzymes histochemistry, immunohistochemistry and electron microscopy.

RESULTS

The morphological study disclosed changes concentrated in the nervous system, skeletal muscle and myocardium in SCO2 dependent manner. Neurodegeneration and neuronal drop out were seen in the central nervous system in all cases. There is evidence that retinal neurons may be seriously affected, too. No typical necrotic lesions in the basal ganglia associated with Leigh syndrome were found. Involvement of motoneurons and peripheral nerves together with cytochrome c oxidase

deficiency in muscle tissues represent ,,double hit" for the skeletal muscle. There were 2 distinct phenotypes in the examined cohort of patients. In 5 patients with dominant neuromuscular phenotype the average life span was 9–12 months. All these children were homozygous for the missense mutation 1541G > A in the SCO2 gene (E140K). The mitochondrial population was not found to be significantly increased or structurally altered. In 2 other cases, the course of disease was more rapid (death at 7 and 11 weeks of life) and featured by early onset progressive cardiac hypertrophy (3- and 4- fold increase in heart weight). One patient was compound heterozygote for mutation 1541G>A and mutation 1280C>T, resulting in a premature stop codon (Q53X). The remaining patient had mutation 1541G>A and deletion 1518delA resulting in a frame shift and giving rise to a truncated protein. Cardiocytes displayed signs of hypertrophy and their mitochondria displayed both abnormal enlargement and alteration of the inner membrane. Similar mitochondrial alteration was identified in neurons of the retina available for study in onees in one. Biochemical and histochemical levels of COX in tissues corresponded with rapidity of clinical course.

CONCLUSION

In terms of pathology the overt structural phenotype of COX deficiency due to mutations in SCO2 gene in the studied cohort of patients can be characterized as neuromuscular degeneration with two subvariants differing in the degree of cardiac involvement. These variants correlate with two main genotypes described.

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A16 IMMUNOHISTOCHEMICAL DETECTION OF ACTIVE CASPASE 3 IN NEOPLASTIC CELLS OF CLASSICAL HODGKIN LYMPHOMA IN PREDICTION OF FAVOURABLE CLINICAL AND THERAPEUTICAL OUTCOME

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Key words: Classical Hodgkin lymphoma/Caspase 3/ Therapy response

AIM

Effective chemotherapy inducing regular apoptosis in Hodgkin lymphoma is linked with the proper function of apoptotic pathways, particularly of apoptotic effector caspase 3. Caspase 3 is activated through either the stress-induced pathway or the death receptor-mediated pathway. In some cases of classical Hodgkin lymhoma (cHL) the resistence to chemotherapy may be caused either by ineffective activation of apoptosis or by inhibition of apoptotic execution. High numbers of active caspase 3 positive neoplastic cells may reflect a regular activation of apoptosis and this phenomenon may result in favourable response to chemotherapy and in a better overall clinical outcome. The aim of the study was to investigate the correlation between active caspase 3 expression and response to therapy.

MATERIAL AND METHODS

We examined samples of primary cHL of 58 paediatric patients diagnosed from 2000–2005. All cases were reevaluated and the diagnosis of cHL was confirmed by morphology and immunohistochemistry (CD30, CD15) in all cases. Formalin-fixed paraffin-embedded tissues were examined immunohistochemically using a primary antibody against the active caspase 3.

RESULTS

The results revealed positivity in 0 % to 13.5 % of the neoplastic Hodgkin and Reed-Sternberg (H/RS) cells. In accordance with other investigators, the cut-off level to

report a patient as caspase 3 positive, was set at 5 % of positive H/RS cells. In the investigated group of patients 17 of 58 (29.3 %) were evaluated as positive. None of the 17 patients relapsed or died in contrast to 6 patients who had a disease relapse (14.6 %) and with 1 patient who died in the group with a low number of positive neoplastic cells.

CONCLUSION

Immunohistochemical identification of chemotherapy sensitive cases of cHL may bring a valuable benefit in a pretreatment stratification of patients with Hodgkin lymphoma and in selecting targeted chemotherapy and radiotherapy.

ACKNOWLEDGEMENT

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A17 EXPRESSION OF ERBB2 AND BCL-2 IN SYNOVIAL SARCOMA

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Key words: Synovial sarcoma/RT RQ PCR/ERBB2 expression/BCL-2 expression

AIM

Synovial sarcomas (SS) are a rare soft-tissue tumor that effect children and young adults. SS are high-grade soft tissue neoplasms often characterized by a biphasic spindle and epitheloid morphology. The majority of synovial sarcomas harbor a specific chromosomal translocation t(X;18)(p11.2;q11.2), which results in a fusion of the SYT gene on chromosome 18 and with SSX genes on chromosome X. Despite advances in the treatment of local disease, distant metastases remain a dominant cause of death. Therefore, there is a need for alternative therapies, targeting receptor tyrosine kinases –ERBB-2 and against cell cycle regulators- for example BCL-2.

MATERIAL AND METHODS

Archival specimens of synovial sarcomas (n=25) were assessed for BCL-2 and ERBB-2 protein expression by standard immunohistochemical techniques (IHC). To validate the results of IHC, real-time quantitative reverse transcriptase plymerase chain reaction (RQ-RT-PCR) assays using fresh and/or paraffin sections were performed. The results of IHC were assessed as diffuse and strong positivity (D3+), diffuse and moderate (D2+), diffuse and weak (D1+) and focal positivity (F1+ – 2+).

RESULTS

BCL-2 protein was detected by immunohistochemistry in 18/18 (100 %) – 3 cases with F1-D1, 10 cases with D2+ and 5 with D3+ positivity. ERBB-2 protein was detected in 9/18 (50 %) synovial specimens but with only weak focal positivity. Coexpression of BCL-2 and ERBB-2 molecules was observed in 9/18 (50 %) synovial sarcomas. We observed a mirror pattern in immunoreactivity of BCL-2 in biphasic synovial sarcomas. The positivity of epitheloid cells and negativity or a very weak positivity of spindle cells was detected in 4 cases, although in one, positivity of spindle cells with negative epitheloid cells was observed. RQ-RT-PCR demonstrated a massive content of mRNA for BCL-2 in 14/25 (56 %) cases, moderate in 28 % of BCL-2 and a weak in 16 % of synovial sarcoma specimens. The presence of mRNA for ERBB2 was detected in 16/24 (67 %) cases in contrast to the results of immunohistochemical techniques. 50 % patients with synovial sarcomas had a strong expression of ERBB2 proven by RQ-RT-PCR. We failed to observe any co-relation between the expression of ERBB2, BCL-2 and histotype or fusion gene SYT/SSX1 or SYT/SSX2.

CONCLUSION

BCL-2 and ERBB2 are expressed in a high percentage of synovial sarcomas. Treatment with antisense BCL-2 may be an appropriate alternative therapy for these patients. The treatment with tyrosine kinase inhibitors in connection with expression of ERBB2 is debatable given the low expression of the ERBB-2 protein.

ACKNOWLEDGEMENTS

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A18 INTRAVASCULAR AMYLOID OF THE GUT WITH CARDIOMYOPATHY IN GENERALIZED SENILE AMYLOIDOSIS.

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Key words: Cardiomyopathy/Amyloid/Gastric biopsy

In 1956, Symmers et al reported that the gastrointestinal tract was involved in 70 % of primary amyloidosis but he did not mention how frequently those patients suffered from cardiomyopathy. Since then the topic has been almost forgotten and the recent restrictions on autopsies in Britain have contributed to limitating further research in this field.

Cardiac amyloid is occasionally considered in patients with restrictive cardiomyopathy diagnosed on echocardiography but definitive diagnosis requires myocardial biopsy.

Our data comprise material from 12 cases of generalised amyloidosis collected over 10 years in the south of England. The majority was sudden death and the age varied from 74–97 years. The clinical information available was usually almost nil. The autopsy and the histochemical results showed typical myocardial changes together with the amyloid in the submucosal arterioles and veins of the digestive tract, particularly the stomach, also in the other organs except for the glomeruli.

The results show that the joint involvement of the blood vessels of the heart and the gut in senile amyloidosis is so common that in cases of cardiomyopathy it would be worth considering the biopsy of stomach rather than that of the myocardium, if amyloidosis is suspected, as the risk of complications is smaller with biopsy of the stomach.

A19 LONG-TERM EVALUATION OF IMMUNOGLOBULINS AND COMPLEMENT IN KIDNEY TISSUES USING Q DOT

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Key words: Renal biopsy/Immunofluorescence/Qdot

OBJECTIVE OF THE STUDY

The aim of the study was to determine how Q Dot conjugates prolong lifetime periods of positivity on sections where A- amyloid, IgG, IgA, IgM, C3, C1q complement, kappa and lambda light chains have been detected as detection of these proteins is used in the diagnosis of renal diseases. We identified these proteins using the one or two-step immunofluorescence detection method. The Q dot primary conjugate has been used for a detection of fluorescein conjugate antibodies for long – term observation of positive results.

MATERIAL AND METHODS

Tissues: 8 μm thick frozen sections were used for detection of A- amyloid, IgG, IgA, IgM, C3, C1q complement, kappa and lambda light chains. Sections were put on slides coated with gelatine and air dried. Detection of proteins: IgG, IgA, IgM, C3, (Sevapharma, Prague) C1q, κ and λ light chains (DakoCytomation, Denmark), were detected by polyclonal antibodies directly conjugated by FITC. Detection of A-Amyloid was made using two-step indirect immunofluorescence technique (monoclonal antibody, DakoCytomation; SwAM-FITC, Sevapharma). Using of Q Dot: We used Q Dot 655 Goat anti Fluorescein Conjugate for detection of antibodies conjugated by fluorescein.

RESULTS

Observation of detected proteins: Observation of proteins was possible in two ways. Either in fluorescence spectroscopy microscope with filter excitation 460–490 nm (FITC – green colour) or with filter excitation 510–550 nm (Q Dot – red colour).

Long-term evaluation: A sequence of pictures was prepared using both filters during 6 month. The evaluation revealed the long-term life of Q Dot detected proteins and on the other hand, as has been described and observed before, the short long-term life of FITC detected proteins.

CONCLUSIONS

Use of Q dot is useful for the study of proteins when there is a need for long time observation, especially when more than one pathologist evaluates it. It is also possible to observe sections repeatedly over time without loss of positivity. It is useful for archiving slides and in the training of pathologists as well.

