B. J. Lü · M. D. Lai · M. Dietel

An overview of the Sino–German symposium on new developments in surgical and basic pathology

Received: 8 December 2004 / Accepted: 8 December 2004 / Published online: 12 April 2005
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Introduction

For more than 100 years, a longstanding intense relationship based on many mutual activities has become part of Chinese and German medical history. For example, in 1907, Dr. Erich Paulun, a German doctor working in Shanghai around the turn of the 19th century, founded the first “Deutsche Medizinschule für Chinesen” (German Medical School for Chinese), later the Medical Faculty of the Tongji University (Shanghai and Wuhan). There, the tradition of a German-based medicine has been upheld until today, leading to student education with lectures in the German language. In Germany, many Chinese doctors have been and still are working while influencing the medical development of many fields. Traditional Chinese Medicine, for example, is very popular in Germany and adds a lot of stimulating ideas to so-called Western medicine. Due to the political situation, there was a period of silence beginning with World War II up to the 1980s, and, only after the policy of openness, could the co-operation be re-established step by step by strong activities from both sides. In the field of anatomical and surgical pathology, the recent years were dominated by the intention to share knowledge and skills as well as to exchange young colleagues. For example, a telepathology project was initiated between Shanghai, Chongching and Berlin, supported by the National Science Foundation of China and the Deutsche Forschungsgemeinschaft. To give these activities a framework, the authorities of the German and the Chinese Society of Pathology agreed to establish continuous interactions, e.g. with the presence of representatives at the annual meetings of each society. Among other reasons, this

was to reflect the dramatically increasing importance of the People’s Republic of China in the scientific community.

As a first step, a Sino–German Symposium on New Development in Surgical and Basic Pathology was held in Hangzhou, China, between 22 April 2004 and 24 April 2004. Attending this symposium were 23 leading pathologists from both countries, supported by the Sino–Germany Science Center and chaired by Professor Maode Lai (Zhejiang University, China) and Professor Manfred Dietel (Humboldt University, Germany). This meeting not only covered a comprehensive update of recent developments in surgical and basic pathology but also provided a general aspect of these fields in both countries. It intended to establish a forum to foster the communication and collaboration for pathologists of China and Germany. This report summarises the essential information from the meeting.

Basic pathology

Professor Heinz Höfler (Technische Universität München, Germany) introduced overall aspects of molecular pathology in Germany. Professor Dietel gave an overview of DNA array technology in diagnostic pathology. This technology may provide molecular signatures for individual tumours as potential powerful indicators for diagnosis, therapy response and prognosis, etc. It will become possible to determine the biological behaviour of a given tumour much more precisely, for example, to predict the potential to metastasise, the resistance to cytostatic drugs or the individual prognosis. The diagnostic of genomic signatures may lead to a new approach of functional surgical pathology. Professor Lai reviewed the state of the art with regard to proteomics in cancer research and briefly mentioned one novel protein that overexpressed in colonic cancers in comparison with corresponding normal colonic mucosa and adjacent mucosa to cancers, detected by 2-D electrophoresis in the up-to-date study of his group on proteomics of colorectal cancer.

Professor Jie Zheng (Peking University, China) showed his group’s extensive and intensive studies on a novel gene-