A20 IMMUNOHISTOCHEMICAL AND GENETIC ANALYSIS OF OSTEOCLASTIC GIANT CELL TUMOR OF THE PANCREAS. A CASE REPORT

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Key words: Osteoclastic giant cell tumor/Histopathology/ Immunohistochemistry/Genetics/ Biological properties

Clinical, histopathological, immunohistochemical and genetic analysis of two osteoclastic giant cell tumors of the pancreas is presented. The neoplasms were composed of osteoclastic giant cells and pleomorphic cells. The tissue-specific markers provided evidence of the mesenchymal nature of the osteoclastic giant cells as well as other components of the tumor but lacked any signs of epithelial differentiation in either patient. The

non-epithelial nature of both components in the osteoclastic giant cell tumors may be associated with a better prognosis and this corresponds to previous reports for similar neoplasms. A positive immunoreactivity to neuron-specific enolase was recorded in patient 2.

The presence of CD68 in oteoclastic giant cells proved their histiocytic nature. Both components of the tumors showed a negative immunoreactivity to desmin and only a scattered reactivity to smooth muscle cell actin, typical markers of myofibroblastic differentiation.

Mutation analysis of the tumor revealed the wild state of both p53 and K-ras oncogenes in both patients. A positive immunoreactivity for p53 in pleomorphic cells of both osteoclastic giant cell tumors was recorded, while osteoclastic giant cells did not express this protein. The expression of p21 was recorded in osteoclastic giant cells in patient 1.

The absence of Ki–67 in the osteoclastic giant cells and its expression in pleomorphic cells gave evidence of a different proliferation rate in the two cell populations.

Different tissue – specific markers, a different proliferation rate, and a different state of oncogene activation in the osteoclastric giant cell tumors contribute to the idea that the tumor derives from a pluripotent cell that may differentiate into an array of phenotypes.

A21 DIFFERENTIAL DIAGNOSIS AND COURSE OF NEUROGENIC AND MYOGENIC CONDITIONS IN CRITICAL ILLNESS NEUROMUSCULAR DISORDERS: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Key words: Critically ill patients/Axoal polyneuropathy/ Myopathy/Course of the disease

Histopathological analysis of 52 biopsies from 43 critically ill adults, was focused on differential diagnosis and the course of illness in intensive care (poly)neuropathy (ICP) or myopathy (ICM). Needle or open biopsies were processed using a set of conventional histological and histo-chemical methods.

The results of electrophysiological examinations were unequivocal: 21 patients axonal polyneuropathy and 6 axonal polyneuropathy with myopathy. The results of the histopathological examination of biopsies evaluated strictly according to the criteria applied to critically ill patients were different. Only 2 patients had unequivocal neurogenic atrophy. The majority of biopsies revealed a myopathic pattern, simple or necrotic myopathy.

The results of histopathological examination, where the presence of scattered, fascicular or diffuse fibre atrophy including angular myofibres are considered signs of possible myogenic as well as neurogenic lesion, brought about a marked shift towards this mixed neuromyopathic pattern and an increased proportion of concordant diagnoses of the IC neuromuscular disorders.

A follow-up study showed that ICM and ICP develop soon after disease onset. In 9 patients, the bioptical examination was repeated and the histological picture of the lesion changed in all cases. When the second biopsy was performed several months or years after the 1st one, all myogenic features i.e. picture of simple or necrotizing myopathy disappeared and either recovery with normal histopathological findings or a picture of a persistent neurogenic lesion was found.

A22 GLEASON GRADING SYSTEM FOR PROSTATE CARCINOMA – OUR EXPERIENCE AND RECENT MODIFICATION.

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Key words: Prostatic carcinoma/Gleason system/Tumour grade

AIM

The Gleason system, the most widely used approach for grading prostate carcinoma, is based on evaluation of the microscopic structure (glandular pattern) of the tumour under relatively low magnification. One advantage of the Gleason system is that not only the highest grade of the tumour is evaluated. The system introduces a Gleason score, the total of primary (predominant) and the secondary (second most prevalent) architectural patterns that are identified and assigned a grade from 1 to 5, with 1 being the most differentiated and 5 being undifferentiated. The original Gleason system created 40 years ago has been refined and modified. The last modification was published in 2005 (Am.J.Surg.Pathol., 2005,29/9:1228). Within the last three years, we have collected and extensively examined a unique set of prostate carcinomas and in this presentation we summarize our experience in Gleason grading in this set of carcinomas. We also introduce the recent modification to the Gleason grading system.

MATERIAL AND METHODS

Tumour specimens from 189 patients who had carcinoma of the prostate were assessed. All patients underwent radical prostatectomy at the Clinical Department of Urology, 3rd Faculty of Medicine, Charles University in Prague and Teaching Hospital Kralovské Vinohrady, Prague, Czech Republic. In all cases, native prostate was transmitted directly from the operating theatre to the Department of Pathology for biopsy examination. After macroscopic description, gross dissection of the whole prostate was performed in accordance with the scheme published in 1994 (Am.J.Clin.Pathol.1994,102:572). Tissue blocks were then fixed with 10 % buffered formalin and embedded in paraffin for following the histological procedure. A Gleason score was assigned for histological slides stained with haematoxylin and eosin, independently by two pathologists (V.E., V.M.). The results were consequently compared and consensually unified.

RESULTS

The tumours were obtained from 189 patients, ranging in age from 50 to 79 years, mean 63 years. Tumours obtained from 113 patients were limited only to prostatic tissue (pT2), while tumours from 76 patients displayed extraprostatic extension into the periprostatic soft tissue (pT3). In the group of pT2 tumours we classified a Gleason score of 3 in 4 patients (4x 2+1), a score 4 in 25 patients (1x 1+3, 24x 2+2), a score 5 in 57 patients (41x 2+3, 16x 3+2), a score 6 in 15 patients (5x 2+4, 8x 3+3, 2x 4+2), a score 7 in 11 patients (10x 3+4, 1x 4+3) and a score 8 in one patient (1x 3+5). In the group of pT3 tumours we classified Gleason score 3 in one patient (1x 2+1), score 4 in 2 patients (2x 2+2), score 5 in 30 patients (14x 2+3, 16x 3+2), score 6 in 15 patients (10x 3+3, 5x 2+4), score 7 in 13 patients (9x 3+4, 4x 4+3), score 8 in 11 patients (5x 3+5, 3x 5+3, 3x 4+4) and score 9 in 4 patients (2x 4+5, 2x 5+4).

CONCLUSION

Our experience in the evaluation of the Gleason score for grading of prostatic carcinoma is in agreement with the data published in recent years. A Gleason score of 2 is in general classified very exceptionally. In our set, no carcinoma of this score was identified. The majority of carcinomas are moderately differentiated (Gleason score 5,6) and in our set, 62 % of tumours belonged to this group. Well-differentiated carcinomas (Gleason score 3,4) were relatively more frequent in the group of pT2 tumours, while poorly differentiated carcinomas (Gleason score 7,8,9) were found more frequently in the group of pT3 tumours. A Gleason score of 10 was not classified in any of tumours of our set. The last modification of the Gleason system for grading prostate carcinoma introduced in 2005 defines clearly the criteria for evaluation of the patterns characteristic for individual grades of tumour differentiation. Exact application of the rules accepted for prostate carcinoma grading in this modified scheme is important in diagnostic practice, to assign an appropriate score for particular tumours and to reduce the differences between the pathologists doing the evaluation of tumour grade.

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A23 HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY OF ARTICULAR CARTILAGE AFTER AUTOLOGOUS CHONDROCYTE IMPLANTATION

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Key words: Human cartilage defect/Autologous chondrocyte implantation/ Immunohistochemistry of chondrocytes/Actin-positive chondrocytes/Myochondrocytes

AIM

Spontaneous healing of focal lesions in cases of adult articular cartilage is very limited. There are two basic approaches to cartilage tissue engineering that have been proposed: Implantation of cell alone or in combination as a cell seeded scaffold to promote regeneration in vivo. The objective was to evaluate the healing of cartilage defects implanted with autologous chondrocyte –seeded onto an acid based scaffold (Hydrograft C) prepared in Italy

MATERIAL AND METHODS

10 patients with articular defects were implanted with Hydrograft C and second-look biopsies were analysed after removal from the joint 10 months following cell implantation. All patients showed clinical improvement and the macroscopic appearance at arthroscopy was normal or nearly normal in all cases. Histological examination and immunohistochemical staining were performed on formalin-fixed paraffin-embedded tissue.

RESULTS

The majority of the new tissue filling the defects was fibrocartilage and hyaline cartilage. Chondrocytes of both components stained positively with antibody for S-100 protein and all specimens also contained numerous alpha-smooth muscle actin(SMA)–positive chondrocytes predominating in fibrocartilage areas. In all samples were observed signs of reparation in the subchondral bone including loss of portions of subchondral bone plate and fibrous deposition in the marrow regions below. In some cases the remains of the degradable scaffold material were observed in the cytoplasm of bone marrow macrophages and rarely also in chondrocytes.

CONCLUSION

These findings confirm that the autologous chondrocytes implantation is a safe and effective option for the treatment of articular cartilage defects. Moreover, our results indicate that actin-positive chondrocytes (myochondrocytes) play important role during the healing of articular defects implanted with autologous chondrocyte.

A24 A CASE OF EXCESSIVE AUTOPHAGY WITH MULTIORGAN INVOLVEMENT AND LOW CLINICAL PENETRANCE. A NOVEL LYSOSOMAL DISORDER?

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Key words: Lysosomal pathology/Autophagocytosis/ Cardiomyopathy/Hepatopathy

AIM

Characterization of the lysosomal lesion in a female patient aged 74 with clinical history of ischemic heart disease with bilateral cardial hypertrophy, hypertension, AV block, chronic bronchitis, dyslipidemia (cured by Simvacard 20 mg/day), corticoid dependent rheumatoid arthritis (Prednison 5 mg/day), dyspepsia and diabetes mellitus type II, without manifest skeletal myopathy.

MATERIAL AND METHODS

Formaldehyde-fixed autopsy samples sent for consultation were processed for histology and electronmicroscopy. Paraffin samples were examined using routine stains and by immunohistochemistry using conventional and confocal microscopy.

RESULTS

A generalized lysosomal disorder with features of excessive autophagy was identified in the heart, liver, and gut smooth muscle cells. The lysosomal compartment (LAMP1, LAMP2 and Cathepisn D positive) displayed ubiquitination in cardiocytes and in smooth muscle cells. We speculate there was an increased lysosomal load caused by permanent autophagy of organelles as could be inferred from gradual shift of mitochondrial epitopes including subunit c of the mitochondrial ATP synthase into the lysosomal compartment.

CONCLUSION

The case may belong to a novel category of autophagic disorders with late-onset and multiorgan involvement (1, 2). Lysosomal membrane dysfunction due to LAMP2 mutation (Dannon disease) was excluded due to normal LAMP2 expression. Whether the process reflects primary dysregulation of autophagocytosis or is induced by a primary defect of mitochondrial membranes (or of membranes of other organelles) cannot be answered.

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A25 LYMFOEPITELIAL LESION OF THE LACRIMAL GLAND

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Key words: Lacrimal gland/Lymphoepithelial lesion/ MALT lymphoma

AIM

Case report describing an interesting case of lymphoepithelial lesion of the lacrimal gland.

MATERIAL AND METHODS

Surgically removed heterotopic and supernumerary lacrimal gland of a 50 years aged woman was bioptically examined by classical staining followed by immunohistochemical technques. Fluorescein in situ hybridisation was later also performed after excision and biopsy of a second lesion of the submandibular salivary gland.

RESULTS

Lymphoepithelial lesion was revealed in the excised lacrimal gland of the patient. Initial suspicion of MALT lymphoma was not confirmed at first by immunohistochemical analysis. Fluorescein in situ hybridisation, performed on the lacrimal gland specimen, later biopsy of submandibular salivary gland where the picture of MALT lymphoma was more apparent, revealed genetic changes characteristic of MALT lymphoma in some cells within the lymphoid infiltrate of the lacrimal gland. The initially uncertain diagnosis of MALT lymphoma in the lacrimal gland was finally confirmed in this way. No signs of local recurrence, nodal involvement or systemic disease were observed in this patient after a more than 1 year follow up.

CONCLUSION

Lacrimal gland is a rare but possible primary site of MALT lymphoma which may affect simultaneously or subsequently the salivary glands. Molecular pathology may become important and helpful in the final diagnosis of cases with an uncertain or doubtful picture using classical and immunohistochemical staining.

A26 COMMENTS ON THE MODIFIED TERMINOLOGY OF SQUAMOUS VULVAR INTRAEPITHELIAL NEOPLASIA (ISSVD, 2004): REVIEW OF 18 VIN I CASES

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Key words: Vulva/Vulvar intraepithelial neoplasia/ Terminology/Grade/Mild dysplasia/Human papillomavirus/Immunosupression/Cervical intraepithelial neoplasia/Lichen sclerosus/Lichen simples chronicus

AIM

Vulvar squamous precancerous lesions are currently classified according to the vulvar intraepithelial neoplasia (VIN) concept proposed by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 1986. The new modified terminology of VIN lesions was worked up and recommended for general use by the ISSVD Vulvar Oncology Subcommittee in 2004. According to this revision, the grading of VIN lesions should be abandoned. The VIN I category will no longer be used and VIN II and VIN III categories will merge. The term VIN will describe high grade squamous lesions only encompassing the VIN, usual type (former VIN II and VIN III) and the VIN, differentiated type (former VIN III). The aim of our study is to review the VIN I cases in our registry, to assess the prevalence of HPV types in these lesions and to discuss their possible biological behaviour with emphasis on the justification that the VIN I category should be abandoned from the classification scheme.

MATERIALS AND METHODS

The total of 18 lesions from 16 patients were enrolled in our study and divided in 3 groups: A. VIN I, usual type (4 cases, 4 patients), B. transition pattern VIN I – VIN II, usual type (6 cases, 4 patients) and C. foci of VIN I appearance in the field of inflammatory vulvar disorders (lichen sclerosus, lichen simplex chronicus) (8 cases, 8 patients). Each lesion was screened for the presence of HPV DNA by means of PCR with GP5+/6+ primers and reverse line blot assay was used for the detection of up to 37 different HPV types. The relevant medical history (presence of HPV associated high-grade VIN, all grades of CIN, carcinoma of the vulva, vagina and uterine cervix and immunological status) of all patients was analyzed.

RESULTS

The prevalence of HPV DNA in groups A and B was 100 %. No HPV DNA was detected in lesions classified under group C. Only high risk HPV types (16, 18, 33 or 45) were detected in HPV positive cases. The mean age of patients in combined HPV DNA positive group A+B was 40.6 years while in HPV DNA negative group C it was 61.9 years. In the combined A+B group 87.5 % of patients showed anamnestical data of high-grade VIN, usual type. No such diagnosis was disclosed in the history of patients in group C. In the combined group A+B 62.5 % of patients and 12.5 % of patients in group C had a history of CIN lesions. Fifty percent of patients from combined A+B group and none from the group C were immunocompromised (2x kidney transplantation, aplastic anaemia and systemic lupus erythematosus).

CONCLUSION

The results suggest that the category of mild vulvar squamous dysplasia may be appropriate to retain in younger women, in patients with a history of high-grade VIN, in women with a history of CIN as well as in immunosupressed patients. In these cases, lesions of VIN I appearance should be classified and managed as dysplastic. The pattern of VIN I in the field of vulvar inflammatory disorders represents more epithelial reactive changes than a manifestation of HPV related carcinogenetic pathway. Therefore, these lesions should not be classified as VIN I, usual type. Their significance in the pathogenesis of the VIN, differentiated type (VIN III) in the environment of lichen sclerosus – lichen simplex chronicus is to be further clarified.

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A27 ADAM8 AS A NOVEL SEROLOGICAL AND HISTOCHEMICAL MARKER FOR LUNG CANCER

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Key words: Adam 8/Lung cancer/Prognosis

AIM

To investigate genes involved in pulmonary carcinogenesis by examining gene expression profiles of non–small-cell lung cancers to identify molecules that might serve as diagnostic markers or targets for development of new molecular therapies. A gene encoding ADAM8, a disintegrin and metalloproteinase domain-8, was se-

lected as a candidate for such molecule. Tumor tissue microarray was applied to examine expression of ADAM8 protein in archival lung cancer symplex from 363 patients. A patient file of 300 lung cancer patients and 72 controls was used.

RESULTS

ADAM8 was abundantly expressed in the great majority of lung cancers examined. A high level of ADAM8 expression was significantly more common in advanced stage IIIB/IV adenocarcinomas than in adenocarcinomas at stages I–IIIA. None of the normal lung tissue including preinvasive lesions expressed ADAM 8. The relationship between expression of ADAM 8 and clinical parameters was being investigated.

DISCUSSION

We found that ADAM8 was abundantly expressed in the great majority of lung cancers examined. A high level of ADAM8 expression was significantly more common in advanced stage IIIB/IV adenocarcinomas than in adenocarcinomas at stages I–IIIA. This is to our knowledge the first study to investigate the immunohistochemical expression of ADAM 8 and correlate it to the clinical outcome in non small cell lung cancer patients.

Several studies have found that serum levels of ADAM8 were significantly higher in lung cancer patients than in healthy controls. The proportion of the serum ADAM8-positive cases defined by our criteria was 63 % and that for carcinoembryonic antigen was 57 %, indicating equivalent diagnostic power of these two markers. (2,3,6). Other authors used a combined assay with the benefits of both ADAM8 and carcinoembryonic antigen increased sensitivity because 80 % of lung cancer patients were then diagnosed as positive,whereas only 11 % of 72 healthy volunteers were falsely diagnosed as positive. In addition, exogenous expression of ADAM8 increased the migratory activity of mammalian cells, an indication that ADAM8 may play a a significant role (1,4,5) in progression of lung cancer.

CONCLUSIONS

These data suggest that ADAM8 should be useful as a diagnostic marker and probably as a therapeutic target.

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A28 WHAT IS THE NEWS ABOUT CARDIOMYOPATHIES

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Key words: Cardiomyopathy/Classification/ Etiology/ Arrhytomagenic right ventricular cardiomyopathy

During the last 20 years major advances have been achieved in our knowledge of cardiomyopathies (CMs), particularly the discovery of new CMs, update of the classification, and the understanding of etiopathogenesis.

Among new entities are arrhythmogenic right ventricular CM, primary restrictive CM, and noncompacted myocardium CM. In 1995, the WHO put forward a new definition of CM – "disease of the myocardium associated with cardiac dysfunction", replacing the previous "heart muscle disease of unknown etiology". What was considered for years to be idiopathic has been largely elucidated as having a genetic background (e.g. hypertrophic CM), or a viral etiology (e.g. dilated CM).

A29 THE RESULTS OF THE STUDY KAPROS IN THE REGION OF OLOMOUC

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Key words: Prostate cancer/Screening/Early detection/PSA

OBJECTIVE

The aim of the study KAPROS (carcinoma of the prostate) was to obtain elementary epidemiological urological data on the male population aged 50 to 70 years in the

Olomouc region with emphasis on prostate cancer, BPH and erectile dysfunction. This is an extensive study, also the first one of its size ever performed in the field of Urology in the Czech Republic.

METHODS

The study was prospective with data collected from 2852 respondents older than 40 years in the Olomouc region in the period June 2004 to September 2005 using a questionnaire.

RESULTS

On the basis of PSA elevation, prostate biopsy was indicated in 101 men out of 2852 respondents. Prostate cancer was subsequently confirmed in 24 samples.

The data showed that PSA level was ≤ 2.5 in 90 % men, 2.5-10 in 9 % and > 10 in 1 % only out of 2852 tested men.

No correlation of family history and PSA level was found.

The number of man with PSA level > 2.5 significantly increased with age.

Index fPSA/PSA ≤ 20 % was detected in 30.5 % men with a PSA level 2.5–10.

It was interesting to discover that there exists a statistically significant difference in distribution of the PSA level in smokers and non-smokers where the PSA level is higher in nonsmokers than in smokers.

CONCLUSION

Several interesting facts emerge from these data. The average incidence of CaP in the Czech Republic is 72/100 000. In our study, the male sample was only informed using a questionnaire. CaP was detected in 24 men which is an of 825/100000. This number may be altered by the selection of men (age 50–70 years), yet the number is striking.

The relatively low distribution of PSA levels in age groups does not exclude the existence of CaP [1]. Changes in PSA-PSA velocity (PSAV)[2] will be surely interesting. The higher rate of non-smokers with the PSA level > 2.5 was surprising. The PSA levels in smokers were statistically proved to be lower. This difference is 0.1 ng/ml and should be considered in the diagnosis of the prostate cancer.

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A30 GLOMANGIOPERICYTOMYOMA OF THE MEDIASTINUM

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Key words: Glomus tumour/Myopericytoma/Perivascular myoma/Mediastinum

BACKGROUND

Perivascular tumours comprise glomus tumour and myopericytoma. Tumours showing features intermediate between glomus tumour and myopericytoma have been designated glomangiopericytomas. Perivascular myoid cells can evolve into smooth muscle cells, tumours with this type of differentiation are known as perivascular myomas. Perivascular tumours are only rarely described in deep soft tissue or viscera.