B. J. Lü · M. D. Lai
Department of Pathology, School of Medicine,
Zhejiang University,
Hangzhou, PR China

M. Dietel (✉)
Institute of Pathology, Charité,
Berlin, Germany
e-mail: manfred.dietel@charite.de

tumour metastasis-related gene (TMSG-1, Genebank accession: NP_037516; gi:7019445), which his group first discovered. This gene was first surrogated by mRNA differential display polymerase chain reaction (PCR) separately on four prostate cancer cell lines or subclones (*BE1/LH7* and *IE8/2B4*), as overexpressions in two subclones (*LH7* and *2B4*) with low invasive and metastatic capacity that had previously been verified by tumorigenesis and spontaneous metastasis in nude mice; then, its full-length cDNA sequence was obtained via sequencing and ESTs assembly, and its chromosomal location (1p) was also disclosed. Eventually, this gene was reconfirmed by Northern-blot technique on the four cell lines or subclones and prime in situ labelling on *2B4* subclone. Monoclonal antibody of TMSG-1 was produced using a standard hybridoma technique. Semi-quantitative reverse-transcription (RT)-PCR on six common human cancers revealed that the expression level was highest in prostate adenocarcinoma (grade IV) and recurrent poorly differentiated breast cancer while lowest in lung cancer. Nevertheless, a higher expression level was documented in poorly differentiated colonic adenocarcinoma than in well-differentiated cancers. Strong positive signals or cytoplasmic stains were found in *2B4* and *LH7* subclones by either Western-blot or immunocytochemical staining in contrast with negative results in the remaining *IE8* and *BE1*. For both breast and colorectal cancers, positive staining (++–+++) was more common in cancers with metastasis than without metastasis (36% versus 7.4% in breast and 52% versus 35% in colorectal). Biological effects of TMSG-1 gene transfection on two prostate cancer cell lines (*2B4* and *IE8*) were also studied. Both transfectants with TMSG-1 sense displayed considerably suppressed cell growth in the curve, obviously lower outside diameter (OD) ratio in mean transit time and reduced clone formation in soft agar clone formation assay, compared with those with vain vector or non-transfectants, while transfectants with anti-sense exhibited more active cell growth, a higher OD ratio and increased clone formation. However, cell apoptotic rate increased only in the sense transfectants and was relatively invariable in anti-sense or vain vector transfectants and non-transfectants.

Professor Weigang Fang (Peking University, China) presented his group's findings, showing the effects of agonists [adenosine triphosphate (ATP) and adenosine monophosphate-purine nucleoside phosphorylase] or antagonists (suramin, PD and SB) of P2 purinoreceptor Y (P2Y), a G protein coupled receptor, on mitogen-activated extracellular signal-regulated kinase-activating kinase (MEK)-mitogen-activated protein kinase (MAPK) or p38 signal transduction pathways in two prostate cancer cell lines, metastatic *IE8* and non-metastatic *2B4*, using Western-blot method. The activities of ERK1/2 and p38 were significantly enhanced by ATP in a time- and dose-dependent manner in both cell lines. However, these effects were dramatically suppressed by suramin, PD and SB, indicating that P2Y activation could incur MEK-MAPK and p38 signal transduction pathways. Furthermore, P2Y receptor-induced ERK1/2 and p38 activation as well as NFB activity were found higher in metastatic *IE8* cells than in non-metastatic *2B4* cells, implying a

potential signalling mechanism in regulating metastatic phenotype. In in vitro invasion assay, the invasion of both cell lines was stimulated by ATP in parallel with ERK1/2 and p38 activation, and this effect by ATP was blocked by pretreatment with SB. While *IE8* and *2B4* both were transfected with dominant-negative MAPK kinase 1 (KA-MEK1), both KA-MEK1 transfectants showed an obvious decrease in passing through matrigel-coated membranes as well as ERK1/2 activities, as compared with pcDNA3 transfectants. MAP kinase phosphatase 5 (MKP5), a novel MKP that inactivates p38 and SAPK/JNK but not MAPK/ERK, was also found to incite cancer invasion by inhibiting ATP-induced p38 activation. As a corollary, activation of both downstream transduction pathways of P2Y receptor, ERK1/2 and p38, might be of great importance in promoting prostate cancer invasion, implying the significance of being potential therapeutic targets for this cancer. Further study revealed that both matrix metalloproteinases (MMPs) 2/9 and tissue inhibitor of metalloproteinases 1/2 might not participate in ATP-induced cancer invasion, as their expressions were not influenced by ATP in *IE8* and *2B4* cells.

Severe acute respiratory syndrome

Severe acute respiratory syndrome (SARS) was a catastrophe in Spring 2003 worldwide, particularly in China, Singapore and Canada. Professor Mingpeng She (Chinese Academy of Medical Sciences & the Peking Union Medical College, China) reported a SARS model in *Macaca Mulata* (Rhesus monkeys), which was generated by giving SARS co-virus or lung tissue lysate from a patient who died from SARS via nasal cavity and bronchus or intravenously, then assessed by clinical symptoms (anal temperature and blood white counts) and virology assays (virus antibody detection, RT-PCR and coronavirus cultivation). The systematic pathological changes were observed up to 60 days after virus inoculation. Definite acute haemorrhagic interstitial pneumonia was seen in the monkey 5 days after virus inoculation, followed by lesions of acute interstitial and bronchial pneumonia in those monkeys 7, 10, 15, 20 and 30 days after virus infection. Even 60 days after virus inoculation, pathological changes, including the destruction of elastic fibres of septa with communication between neighbouring air sacs, expansion of alveoli and chronic pleuritis still appeared in monkeys, but the number of CD4+ or CD8+ infiltrating cells decreased 30 days and 60 days after virus infection. Hyaline membrane and desquamation of lining cells were seen in damaged alveoli but not so remarkable as that described in human autopsy findings. In addition to lung and pleural lesions, congestion of red pulp in spleen, proliferation of epithelial and endothelial cells in renal glomeruli and focal liver necrosis were also discovered during the SARS development. Professor Bingquan Wu (Peking University, China) introduced a method for gene detection of SARS coronavirus that had broad application for clinical diagnosis and surveillance investigation. They devised a pair of primers and a probe (molecular beacon), which were specific for the recognition of a highly conserva-

tive region between 15301 and 15480 of the SARS-related coronavirus polymerase gene sequences obtained from Genbank. In the real-time RT-PCR assay, the extent of SARS-related coronavirus amplification was measured in terms of the increase in fluorescence during the amplification process. The 145-bp fragment of PCR product was further confirmed using conventional PCR assay and proved using DNA sequencing to be identical to the target sequence to which the probe was hybridised. In 25 of 85 clinical specimens from patients with suspected SARS and all 6 SARS post-mortem cases during the epidemic in Beijing, SARS-related coronavirus was identified using this method.

Colorectal cancer

Professor Thomas Kirchner (University of Erlangen-Nuremberg, Germany) showed his group's recent studies on colorectal cancers. The progression of colorectal cancer is non-linear, which is driven by morphogenetic programs that derived from normal development (gastrulation). β -Catenin, an important molecule in the Wnt signalling pathway, had more prominent nuclear staining at the invasive front (usually with epithelial mesenchymal transition) than in the centre of the tumour, implicating a critical role in the morphogenesis by up-regulating its downstream target genes, including p16, MMP7, UPA and Lam-5 γ 2, etc. A study of invasion-associated gene expression profiles on 11 colorectal cancers (Affymetrix U133A chip containing 22283 genes) disclosed an intratumourous heterogeneity in colorectal carcinomas as 127 genes up and 49 down were found at the invasive fronts. The up genes could be separated into three subgroups, including the aforementioned known β -catenin target genes, lymphocyte genes and factors of angiogenesis. The gene expression profiles can be defined to identify targets for an interference of morphogenesis and colorectal cancer progression. Microsatellite instability accounts for 10–15% colorectal carcinogenesis. In comparison with microsatellite stability and microsatellite instability (MSI)-L cancers, MSI-H colorectal cancers are more frequently associated with right hemicolon location, mucinous, signet ring cell, cribriform or medullary subtypes, prominent intraepithelial lymphocytes and a Crohn's-like reaction, frameshift mutations in transforming growth factor (TGF) β -RII, insulin-like growth factor (IGF)-IIR and BAX, and relatively better prognosis despite poor differentiation. MSI-H colorectal cancers could occur in familial [hereditary nonpolyposis colonic cancer (HNPCC)] and sporadic settings. Different clinicopathological and molecular features were discovered in the two parallel pathways. HNPCC cancers, displaying germline mutations in mismatch genes (mainly hMLH1 and hMSH2), were associated with the traditional adenoma–carcinoma sequence and less concurrent serrated adenomas (2%) and mucinous adenocarcinomas (14%), whereas sporadic MSI-H cancers, characterised by hypermethylation in the promoter region of hMLH1 (methylator phenotype), were associated with serrated pathways and more concurrent serrated adenomas