DESIGN

We demonstrate a case of a 68 year old woman with perivascular myoma in the superior mediastinum which showed features of glomangioma, myopericytoma and angioleiomyoma.

RESULTS

A 68 year old woman was admitted to hospital for cough and thoracic pain. CT scan revealed a tumour in the superior mediastinum and the patient underwent surgery. The tumour was circumscribed and unencapsulated. The cut surface was fibromyxoid. Microscopically, the tumour showed a biphasic pattern that consisted of vascular areas with prominent branching vessels lined by small endothelial cells and myoid areas with fascicles of eosinophilic spindle cells. In vascular areas we found clusters of epitheloid cells with a peripheral rim of PAS positive material as well as elongated cells concentrically arranged around vessels. Immunohistochemically, these cells were positive for smooth muscle actin but not for desmin. Eosinophilic spindle cells in myoid areas showed coexpression of both markers. Mitosis and cytologic atypia were not seen.

CONCLUSION

To our knowledge this is the first case of mediastinal perivascular myoma described to date. Perivascular cells showed features of glomus cells and myopericytes: For this reason we prefer the designation glomangiopericytomyoma. Although these tumours can recur, their benign behaviour is assumed.

A31 PSORIATIC NEPHROPATHY

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Key words: Psoriasis/IgA nephropathy

BACKGROUND

It is generally is accepted that there no higher prevalence of renal diseases occurs in psoriatic patients with the exception of secondary amyloidosis in psoriatic arthropathy. In contrast it has been suggested that kidney diseases in psoriasis vulgaris may be more common than thought and this presumes the existence of psoriatic nephropathy.

DESIGN

We report a case of IgA nephropathy in a patient with psoriasis vulgaris as a contribution to the ongoing discussion of around the entity, psoriatic nephropathy.

RESULTS

A 62 year old man with a history of psoriasis vulgaris without evidence of psoriatic arthropathy was admitted to hospital for nephrotic proteinuria 6.74 g/day and moderate decrease in glomerular filtration rate with creatinine 213umol/l and creatinine clearance 0.95 ml/s. Kidney biopsy revealed IgA nephropathy, a type with diffuse mesangial proliferation and focal glomerulosclerosis, severe interstitial nephritis and arteriolosclerosis (CsaIV). After one month of treatment with Prednisone 1 mg/kg/day proteinuria decreased to 2.45 g/day and the skin lesion almost completely resolved.

CONCLUSION

About 10 cases of IgA nephropathy accompanied psoriasis were referred to in the literature. We report another interesting case of IgA nephropathy in a psoriatic patient as contribution to the discussion about the still hypothetical concept, psoriatic nephropathy. We recommend careful examination of kidney function and wider application of renal biopsy in psoriatic patients.

A32 PROGNOSTIC FACTORS IN OVARIAN CANCER AND CORRELATION WITH CLINICAL HISTORY OF THE DISEASE

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Key words: Ovarian carcinoma/Prognosis/Steroid receptors/Proliferative activity/Apoptosis/ Angiogenesis

INTRODUCTION

Ovarian carcinoma is the sixth most common female cancer in the world. This carcinoma carries the highest mortality of all gynecological malignancies. The high mortality is due mostly to the fact that the tumour is frequently diagnosed late, in advanced stages (III, or even IV). The most important prognostic factors in ovarian carcinoma are the extent of the tumour (stage), the size of residual tumour following operation, the presence vs. absence of ascites, the age and the general condition of the patient at the time of diagnosis, the histology of the tumour, and in the early stages, also the histological differentiation of the tumour (grade).

AIM

The aim of this study was to find immunohistochemically detectable significant prognostic and predictive markers for invasive ovarian carcinoma. There were three areas of research: (1) the expression of hormonal receptors by tumour cells; (2) the assessment of cell growth kinetics in ovarian carcinoma by examination of proliferation activity by means of antibodies Ki-67 and topoisomerase IIα, and (3) the assessment of the angiogenic potential of ovarian carcinoma by means of immunohistochemical detection of vascular endothelial growth factor, and by

quantification of microvessel density at sites of the highest angiogenic activity (hot spots).

MATERIAL AND METHODS

A retrospective study was carried out of 116 patients with histologically proved invasive ovarian carcinoma diagnosed at the Fingerland Department of Pathology during the period 1996–2003. The available clinical data on tumour stage according to the FIGO classification, the size of the residual postoperative tumour, and the length of survival after operation were summarized. The surgical specimens were routinely fixed in 10 % formalin, processed, embedded in paraffin and stained with hematoxylin-eosin. The histological type of the tumour and its grade were reviewed. The following panel of immunohistochemical reactions was applied: estrogen (ER) and progesterone (PR) receptors, proliferation antigens Ki-67 and topoisomerase IIα., and vascular endothelial growth factor (VEGF). The tumor angiogenic capacity determined by density of the capillaries was visualized by antibodies against von Willebrand factor – FVIII and against antigen CD34. An indirect immunohistochemical method using avidine–biotine visualisation system LSAB+ was used. The positivity of the reactions was evaluated by a light microscope. For ER and PR quantification a semiquantitative evaluation was used. The expression of Ki-67 and topoisomerase IIα was evaluated quantitatively in peripheral parts of the tumor. The VEGF was evaluated semiquantitatively. The quantification of capillaries comprised two steps: first, foci with the highest density of capillaries ("hot spots") were identified in slides stained with antibody against FVIII and CD34, under low magnification. This was followed by quantification of individual capillaries within the „hot spots". For statistics, the highest number of capillaries in the tumour and the average density of capillaries were considered. For statistical evaluation the program NCSS was applied. The time of overall survival was evaluated by Kaplan-Meier method, the individual factors were compared with log-rank test, and Cox method was used for multivariate analysis.

RESULTS

A total of 116 patients, aged 27–55 years (mean age 55.2 y.) were evaluated. The estrogen receptors were positive in 76 carcinomas (65.6 %), the progesterone receptors in 52 carcinomas (44.8 %). The proliferation antigen Ki-67 was expressed in 6.9 – 98.1 % of the tumour cells (median 41.9 %), and the proliferation antigen topoisomerase IIα in 4.1 – 92 % of the tumour cells (median 35.2 %). VEGF was positive in 80 carcinomas (69 %). The mean microvessel density was 27.8 – 213.9 capillaries per mm^2 (median 77.8) when using antigen FVIII, and 43 – 379.2 capillaries per mm^2 (median 150) when using antigen CD34. The maximal microvessel density was 33.3 – 229.2 capillaries per mm^2 (median 83.3) when using antigen FVIII, and 45 – 387.5 capillaries per mm^2 (median 164.6) when using antigen CD34.

In univariate analysis, age ($p = 0.02$), FIGO stage ($p = 0.000004$), residual tumor ($p = 0.0002$), grade ($p = 0.02$), Ki-67 ($p = 0.005$), topoisomerase IIα ($p = 0.04$), progesterone receptors ($p = 0.0008$), CD34 mean microvessel density ($p = 0.01$) and CD34 maximal microvessel density ($p = 0.05$), were all associated with survival. In multivariate analysis, the factors that were independent predictors of survival were only age ($p = 0.01$), residual tumor ($p = 0.0004$), FIGO stage ($p = 0.02$) and CD34 mean microvessel density ($p = 0.004$).

CONCLUSION

The presented data suggest that the only immunohistochemically detectable significant prognostic marker for invasive ovarian carcinoma is microvessel density by using antigen CD34.

ACKNOWLEDGEMENT

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A33 GENE EXPRESSION PROFILES IN MICRODISSECTED CELLS OF INVASIVE LOBULAR AND DUCTAL BREAST CARCINOMAS

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Key words: Breast/Lobular carcinoma/Ductal carcinoma/ Microarray/Laser/Microdissection

AIM

Invasive ductal and lobular breast carcinomas account for 80 % and 15 % of all invasive breast tumors, respectively. Both tumor types are derived from the terminal ductal-lobular unit of the mammary gland. However, despite a number of similarities, some clinical follow-up data and metastatic patterns suggest that they are biologically

distinct and some studies report differences in gene expression profiling. A major difference exists for example in expression of E-cadherin which is almost completely absent in lobular cancer. A few papers suggest several genes are differentially expressed in ductal and lobular tumors. The aim of this study was to extend knowledge of differential gene expression profiles in these carcinomas.

PATIENTS AND METHODS

We examined 10 mastectomy specimens from postmenopausal breast cancer patients. Of these 5 were invasive ductal, and 5 were lobular carcinomas. Tumor and normal tissues were snap-frozen in liquid nitrogen. Frozen sections were stained by hematoxylin in RNase-free conditions. Ductal and lobular tumor cells were microdissected from cryosections using Veritas™ Laser Capture Microdissection System. Fifty nanograms of total RNA were amplified by combined PCR and in vitro transcription. Samples were analysed by Affymetrix Human Genome U133 Plus 2.0 Arrays. Gene expression analysis was performed by Affymetrix Gene Chip Operating Software, dChip software and Internet databases.

RESULTS

Upregulated genes were as follows: ASPN (asporin), leucine-rich repeat protein with porin activity; EMP1 (epithelial membrane protein 1) involved in cell growth and proliferation; DVL1 (Dishevelled, dsh homolog 1) with signal transducer activity, involved in Wnt signaling pathway; ENC1 (ectodermal-neural cortex) participating in actin and protein binding; THBS4 (thrombospondin-4), adhesion molecule involved in calcium ion, protein and heparin binding; ANGPTL2 (angiopoietin-like 2) involved in receptor binding; ACACB (Acetyl-Coenzyme-A carboxylase-beta) important in fatty acid synthesis, nucleotide, biotin and ATP binding; FOXP1 (Forkhead box P1) participating in nucleic acid, zinc and metal ion binding, with transcription factor activity. Downregulated genes include PPP3CB (protein phosphatase 3), PCSK6 (proprotein convertase subtilisin), YWHAB (tyrosine 3-monooxygenase) and DDR1 (discoidin domain receptor family member 1) which are involved in calcium signaling pathway; IRAK1 (interleukin-1 receptor-associated kinase 1) possessing NF-kappaB-inducing kinase activity; PRKCI (protein kinase C, iota) participating in G protein and Wnt signaling pathways; CHML (Rab escort protein 2) and RHOU (RAS homolog gene family, member U) are important in GTP binding; ADNP (activity-dependent neuroprotector) with transcription factor activity and NRIP1 (nuclear receptor interacting protein 1) with transcription coactivator/corepressor activity, capable of nuclear hormone receptor binding. CDH1 (E cadherin) was downregulated in all lobular carcinomas.