(17%) and mucinous adenocarcinomas (44%). Professor Kirchner also introduced three new routes for colorectal carcinogenesis, serrated route, juvenile polyp route and colitis-dysplasia-carcinoma route, which were inferred in the new World Health Organization (WHO) classification of tumours of the digestive system, in addition to traditional adenoma–carcinoma route. A multi-gene and multi-step process as the accumulation of mutations/loss of heterozygosity (LOH) at 5q, 12p, 18q and 17p, etc. has been widely accepted in the traditional adenoma–carcinoma sequence. Nevertheless, the combined mutations could only interpret 7% of colorectal cancers, implying the presence of other molecular models. Accumulating evidence suggested that MSI might be critical in the serrated pathway. In addition to an increased frequency of serrated polyps and adenomas adjacent to sporadic MSI-H colorectal cancers, molecular alterations, including hMLH1 inactivation and shift mutations in TGF β RII, BAX and IGFIR were also uncovered. About 5–10% or 15–20% ulcerative colitis patients can develop colonic cancers in 20 years or 30 years, respectively. As invasion could occur in intraepithelial neoplasia in ulcerative colitis exhibiting relatively mild morphological changes, the criteria for high-grade intraepithelial neoplasia complicating colitis should be less severe than in adenomas. Both dysplasia-associated lesion or mass (DALM) and high-grade flat dysplasia are commonly associated with concurrent invasive cancers, frequently leading to total colectomy. Hence, the discrimination between sporadic adenomas and DALM is pivotal. Despite frequent villous structure, high nuclear p53 and low nuclear β -catenin in DALM, the discrimination remains difficult.

Identification of new molecular markers associated with colorectal adenoma and cancer may uncover critical events involved in the initiation and progression of colorectal cancer and may be of great significance in the prevention, diagnosis and treatment of this cancer. Professor Lai and his group have done some intriguing work in this field. They generated three differentially expressed cDNA libraries from the same patient, A-N (colonic adenoma-normal mucosa), T-N (colonic adenocarcinoma-normal mucosa) and T-A (colonic adenocarcinoma-adenoma) using the suppression subtractive hybridisation method in 1999. Then, 109 clones were obtained in the A-N library, 95 in T-N and 98 in T-A. The insert sequences of all clones were amplified and verified by means of a reverse Northern-blot technique using digoxigenin (DIG)-labelled probes. A great number of genes, separated into known genes, hypothetical proteins and novel genes or unknown expressed sequence tag (EST), were obtained by traditional methods, such as sequencing, ESTs assembly and BLASTing with a public database in combination with bioinformatics analysis based on a self-devised Linux-based platform, analysing nucleic sequences automatically. Unknown ($n=25$) EST sequences were submitted to GeneBank. The known genes were found to be associated with important biological functions, fundamentally associated with cytoskeleton, cell cycle and proliferation, immunity, metabolism and signal transduction pathways. Two candidate genes, IGFBP-rp1/B2 and RegIV, were further investigated. The known sequence of B2 gene

was separated from T-N library, and, by combining with the sequence from 5'RACE (rapid amplification of cDNA end) product, a sequence of 1125 bp was obtained which shared 1122/1125 identities with IGFBP-rP1, indicating that both genes were the same. Semi-quantitative RT-PCR and immunohistochemistry were used to detect the expression of B2 in colorectal cancer tissues and cell lines (*SW480*, *SW1116*, *SW620*, *HCT8*, *CoLo205* and *LoVo*). At the level of mRNA, the expression of B2/IGFBP-rP1 was high in colorectal carcinomas, moderate in adenomas and tissues adjacent to tumours and low in normal tissues. No expression of B2/IGFBP-rP1 was found in the five cell lines except *SW480*. Immunostaining of B2/IGFBP-rP1 was stronger in cancer tissues than in paired normal tissues. A stronger staining of B2/IGFBP-rP1 was seen at the invasive front of cancer than in the centre in 28.9% (22/76) of cancers, which were also associated with an increased frequency of lymph-node involvement, deeper invasion and stronger staining of B2/IGFBP-rP1 than the remaining cancers. Further exploration uncovered that the fasting glucose level was significantly correlated with IGFBP-rP1 expression in cancer tissues. In conclusion, B2/IGFBP-rP1 overexpression might play an important role in the initiation and progression of colorectal cancer and may be linked with an increased potential of invasion. Its expression may also be associated with fasting glucose level and the presence of diabetes mellitus. RegIV, a novel gene first isolated from a large inflammatory bowel disease library in 2001, was obtained in A-N library in a manner of contigs assembly using electronic cloning (in silico cloning) with the EST database. Semi-quantitative RT-PCR in 12 colorectal adenomas and 10 concurrent carcinomas indicated that Reg IV mRNA level was higher in all adenomas and in 9 of 10 concurrent colorectal carcinomas when compared with paired normal colorectal mucosa. Northern-blot analysis further supported these findings. Subsequent study of in situ hybridisation with DIG-labelled cRNA in 32 colorectal adenomas with varying degrees of dysplasia disclosed that Reg IV was overexpressed in 74% (14/19) of adenomas with mild or moderate dysplasia and 100% (13/13) in cases of adenoma with severe dysplasia, compared with paired normal tissues. In addition, higher levels of Reg IV mRNA were consistently scored in regions with more severe dysplasia within the same adenoma sample displaying varying degree of dysplasia. The strongest staining was seen within carcinomatous areas of the 12 adenoma cases. All these results support that overexpression of Reg IV may be an early event in colorectal carcinogenesis. Detection of Reg IV overexpression may be useful in the early assessment of carcinomatous transformation of adenoma.