CONCLUSION

Gene sets, differently expressed in invasive ductal and lobular breast carcinomas, are involved in cell growth and proliferation, calcium, G-protein and Wnt signaling pathways, nucleic acid, nuclear hormone receptor, zinc ion binding and transcription factor activity suggesting that invasive growth of ductal and lobular tumor cells may be mediated by different genetic mechanisms. Some of these genes may prove to be important for diagnostic purposes and as potential targets for therapy against specific tumor types.

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A34 P21^{WAF1} GENE ACTIVATION VIA SP1 FAMILY MEMBERS IN TGFBET A1-ARRESTED FOLLICULAR LYMPHOMA

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Key words: Cyclin-dependent kinase inhibitor/Follicular lymphoma/Transforming growth factor beta 1/Sp1/Sp3 transcription factor

AIM

Transforming growth factor beta1 (TGFbeta1) induces growth arrest in many cell types, including B lymphocytes. The inhibitory action of TGFbeta1 is mediated by deactivation of cell cycle machinery. Several feedback-sensitive pathways determine whether the cells are stopped in G1 phase or are allowed to leave the G1 phase and enter the S phase. The cell-cycle engine is tightly controlled by cyclin-dependent kinase (cdk) inhibitors which mediate extracellular negative signals, resulting in cell-cycle arrest at different G1 points. In this study, we tried to elucidate whether cyclin-dependent kinase inhibitors (CKIs) may become targets for the inhibitory signalling pathways induced by TGFbeta1.

MATERIAL AND METHOD

Our experimental DoHH2 cell line model was derived from a patient with malignant non-Hodgkin's lymphoma of follicular origin. The effect of TGFbeta1 on cell cycle progression was studied by flow cytometry. We examined

the effect of TGFbeta1 on the expression of $p21^{WAF1}$ by immunoblotting and RT-PCR. The binding activity of transcription factors to promoter of p21 gene was determined by gel mobility shift assay (GMSA).

RESULTS

Our results showed that the treatment of TGFbeta1 increased the number of cells which are arrested in the G_0/G_1 phase compared to untreated control cells. Moreover, we found that expression of $p21^{WAF1}$ was significantly up-regulated on the protein level after TGFbeta1 treatment. Similar to the protein level, the expression of p21 mRNA was increased in TGFbeta1 treated cells. We further examined binding activity of Sp family of transcription factors to examine their role in $p21^{WAF1}$ up-regulation.

CONCLUSIONS

The results indicate that $p21^{WAF1}$ overexpression in TGFbeta1-arrested malignant B cells is mediated by binding of Sp1 and/or Sp3 transcription factors to the (-92/-71), (-77/-58) and (-65/-45) elements of the promoter region of the p21 gene.

ACKNOWLEDGEMENT

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A35 CASE REPORT: 82-YEAR OLD MAN WITH MYELODYSPLASTIC SYNDROME PRESENTING AS A GENERALIZED CRYSTAL STORAGE HISTIOCYTOSIS

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Key words: Plasmocytoma secerning paraprotein/Crystal storage histiocytosis/Proper diagnostic approach

AIM

Would a proper diagnostic approach to an elderly patient reveal the diagnosis ante mortem?

BACKGROUND

82-year old man with clinical diagnosis of myelodysplastic syndrome lasting two years was admitted to the hospital dehydratated, febrile, with anaemia, leukopenia, thrombocytopenia, hypogammaglobulinemia and upper dyspeptic syndrome. The patient died after 3 days of bilateral bronchopneumonia and a consequent sepsis.

MATERIAL AND METHODS

The diagnosis of the myelodysplastic syndrome was made by an outpatient-department hematological specialist 2 years ago and this diagnosis was based on the clinical status and examination of the peripheral blood (hematocrite, vitamin B12, ferritin). Specifically, trepanobiopsy and bone marrow examination was not perfomed. The patient was treated with blood transfusions and corticosteroids. The autopsy was performed 17 hours after the patient's death. The patient was cachectic, had excentric heart hypertrophy with a diffuse myofibrosis, and bilateral bronchopneumonia. Hepatosplenomegaly (liver 2200 g, spleen 600 g) and moderate enlargement of the kidneys (350 g) were the most conspicuous gross findings. Microscopical examination of formalin-fixed, paraffin-embedded tissue from the bone marrow (vertebral body and femoral bone marrow), liver, spleen and kidneys stained by hematoxylin-eosin revealed excessive numbers of macrophages phagocytosing a crystalic material. Various staining methods including PAS, AB-PAS, Giemsa, Gram, Masson's trichrom and mucicarmine, immunohistochemical (light immunoglobulin chains kappa and lambda, CD68, MUM1, CD138) and electronmicroscopical examinations were utilized to identify the origin of the crystals. The bone marrow was also examined using Gömöri silver impregnation.

RESULTS

The bone marrow was hypocellular, trilinear without dysplastic changes and without fibrosis. The crystals were found phagocytosed in macrophages, and also extracellularly inside the renal tubules. The crystals were of a proteinaceous origin, had a shape of hexagonal biprismas. They revealed no autofluorescence and the polarized light showed no signs of birefringence. The crystals were positive in all stainings except for mucicarmine. The immunohistochemical examination of bone marrow revealed a disperse population of monoclonal plasma cells with a kappa light chain restriction. Their amount was estimated to form, at least focally more than 15 % of cells of the bone marrow.

CONCLUSION

The autopsy findings, together with extensive microscopical and immunohistochemical examinations identified the proper cause of the disease and contributed to an appropriate diagnosis: plasmocytoma secerning paraprotein and causing a generalized crystal storage histiocytosis. In case, if the patient had had the bone marrow investigation and appropriate biochemistry tests performed at the beginning of his initial symptoms, this would have led to a proper diagnosis and treatment.

ACKNOWLEDGEMENT

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A36 IS THERE ANY DIFFERENCE IN GLEASON SCORE BETWEEN PROSTATE BIOPSY AND PROSTATECTOMY SPECIMEN?

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Key words: Prostate carcinoma/Biopsy/Retropubical radical prostatectomy/Gleason score

OBJECTIVE

The growth in incidence of prostate carcinoma is evident as the population grows older.

If the CaP is detected in time, there are various treatment strategies. One curative treatment possibilities is retropubic radical prostatectomy (RRP). Apart from the clinical stage, histopathological grading (Gleason score) plays a crucial role in the indication for this surgical treatment and potential subsequent therapy (hormonal/radiotherapy).

The aim of this study was to evaluate the correlation of Gleason score from the biopsy of the prostate and the specimen after retropubical radical prostatectomy (RRP) in patients with carcinoma of the prostate.

METHODS

We retrospectively reviewed the records of 364 patients (age 35–74 years) who underwent RRP for prostate cancer from 1997 to 2004: 339 patients were evaluated. Gleason score data were collected from the prostate biopsy and subsequent retropubical radical prostatectomy specimen.

RESULTS

Correlation GS from biopsy and RRP specimen (n = 339)

GS – biopsy	correlation	higher	lower
2–6 (n =273)	47 (17,2 %)	206 (75.5 %)	20 (7.3 %)
7–10 (n = 66)	31 (47 %)	20 (30 %)	15 (21 %)
Total (n = 339)	78 (23 %)	226 (66.7 %)	35 (10.3 %)

CONCLUSION

Correlation of the GS is only 23 % which raises the question whether the pre-operative Gleason score is a reliable predictive factor. Only the Gleason score made on a specimen after RRP allows us to prediction the possible future tumor progression. The outcomes of the non-surgical methods of treatment (radiotherapy, brachytherapy, HIFU) are also probably influenced by the low correlation.

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A37 EXPRESSION OF MISMATCH REPAIR PROTEINS (MLH1, MSH2) DOES NOT PREDICT MALIGNANT POTENTIAL OF COLON ADENOMAS.

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Key words: Colorectal carcinoma/Adenoma/Mismatch repair system/Immunohistochemistry

AIM

Mismatch repair gene defects are the mainstay of the hereditary non-polyposis colorectal cancer syndrome. However, they are also present in aproximately 10–15 % sporadic cancers. The literature on these findings in sporadic adenoma of the large bowel is scarce.

PATIENTS AND METHODS

We analysed 118 sporadic colorectal adenomas from 118 patients. Basic histology was followed by an immunohistochemical analysis of both main proteins of MMR system: MLH1 and MSH2. The immunohistochemistry results were compared with the following parameters of adenomas: malignant potential (assessed by the size of adenoma, histology and grade of dysplasia), location of adenoma in the bowel, type of growth, and age of patient. We also compared the results with those obtained in a small group of patients with colorectal carcinoma.

RESULTS

Immunohistochemical examination revealed loss of MLH1 protein in 11 adenomas (9.3 %) and loss of MSH2 in 6 adenomas (5.1 %). We observed two cases of synchronous absence of both MLH1 and MSH2. Therefore, the total number of adenomas without MLH1 and/or MSH2 was 15 (12.7 %). In 15 cases of patients with colorectal cancer, loss of MMR proteins was found in two cases (13.3 %). When we compared the results of immunohistochemistry with the characteristic of adenomas, we found no correlation between expression of MMR proteins and grade of malignant potential, location of adenoma in the bowel, type of growth, or age of patient. The results of immunohistochemistry were comparable with analysis of microsatellite instability in a small subgroup of patients analysed for this parameter.

CONCLUSION

We found no relationship between MLH1 and MSH2 expression in colorectal adenomas and their malignant potential, location in the bowel, type of growth, and age of patient. In this regard, adenomas differ from findings described for colorectal carcinomas.

A38 "POLYMYOSITIS" IN PATIENTS WITH MYASTHENIA GRAVIS AND THYMOMA – RATHER A PARANEOPLASTIC EVENT THAN A REAL PRIMARY AUTOIMMUNE INFLAMMATION

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Key words: Myasthenia gravis/Polymyositis/Thymoma/ CD45RA

AIM

Lymphocytic infiltrate in muscle of patients with myasthenia gravis (MG) and thymoma was reported repeatedly as a coincidental autoimmune polymyositis. In this study we aim at providing evidence that most cases of such a "polymyositis" do not represent a myositis *per se,* but only a paraneoplastic event.

MATERIAL AND METHODS

An excision from the sternothyreoid muscle taken during thymectomy was examined histopathologically in 28 patients with diagnosed MG and thymoma. Provided that lymphocytic infiltrates were found, we performed immunohistochemistry to establish their immunophenotype (CD20, CD3, CD4, CD8, TdT, CD68). Further, an antibody against CD45RA antigen (physiologically present in B-cells and mature but naive T-lymphocytes) was applied. We also searched immunohistochemically for the expression of HLA-ABC antigens on the surface of the muscle fibers. The immunophenotype of thymoma-lymphocytes was also analyzed. The findings in muscles were compared with those obtained in 14 patients with definite polymyositis.

RESULTS

In all polymyositis cases, the inflammatory infiltrate was composed mostly of CD8+CD4- T-lymphocytes and scat-

tered macrophages and there was a strong diffuse expression of HLA-ABC antigens found on the surface of muscle fibers. All the CD8+ cells were CD45RA negative.

In 16 patients with MG and thymoma the lymphocytic infiltrates were identified in muscles and they were morphologically indistinguishable from those in polymyositis. However, the expression of HLA-ABC was limited to the muscle fibers close to the lymphocytic infiltrates, all other fibers were negative. The lymphocytes were CD8 positive, but a small proportion of them co-expressed the CD4 antigen. The CD8+ T-lymphocytes were simultaneously CD45RA positive. The thymic tumors of the 16 patients were all thymomas of type B or AB. No lymphocytes were found in muscles in MG patients with type A thymoma. Although most of the intratumoral lymphocytes were TdT+ immature T-cells, we have identified a proportion of CD8+CD45RA+ cells admixed to the thymocyte-population. Clinically, the MG patients with the lymphocytic infiltrates in muscle did not differ significantly (both in the preoperative presentation and in the follow-up analysis) from those without the infiltrates. In both groups, the muscle weakness improved after the thymoma removal.

CONCLUSION

It has been shown previously that the proportion of CD8+CD45RA+ lymphocytes is significantly increased in the blood of patients with thymomas (Hoffacker et al., 2000). We identified CD8+CD45RA+ T-cells in cortical thymomas and demonstrated that the cells of the polymyositis-like lymphocytic infiltrates in muscles have the same immunophenotype, different from that of polymyositis. Therefore, we suggest that the lymphocytic infiltrates in patients with MG and thymoma represent more likely a paraneoplastic event due to the "spillover" of thymoma-derived mature naive T-cells than a real cell-mediated autoimmune disorder. The finding CD8+ CD45RA+ lymphocytes in muscle biopsies (especially in MG patients) should not be interpreted as a polymyositis, but should lead to the exclusion of an underlying thymic neoplasm.

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A39 PRIMARY LYMPHOMA OF THE SMALL INTESTINE

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Key words: Primary lymphoma/Small intestine

AIM

To evaluate the frequency and histological types of lymphomas of the small intestine in the biopsy register at the Institute of Pathology in Brno over the last five years.

MATERIAL AND METHODS

A retrospective study was performed on 9 surgical pathology cases of the small intestine. In seven cases there were lymphomas of the jejunum, in one case the terminal ileum was infiltrated and in one case the lymphoma infiltrated the ileocoecal region. Tumors were examined by light microscopy, immunohistochemistry and the FISH method. The patients were admitted mostly with recent abdominal symptomatology, intestinal obstruction or perforation.

RESULTS

DLBCL (difuse large B-Cell lymphoma) predominated in the series and had the same histological pattern as lymphomas of the lymph nodes. Bcl-2 was positive in a range from < 1 % to 80 % of cells. Bcl-6 was positive (> 10 % of tumor cells) in 3 of 5 cases.

In 2 cases of follicular lymphoma, infiltration of surrounding lymph nodes and soft tissues around the intestine were found. Histologically, with follicular arrangement of the tumor cells, grade 1. Immunohistochemistry showed CD20, CD79a, CD10, Bcl-2 positivity; CD5, CD45RO negativity. FISH method for translocation t(14;18) was positive in more than 90 % of cells in both cases. One case had MCL (mantle cell lymphoma) morphology in duodenal region. The lymphoma cells were immunoreactive for CD20, CD79a, CD5, cyclin D1 and negative for CD20, CD23, CD10. One case of ALCL (anaplastic large cell lymphoma) was diagnosed with the immunoprofile: CD3, CD45RO, CD43, CD30 positivity; CD20, CD79a, CD23, CD10 negativity. More than 90 % of tumor cells were Ki-67 positive.

CONCLUSIONS

Nine primary lymphomas of the small intestive were examined by light microscopy, immunohistochemistry and FISH method. The most frequent lymphoma type was DLBCL. Lymphomas were Bcl-6 positive in 3 cases and all CD10 negative. They are probably derived from germinal and non-germinal center B-cells. Rarely, 2 follicular lyphomas penetrated to the lymph nodes and soft tissues around the intestinum. According to literature prognosis is better than for lymph node localization. In our cases the clinical follow-up was only several months.

CARCINOMA PRECURSORS IN LARYNGEAL RESECTIONS

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Key words: Larynx/Histopathology/Dysplasia/Immunohistochemistry

In a group of 30 laryngeal resections with invasive spinocellular carcinoma the authors studied the unchanged mucous structures in the supraglottic, glottic and subglottic areas. Mucosal samples were taken and their examination was focused on standard dysplastic changes using basic colouring hematoxylin-eosine and other special methods. Immunohistochemical examination (p53, Ki 67, p16) showed positivity only in advanced stage dysplasia and in carcinoma in situ. Chronic inflammatory changes were commonly found. The authors suggest that viral aetiology should not be underestimated along with other risk factors.

INTRODUCTION

Human papillomaviruses (HPV) are associated with a range of epithelial localisations, especially in the areas of the cervix uteri, skin, oropharynx and larynx. Laryngeal tumours are among the most common ones in ENT. Condyloma formations and condyloma-like lesions can be found, as well as squamous papilloma, focal epithelial hyperplasia, dysplastic and neoplastic changes. In the laryngeal area, neoplasia occurs most commonly in the supraglottic and glottal areas and subglottal localisation was found to be very rare[7]. Laryngeal carcinoma accounts for 1 % of all tumours and 0.73 % of all lethal malignant diseases are due to laryngeal carcinoma[13]. The average annual incidence of laryngeal carcinoma in the Czech Republic is 5.09 cases per 100,000 of the total population. The ethiopathogenesis of the laryngeal cacinoma is multifactorial with multicentric occurence and „field cancerization"[15].

MATERIALS AND METHODS

The selected study group (cohort) consisted of 30 individuals with laryngeal carcinoma – 29 men and 1 woman aged 54. The age of the men ranged from 41 to 80 years with an average age 57.3 years. The majority of the selected individuals were smokers who occasionally used alcoholic beverages.

Samples from the 30 laryngeal resections were examined histopathologically and were described both macroscopically and microscopically following fixation in 10% formalin (Pictures 1, 2). The tissues were processed by the paraffin method and other special methods (PAS, Gomori, VG) and immunohistochemical methods focused on showing changes suggestive of tumor proliferation (p52, Ki67, p16) (Pictures 3–5).

Following the diagnosis of a tumor lesion (in all these cases this was spinocellular carcinoma with invasion of varying degrees of differentiation), histopathological examination focused on macroscopically intact mucosal parts with standard samples taken from the supraglottis, glottis and subglottis. The distance of the site where samples were taken from to the localisation of carcinoma was at least 10 mm. Cases not complying with this criterion were excluded.

Mucosal samples from macroscopically nontumorous sites were examined in detail with the focus being on epithelial changes as hyperplasia, metaplasia and dysplasia, and examined using basic, special and immunohistochemical methods.

RESULTS

The histopathological findings in individual laryngeal areas are shown in Table 1. Assessment of the histopathological examinations was focused on chronic inflammatory changes, the occurence of acanthosis, parakeratosis, hyperplasia and squamous-cell metaplasia. The frequency of the aforementioned changes shows the prevalence of chronic inflammation in the glottal area while chronic inflammatory changes in the supraglottal area were found to be significantly lower. Other changes – acanthosis, parakeratosis and epithelial hyperplasia were more common in the supraglottal area. Squamous-cell metaplasia was assessed only in those places where it replaced normal respiratory epithelium. The occurrence of squamous-cell metaplasia in glottal and subglottal areas was 15.6 %.

Dysplasias were found 56 % of all samples in supraglottal areas while in glottal areas only 15.2 %. This difference was highly statistically significant (Table 2). It was not possible to statistically assess the other comparisons due to the presence of zero occurences in one area.

Table 1. The frequency of finding specifications in individual sites.

Finding specification	Supraglottis		Glottis		Subglottis	
	N	%	N	%	N	%
Normal finding	0	0.0	1	3.0	3	9.3
Chronic inflammatory changes	2	5.8	20	60.6	24	75.0
Acanthosis	2	5.8	0	0.0	0	0.0
Parakeratosis	3	8.8	1	3.0	0	0.0
Hyperplasia	6	17.6	0	0.0	0	0.0
Squamocellular metaplasia	0	0.0	6	18.2	5	15.6
Dysplasia total	19	55.9	5	15.2	0	0.0
light	15	44.3	3	9.1	0	0.0
medium	2	5.9	2	6.1	0	0.0
heavy	2	5.9	0	0.0	0	0.0
Carcinoma in situ	2	5.9	0	0.0	0	0.0
Number of findings total	34	100.0	33	100.0	32	100.0

Table 2. The results of statistical comparison of dysplasia findings in individual sites.