Professor Lirong Chen (Zhejiang University, China) explored the histogenesis and neural differentiation in 20 gastrointestinal stromal tumours (GIST, all c-kit+) using electron microscopy and immunocytochemistry. The ultrastructural features for neural differentiation were observed in 7 GISTs, myogenic differentiation in 1 GIST and neither in 12 GISTs. Among 7 GISTs with ultrastructural features for neural differentiation, all were positive for both neuron-specific enolase (NSE) and CD99, and 5 or 4 cases were

positive for S-100 and CD56, respectively. Hence, gastrointestinal autonomic nerve tumour should be regarded as a subtype of GIST, due to their overlapping morphological features, with GIST accompanying neural differentiation.

Lymphoid tumours

Professor Harald Stein (Berlin—Free University, Germany) introduced the development history and general features of the present WHO classification of lymphoid tumours and discussed recent molecular issues on non-Hodgkin's lymphoma. To achieve an international standard and to assess the diagnostic value of IgH rearrangement, a collaborative study of 48 European institutes (including 30 PCR laboratories) in seven national networks was carried out on 110 gastric biopsies. The histological diagnoses were reassessed by seven pathologists, without knowledge of the PCR results. Meanwhile, IgH PCR analysis was done without knowledge of the histological diagnoses using three Biomed-2 IgH primers, FR1, FR2 and FR3. Monoclonal IgH was identified in 2% (1/53) of gastritis cases, 22% (4/18) of borderline cases and 92% (24/26) of mucosa-associated lymphoid tissue (MALT) lymphomas, respectively. Thus, Professor Stein concluded that the distinction between true clonality from pseudoclonality was essential for a correct interpretation and possible when the PCR assay was repeated at least two times using primers of high quality. Mantle cell lymphoma (MCL), the unique small cell lymphoma with poor prognosis, was characterised by cyclin D1 overexpression as a result of t(11;14) leading to IgH-cyclin D1-fusion protein. However, an MCL gene expression signature defined a large subset of MCLs that expressed cyclin D1 and a novel subset that lacked cyclin-D1 expression. The more than 5 years in median survival was better in cyclin D1+ MCLs than in cyclin D1- MCLs. Furthermore, the gene expression profiles of leukaemic MCL cells was otherwise identical to the cyclin-D1-positive MCLs in a comparative study among MCL cell lines, leukaemic MCL cells and nodal MCL cells. Three chromosomal aberrations, trisomy 3, t(1;14) and t(11;18), were found in only a fraction of gastric MALT lymphomas as demonstrated by a study on molecular-cytogenetic comparison of MALT lymphoma and large B-cell lymphoma, which proposed that MALT lymphomas can be separated into different genetic subgroups. Furthermore, a clonal lymphoma progression occurred from the small to the large cell component was identified with accumulation of gains and losses of chromosomal material in the large cell component in MALT lymphoma plus large B-cell lymphoma. Aberrations overlapping with MALT lymphoma and MALT lymphoma plus large B-cell lymphoma included losses on chromosome 13, amplifications of the REL proto-oncogene or gains on chromosome 12. In addition, the large-cell component revealed gains on 8q24, including amplifications of the MYC proto-oncogene and losses on 2q. The large B-cell lymphoma had frequent gains on chromosomes 12 and 9, as well as on 11q, and losses on 6q. Diffuse large-cell B-cell lymphoma (DLBCL) is one of the most complicated lymphoid tumour

entities and has many WHO morphological variants and types. Recently, three gene-expression subgroups—germinal-centre B-cell-like (GCB type), activated B-cell-like (ABC type) and type-3 DLBCL—were identified in a DNA microarray analysis on 240 DLBCLs, among which patients in GCB subgroup had the highest 5-year survival rate.