Comparison of dysplasia findings in individual sites		
Supraglottis: glottis	Glottis: subglottis	Supraglottis: subglottis
$X^2_{(1)} = 15.322$ **P = 0.0000**	Not possible to evaluate	Not possible to evaluate

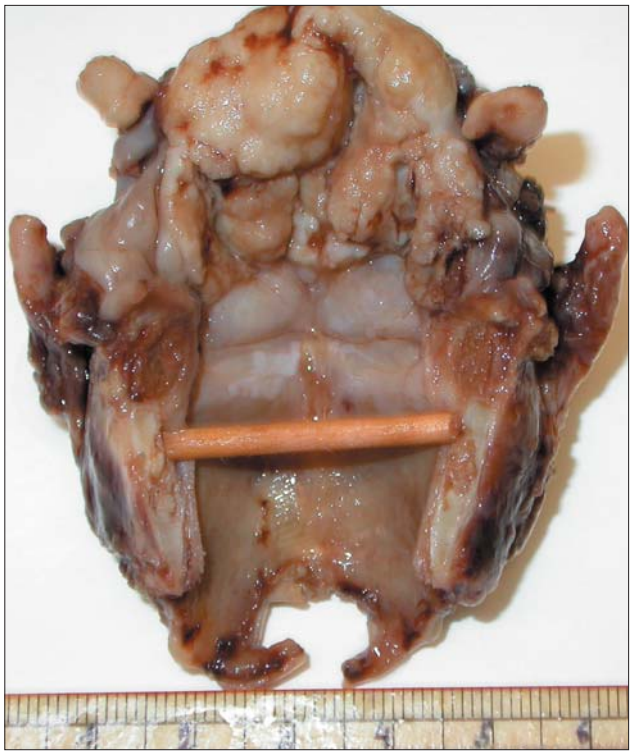

Fig. 1. Laryngeal resection – supraglottic form of carcinoma

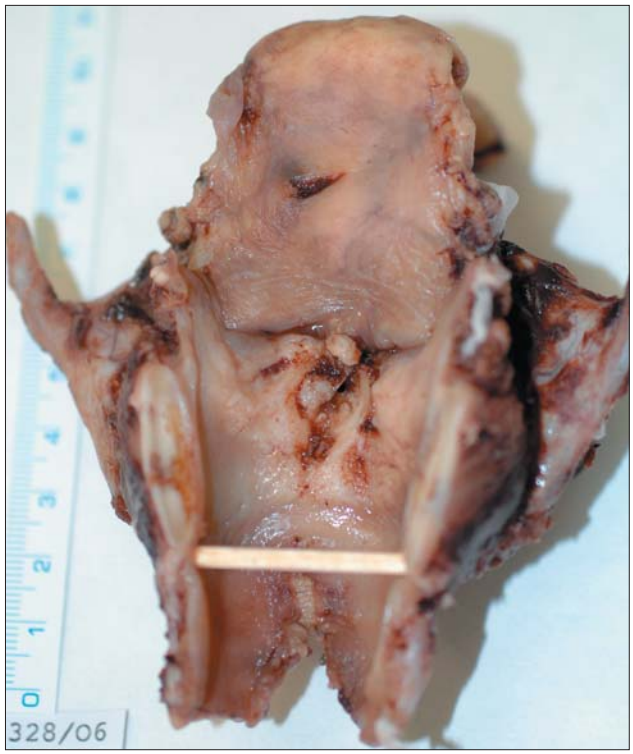

Fig. 2. Laryngeal resection – glottic form of carcinoma

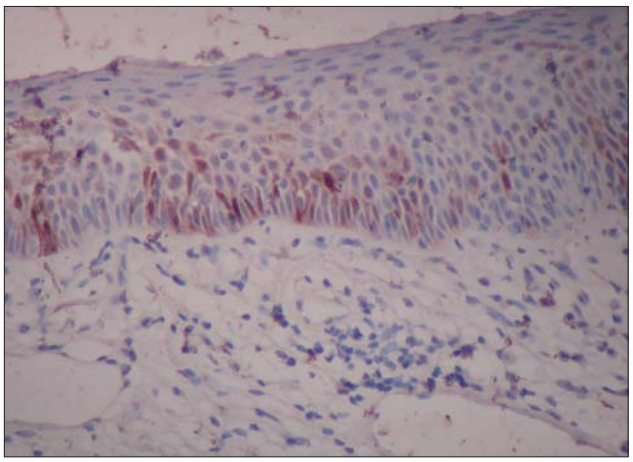

Fig. 3. Laryngeal mucosa, medium dysplasia, p16, 200x

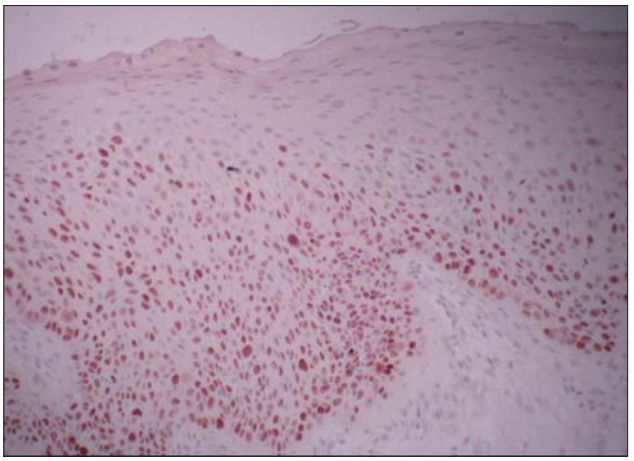

Fig. 4. Laryngeal mucosa, heavy dysplasia, p53, 200x

Immunohistochemical examinations (p53, Ki67, p16) were applied to tissue samples with histologically proved dysplastic changes using hematoxylin- eosine. Immunohistochemical examination in dysplastic lesions including carcinoma in situ (CIS) revealed positivity in 10 cases: using applied antibodies, p53 positivity was found in 5 cases, Ki67 positivity in 3 cases and p16 positivity in 2 cases. Chronic inflammatory changes were common in the total laryngeal areas studied and round cell cellulisation with pevailing presence of plasmocytes was noted. Squamous cell epithelium showed, especially in supraglottal parts with hyperplastic changes in basal layers, and parakeratosis and acanthosis in 5 cases. Koilocytar changes were found in one case. Squamous-cell metaplasia replacing normal respiratory epithelium was found in 11 cases of larynxes examined.

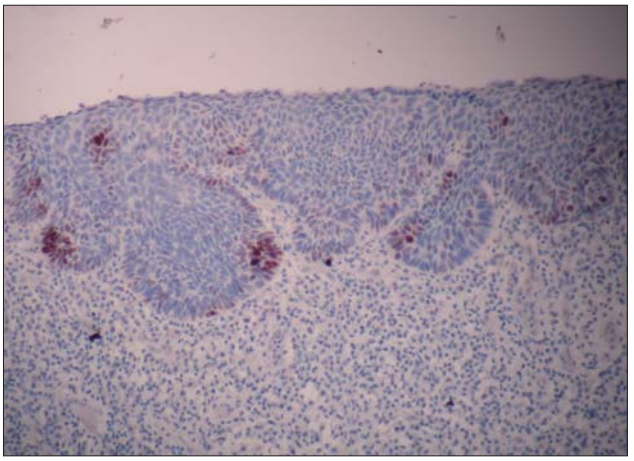

Fig. 5. Laryngeal mucosa, carcinoma in situ, p53, 200x

DISCUSSION

Analysis of the histopathological findings follows from previous studies which assess histopathological findings in laryngeal carcinoma and mucosal changes[4,5]. Amongst factors that play an important role in dysplastic and neoplastic changes of the larynx, HPV infection is to be ranked alongside those of age, mouth hygiene, cancerogenic substances, tobacco and alcoholic beverage consumption[2]. Studies assessing dysplastic laryngeal changes published in the literature are well-known and distinct using hematoxylin-eosine[12]. With further study of these lesions, immunohistochemical examination has been increasingly used in both tumorous and nontumorous lesion analysis. It is important to detect HPV DNA in papillomatous carcinoma structures, where HPV 11 is confirmed[14]. Immunohistochemical changes show frequent positivity of p53 and p16 detection[16]. By using Ki67 and p53 in the diagnosis of adenosquamous carcinoma, squamous and glandular components can be differentiated[1]. Using p53 expression, a prognosis of squamous cell carcinoma can be made from examination of the oral region[8]. Tumorous changes of the laryngeal mucosa are studied using of molecular genetics that have shown loss of heterozygote status and thus the period of survival can be more precisely determined. Accumulation of p53 is in accordance with survivin expression an apoptosis inhibitor in laryngeal squamous carcinomas. Expression lies in protein inhibition[11]. Stromal fibromyoblasts positive in SMA and CD34 are suggested for differentiation of stromal mucosa changes in atypical hyperplasia and squamous laryngeal carcinoma[13]. Maspin, a tumor suppressor gene correlates with p53. It plays an important role in laryngeal carcinoma especially in making a prognosis. Cytoplasmatic expression of maspin is found in 47 % of cases. Nuclear localisation of maspin is a good prognostic factor in the diagnosis of spinocellular carcinoma[9].

Our findings revealed using hematoxylin-eosine dye, the histopathological changes with dysplasia that have taken place and we were able to determine the degree of disease progression. The benefit of immunohistochemical methods has been proved only for dysplastic lesions of a higher degree (in advanced dysplasia and CIS). Dysplastic changes are more common in the supraglottal areas, koicylocytar changes were found in supraglottal area in one case only.

Chronic inflammatory changes prevail especially in glottal and subglottal areas. These histopathological findings corresponding to acanthosis and parakeratosis do not exclude a viral aetiology. These findings were present especially in the supraglottal areas. Squamous cell metaplasia was found most commonly in the glottal areas. In association with these findings other aetiologic agents presenting as risk factors in the larynx can be taken into account, especially smoking, the mutagenic effects of tobacco, especially the smoke from cigerettes and the drinking of alcoholic beverages, which are also suggested by the results of our study. The effect of spirits (drinks with higher alcohol content) is obvious especially in supraglottic carcinoma[3].

Based on the results it is possible to predict that cancerogenesis of the epithelium in the supraglottic areas can be greatly influenced by risk factors that dominate over and above the viral aetiology.

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BONE MARROW ANGIOGENESIS IN MULTIPLE MYELOMA AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOETIC CELL TRANSPLANTATION.

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Key words: Multiple myeloma/Angiogenesis/Bone marrow/High-dose chemotherapy

We examined 40 cases of multiple myeloma (MM) treated with high-dose chemotherapy and autologous transplantation of hematopoetic stem cells. We focused on comparing micro vessel density of bone marrow tumor infiltration at preliminary trepanobiopsy and after treatment in a 4–6 month time interval. The blood vessel sections (2–3 μm thick) were labeled with anti CD34 (DAKO, clone QBEnd 10) antibody, counted using a calibrated counter at 400x magnification on a 0,141 mm^2 surface area for each sample, using three different locations which at general magnification showed greatest micro vessel density (hot spots). The average vessel number was then correlated to 1 mm^2 surface area. The SPSS, v. 10.1 (SPSS Inc., Chicago, USA) software was then used for statistical analysis. The results were compared using a two-sample t-test at the 0.05 confidence level. A significant decrease in vessel number following therapy was discovered during the control trepanobiopsy compared to the preliminary examination in patients in remission and with residual disease, regardless of MM differentiation level. In contrast, patients with disease progression and relapse following a transitory remission showed a significant increase in angiogenesis. Decreased micro vessel density is believed to be due to the chemotherapeutic reduction of the tumor infiltration.

INTRODUCTION

Angiogenesis in the stroma of solid tumors has long been a topic of interest. It plays an important role in the development, progression and dissemination of malignant tumors. Within the group of hematological malignities, the issue of angiogenesis is extensively studied in multiple myeloma (MM), especially in relation to disease progression[4, 8, 10, 11, 14, 17, 19]. We ourselves compared the micro vessel density in MM depending on its morphological differentiation level and bone marrow infiltration type[15].

It is assumed that the induction of angiogenesis from a pre-existing vascular network depends on the balance between a number of angiogenic activators and inhibitors in the microenvironment of the tumor. To date, more than 20 endogenous angiogenesis promoters are known, and only a few less factors with an inhibitory effect. These are produced by tumor cells, as well as by stromal and inflammatory elements[2, 5, 7, 18].

It seems that in MM, VEGF (vascular endothelial growth factor), which is secreted by myeloma cells, plays a key role[3, 5, 6, 9, 12]. All isoforms of VEGF have tyrosine kinase VEGFR (vascular endothelial growth factor receptor) 1, 2, 3 receptors which are expressed on the endothelial surface of blood and lymphatic vessels as well as hematopoetic progenitor cells. It is likely that angiogenesis is the result of interaction between malignant cells and endothelium by means of paracrine production of angiogenic factors. In our work, we focused on evaluating the effect of high-dose chemotherapy and autologous transplantation of hematopoetic stem cells on micro vessel density in MM.

MATERIALS AND METHOD

We studied a set of 40 patients with newly diagnosed MM. After high-dose chemotherapy and autologous transplantation of hematopoetic cells, a control biopsy was performed within 4 to 60 months after transplantation. The patients were divided into 4 groups based on their reaction to therapy: remission, residual disease, without morphological response, disease progression. As well, 4 patients who relapsed after a transitory remission were evaluated.

All trepanobiopsy samples taken from the hipbone were fixated in 10% formalin, decalcified with 10 % chelatone and embedded in paraffin. Sections of 2–3 μm were stained with HE and a series of special stains (Giemsa, PAS, Fe, Gomory, naphthol ASD chloroacetate esterase). Immunohistochemical analysis was performed using the monoclonal antibodies CD138 (DAKO, clone MI-15), light chains of Ig kappa (IMMUNOTECH, clone KP-53) and lambda (IMMUNOTECH, clone HP-6054) for determination of the type, quantity and clonality of the myeloma cell infiltration. The capillaries were labeled with anti CD34 antibody (DAKO, clone QBEnd 10). The DAKO Cytomation En Vision + system labeled Polymer – HRP set was used for visualization.

Table 1: Remissio

		AGE (y.)	Tim since month	Vessels. / mm^2 biops	Vessels. / mm^2 remissio
G	N	9	9	9	9
	Minimum	49	9	45.0	26.0
	Maximum	62	33	111.2	42.6
	Median	56.00	11.00	61.50	35.50
	Mean	55.67	16.22	74.14	34.71
	Std. deviation	4.56	9.40	26.12	5.42
G	N	3	3	3	3
	Minimum	34	9	59.2	33.1
	Maximum	64	16	78.1	47.3
	Median	62.00	12.00	71.00	37.90
	Mean	53.33	12.33	69.43	39.43
	Std. deviation	16.77	3.51	9.55	7.22
G1 + G	N	12	12	12	12
	Minimum	34	9	45.0	26.0
	Maximum	64	33	111.2	47.3
	Median	56.00	11.50	66.25	35.50
	Mean	55.08	15.25	72.97	35.89
	Std. deviation	8.21	8.35	22.74	5.95

Table 2: Residual Disease

		AGE (y.)	Time since trans months	Vessels / mm^2 prelim biopsy	Yessels / mm^2 – resid. Dis.
G1	N	7	7	7	7
	Minimum	4	6	59.2	42.6
	Maximum	6	4	99.4	56.8
	Median	55.00	11.00	75.70	47.30
	Mean	55.29	14.29	76.09	48.67
	Std. deviation	6.05	11.94	13.68	5.08
G2	N	3	3	3	3
	Minimum	4	6	85.2	42.6
	Maximum	5	3	146.7	56.8
	Median	52.00	8.00	104.10	54.40
	Mean	52.33	16.67	112.00	51.27
	Std. deviation	6.51	16.77	31.50	7.60
G3	N	5	5	5	5
	Minimum	4	8	106.5	33.1
	Maximum	6	3	149.1	142.0
	Median	56.00	9.00	127.80	63.90
	Mean	54.60	13.80	128.26	71.46
	Std. deviation	8.02	11.86	19.62	43.38
G1+G2	N	1	1	1	1
+G3	Minimum	4	6	59.2	33.1
	Maximum	6	4	149.1	142.0
	Median	55.00	9.00	99.40	47.30
	Mean	54.47	14.60	100.66	56.79
	Std. deviation	6.44	11.95	30.55	25.95

Tab. 3: Without Morphological Reaction to Therapy

		AGE	Time since trans	Vessels /mm^2 prelim	Vessels / mm^2 post th.
G	N	5	months 5	biopsy 5	5
	Minimum	45	4	80.5	71.0
	Maximum	65	28	101.8	106.5
	Median	52.00	9.00	92.30	82.80
	Mean	54.60	14.20	92.32	87.56
	Std. deviation	8.20	11.01	9.62	14.09
G	N	3	3	3	3
	Minimum	51	6	89.9	56.8
	Maximum	65	60	106.5	120.7
	Median	55.00	27.00	99.40	77.30
	Mean	57.00	31.00	98.60	84.93
	Std. deviation	7.21	27.22	8.33	32.63
G	N	1	1	1	1
	Minimum	56	35	146.7	142.0
G1 + G2 + G	N	9	9	9	9
	Minimum	45	4	80.5	56.8
	Maximum	65	60	146.7	142.0
	Median	55.00	24.00	99.40	82.80
	Mean	55.56	22.11	100.46	92.73
	Std. deviation	6.93	18.31	19.33	26.62

Tab. 4: Disease Progression

		AG	Time since month	Vessels / mm^2 biops	Vessels / mm^2 post th.
G	N	4	4	4	4
	Minimum	56	12	42.6	99.4
	Maximum	64	24	63.9	108.9
	Median	61.00	20.50	50.90	102.95
	Meann	60.50	19.25	52.08	103.55
	Std. deviation	3.42	5.25	8.86	4.89

Capillary density was evaluated using the calibrated counter at 400x magnification over a 0.141 mm^2 surface area. Each sample was evaluated this way in three different locations, which under general magnification displayed the greatest micro vessel density (so-called hot spots). The average vessel number was then correlated to a 1 mm^2 surface area. Histological grading of the myeloma cells was performed according to criteria set by Bartlem et al.[1] for G_1, G_2, G_3. The prevailing myeloma cell infiltrated marrow type was then evaluated. 2 basic infiltration types were seen in the patients studied: interstitial and nodular. Interstitial infiltration was commonly seen as part of the nodular infiltrations. However, it was deemed insignificant when evaluating micro vessel density because vascularization here was always significantly lower than in the nodular zones.

SPSS, v. 10.1 (SPSS Inc., Chicago, USA) software was used for statistical analysis. Angiogenesis at preliminary biopsy and at control biopsy after treatment were compared using a two-sample t-test at a 0.05 significance level.

RESULTS

Results are summarized in tables 1–4.

1. Remission at the time of control biopsy was present in 12 patients (9 – G_1, 3 – G_2). The average vessel number at preliminary biopsy was 73/mm^2, at control 36/mm^2. This difference is statistically significant (0.0002).
2. In all 15 cases of residual disease (7 – G_1, 3 – G_2, 5 – G_3), a significantly lower vessel number was observed in comparison to preliminary examination (0,0001). The average vessel number at preliminary biopsy was 101/mm^2, at control 57/mm^2.
3. In the group of 9 patients (5 – G_1, 3 – G_2, 1 – G_3), where no morphological change in the quantity and type of infiltration was observed after therapy application, the two-sample t-test did not show a statistically significant difference in vessel number (0.325). The average vessel number at preliminary biopsy was 100/mm^2, at control 93/mm^2.
4. A morphological progression of the disease was observed in 4 patients. These were mostly G_1 MM cases, where at preliminary biopsy interstitial infiltration was present and after therapy nodular infiltrations were observed. The average vessel number at preliminary examination was 52/mm^2, at post-therapy control 103/mm^2. The difference is statistically significant (0.004).
5. Disease relapse after transitory remission was observed in 4 patients with G_1 MM. In remission, the average vessel number was 36/mm^2, in relapse 73/mm^2. This difference is also statistically significant (0.010).

It can therefore be stated that the micro vessel density in bone marrow infiltrations proportionally increases or decreases in direct correlation to morphological changes in the infiltrations induced by chemotherapy. Morphological remission and residual disease with quantitative infiltration decrease was characterized by a significantly lower micro vessel density; contrarily, disease progression and relapse led to a significant increase in vascularization.

DISCUSSION

The effort to refine the prediction and prognosis of patients with malignant tumors leads to searching for new factors, which may bring new knowledge in this sense. In the last years, considerable attention is focused on angiogenesis, not only in solid tumors, but also in hematological malignancies. It seems that similarly to solid tumors, hematooncological diseases exhibit more pronounced neovascularization in morphologically less differentiated and thus biologically more aggressive tumors[8, 10, 11, 13, 14, 19].

When we evaluated micro vessel density in MM in our previous work[15], we observed that neovascularization is greater in less differentiated forms of MM, which are usually manifested by the nodular type of infiltration. In well-differentiated MM with interstitial infiltration, the vessel density is much lower. Evaluating micro vessel density is therefore, along with the differentiation level of myeloma cells and infiltration type, another morphological prognostic marker.

This observation is also in accordance with the results of our study, which focused on evaluating the reaction of high-dose chemotherapy and autologous transplantation of hematopoetic stem cells on micro vessel density. It is almost surprising how unambiguously it may be stated that in patients where therapy induced remission, or at least decreased the quantity of infiltration, there was a significant decrease in micro vessel density. Contrarily, disease progression and relapse always led to a significant increase in vessel number. The question remains whether chemotherapy acts directly on vascular endothelium, or whether the effect is mediated by a reduction in tumor infiltration with decreased paracrine production of angiogenic factors. We can state that the degree of angiogenesis may also be of potential therapeutic value. Thalidomide and a number of other newly developed substances, which are currently being evaluated in relation to therapeutic benefit in namely refractory or relapsing forms of myeloma, are characterized by their antiangiogenic effect. Evaluating angiogenesis in trepanobiopsy may contribute to the determination of patients suitable for antiangiogenic therapy in the future.

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