Another microarray study focused on centroblastic lymphoma (CBL) and Burkitt lymphoma confirmed that DLBCL of centroblastic morphology can be separated into a GCB type and ABC type. However, more than 50% of CBL was of the ABC type and all Burkitt lymphomas were the GCB type in contrast to the study above. The latter difference is probably due to a highly variable percentage of the tumour cells in biopsies. Reliable distinction between primary mediastinal (thymic) large B-cell lymphoma (PMLBCL) and common types of DLBCLs is only obtained via clinical information. However, this distinction can be reliably made by gene expression profiling without clinical information. Intriguingly, PMLBCL shared several features for down-regulating Stat1, AK2, TRAF1 and IL13R, resembling classical Hodgkin's lymphoma. Another rare entity of DLBCL, plasmablastic lymphoma, can also be distinguished from DLBCL of ABC type and GCB type by gene expression profiling. Thus, at the close of his report, Professor Stein concluded that new scientific data should be integrated into the WHO classification concept, which would lead to the identification of new lymphoma diseases and sharpening of existing definitions.

Professor Gandi Li (Sichuan University, China) investigated 42 intestinal T-cell lymphomas (ITCL) in Chinese, including a broad pattern of clinicopathological features and molecular alterations. Neither immunosuppression nor clinical evidence of celiac disease at the time of diagnosis was implicated in all 42 patients. The prognosis was generally poor, as indicated by a short relapse period (4 patients relapsed within 24.3 months) and survival time (23 patients died with a median survival of 3.0 months), despite the fact that 6 patients with one site involvement had survived for 156 months. In comparison with enteropathy-type intestinal T-cell lymphomas (ETCLs), ITCLs had unusual clinical features, such as male predilection (33/42), young age (mean 30.4 years) and predominantly ileocecum and colon location (33/42). Histological patterns were similar in ITCLs and ETCLs, except that massive zonal necrosis and the absence of enteropathy were seen in ITCLs. Both immunophenotyping and Epstein-Barr virus (EBV) detection showed similar patterns as found in nasal NK/T cell lymphoma, including invariably CD56+ (12/42), TIA-1+ (39/42) and EBER+ 41/42 (97.62%). Owing to a peculiar EBV-associated entity with distinct clinicopathological features, ITCL in Chinese should be put into a catalogue separate from ETCL.

Professor Weiping Liu (Sichuan University, China) studied 185 cases of nasal NK/T-cell lymphoma. A routine diagnostic protocol for nasal NK/T-cell lymphomas was put forward, including morphological features for massive coagulative necrosis, mostly medium-sized atypical cells and increased vessels, immunophenotype displaying CD3 ϵ +, CD20-, CD56+, TIA-1+ and granzyme B+, and EBER+

using in situ hybridisation. It was addressed that EBER in situ hybridisation was a more sensitive method for EBV detection (58/64) in this lymphoma on paraffin tissues than both PCR (19/28) and LMP-1 immunostaining (2/20). A follow-up study of 120 cases revealed that the general 1-year, 3-year and 5-year survival rates of NK/T-cell lymphoma were 71.80%, 55.40% and 54.10%, respectively. Lower tumour stage (I–II), free of recurrence and combined therapies were the most favourable prognosticators. In stage-I to -II tumours, the 1-year, 3-year and 5-year survival rates were 76.40%, 65.50% and 62.70%, respectively, in comparison with 38.20%, 23.60% and 0 in stage-III to -IV tumours. A nude mouse model and the cell line of NK/T-cell lymphoma were successfully generated from a secondary lymphoma in stomach, both of which might be of great significance for further study on this tumour. Afterwards, a cDNA microarray analysis on the lymphoma was carried out. A total of 265 differential expressed genes were identified, of which 178 were upregulated and 87 downregulated. Preliminary assessment suggested that 25 downregulated or 21 upregulated genes might be closely correlated with nasal NK/T-cell lymphoma.

Gynaecological tumours

Professor Manfred Dietel (Charité, Berlin, Germany) gave an overall aspect of considerable improvements of 2003 WHO Classification of Gynaecologic Tumours in comparison with the 1999 version. He explained changes of the diagnostic terminology in new the WHO classification. As an important example, the former category of “epithelial tumours of borderline malignancy (low malignant potential)” is condensed to the term “borderline tumour”, and the term “low malignant potential” is avoided due to the considerable misunderstandings among pathologists and clinicians, resulting in distinct overtherapy. The serous borderline tumour of the ovary was particularly addressed. Immunohistochemistry, as well as new diagnostic procedures such as DNA cytophotometry, was recommended in the assessment of invasive foci ($\leq 10 \text{ mm}^2$) for the diagnosis of serous borderline tumours with microinvasion, although no prognostic relevance can be attributed to microinvasion. As a new type, micropapillary serous borderline tumour was mentioned but not fully accepted by WHO 2003. Surface proliferations and peritoneal implants were common in serous borderline tumours, however, only invasive or destructive implants are associated with unfavourable outcomes, whereas surface proliferations per se and non-invasive peritoneal implants are not strikingly correlated to poor prognosis. Therefore, it should be addressed that peritoneal implants be distinguished into an invasive and a non-invasive type according to the criteria recommended by WHO 2003.

With regard to endometrial carcinoma, a type I and II have to be separated, due to different clinicopathological features. Type-I cancer was low-grade prototypical endometrioid adenocarcinoma, which is associated with exogenous or endogenous hyperoestronism, endometrial hyperplasia, in particular, atypical hyperplasia, relatively good clinical

prognosis and higher oestrogen receptor or progesterone receptor overexpression, whereas type-II carcinoma exhibits a more aggressive behaviour, primarily in elderly patients, and higher p53 overexpression. A three-tiered grading system is adopted for type-I endometrial carcinoma, based on the component of solid growth pattern (without glandular formation). But WHO also supplemented that solid nests of cells showing squamous or morular differentiation do not increase the tumour grade, and bizarre nuclear atypia should raise the grade by one type, e.g. from 1 to 2 or 2 to 3. The new WHO classification defined the endometrial intraepithelial neoplasia in the uterine corpus, however, there is limited experience in clinical implementation at the present time.

In addition, the cervical squamous cell carcinoma with microinvasion has to be distinguished from high-grade cervical intraepithelial neoplasia (CIN 3) in the uterine cervix. Early invasion is indicated by more mature squamous cells extending from a focus of in situ carcinoma, luminal necrosis and intraepithelial squamous maturation as well as stromal oedema and a stromal desmoplastic and lymphocytic response, features that aid in its distinction from crypt involvement by CIN.

With regard to the CIN system, there is an increasing tendency to use a two-tiered classification of low- and high-grade CIN that equates to CIN 1 versus CIN 2 and 3, respectively (WHO Classification of Tumors, 2003, p 11). Further, the International Federation of Gynecology and Obstetrics definition of stage-1A carcinoma of the cervix was outlined in that the depth of invasion may not be greater than 5 mm, and the horizontal spread should measure 7 mm or less.

Professor Joachim Diebold (Ludwig-Maximilians-University München, Germany) focused on selected issues concerning the WHO Classification of Ovarian Tumours' classification of mucinous tumours, classification of extra-ovarian lesions associated with serous tumours and classification of primary extraovarian (peritoneal) serous tumours. A three-tiered grading system of ovarian carcinomas scored on growth pattern, nuclear pleomorphism and mitotic activity was recommended by the new WHO classification, as proposed by Silverberg in 2000. A prominent prognostic significance of the Silverberg grading was disclosed by multivariate Cox analysis. However, as listed in the WHO, clear cell carcinomas and mucinous carcinomas are not graded, due to absence of prognostic and/or therapeutic relevance, and endometrioid carcinoma or transitional carcinomas can be graded by use of the criteria for endometrial carcinomas or transitional carcinomas of the urinary tract. Mucinous adenocarcinomas of the ovary are rare, despite the presence of many subtypes. It was emphasised that mucinous metastases in the ovary from colorectal, appendiceal, gastric, pancreaticobiliary, and endocervical carcinomas are more common than primary ovarian mucinous carcinomas, and the differential diagnosis may be extremely difficult due to shared gross and microscopic features and overlapping immunophenotype. The two subtypes of mucinous borderline tumours of the ovary, the endocervical type and the intestinal type, have different clinical and morpholog-

ical features. Intraepithelial carcinoma is defined as the presence of areas with severe atypia or more than three cell layers in the intestinal type of mucinous borderline tumours. Extraovarian lesions associated with serous tumours are not uncommon. They have clinicopathological features similar to their ovarian counterparts. Particular attention should be paid to Mullerian inclusion cysts as potential diagnostic pitfalls, due to their presence in 25–30% of pelvic and para-aortic lymph nodes. According to a recent report, primary peritoneal serous carcinomas are not rare (31 of 220, 14.1%). These carcinomas are defined using histology that is indistinguishable from primary ovarian carcinoma but normal-sized ovaries. In his review on molecular approaches to gynaecological tumours, Professor Diebold concluded that a molecular basis for the multi-step process of neoplastic transformation is only established for some gynaecological carcinomas, and the genetic changes identified so far only partially explain the phenotypic variability of gynaecological tumours. Type-I endometrial carcinoma is a good example for the paradigm of multi-step carcinogenesis. During the process of transformation from normal endometrium, simple hyperplasia, complex hyperplasia to low-grade and high-grade carcinoma under the estrogenic stimulation, underlying genetic alterations, including microsatellite instability and mutations involving PTEN, k-ras, β -catenin and p53, etc., are accumulating. Specifically, MSI-H endometrial carcinomas are associated with mucinous differentiation, favourable prognosis and PTEN mutation. Using comparative genomic hybridization (CGH), array accumulation of genetic aberrations was also found in the transformation of serous borderline tumours into invasive serous carcinoma, which is a rare event ($\leq 1\%$). Most genetic aberrations in endometrial and ovarian tumours affect members of signalling pathways, growth factors and their receptors and regulatory molecules for cell cycle, differentiation and apoptosis. The activation of members of RAS and Wnt pathway is frequent in type-I endometrial carcinoma. For example, k-ras and β -catenin mutations were found in 16% or 31% type-I endometrial carcinomas, respectively. Several genes in the signalling pathways of receptor tyrosine kinases are frequently activated in epithelial ovarian tumours, including Her2/neu, Dab-2, Ras, Raf and PTEN, etc. Moreover, serous borderline tumours are characterised by activation of the RAS-BRAF pathway, suggesting a different molecular developmental pathway in contrast to invasive serous carcinomas. Professor Diebold also discussed the role of chromosome 20q for ovarian carcinogenesis, which harbours a high density of putatively relevant genes, such as CSE1L/CAS, EEF1A2, ZNF217, etc. Some studies proved that gains of transcription factor ZNF217 on 20q13.2 using fluorescent in situ hybridisation (FISH) and reduced overexpression of CAS/CSE1 on 20q13 were unfavourable prognostic factors in ovarian carcinomas.

Urothelial tumours

Professor Ferdinand Hofstädter (University Regensburg, Germany) compared the 2004 WHO Classification of Tu-

mours of the urinary system with the 1999 version and focused on several topics regarding urothelial tumours. Based on molecular and clinical data, urothelial tumours were separated into infiltrating tumours, which are defined as urothelial tumours that invade beyond the basement membrane, and non-invasive tumours, in which papilloma, papillary urothelial neoplasm of low-malignancy potential (PUNLMP) and non-invasive cancer are included. PUNLMP is not supposed to carry the label of cancer. A two-tiered grading system for urothelial epithelial cancer (low/high grade) substituted for the former three-tiered (G1–3) system to avoid G1–2 or G2–3 diagnosis, as was the case previously. In the 2004 WHO classification, flat lesion is not an independent type but a comprehensive entity exhibiting hyperplasia, reactive atypia, atypia of unknown significance, dysplasia (low-grade intraurothelial neoplasia) and carcinoma in situ. Hyperplasia and dysplasia, both as precursor lesions of urothelial tumours, might be representative of two different pathways in carcinogenesis, the papillary or invasive pathway. The papillary pathway is genetically stable in most cases and associated with chromosome-1 (frequently the whole chromosome) loss and other rare genetic alterations, whereas the invasive pathway is genetically unstable and frequently shows p53 mutations. Both lesions in cancer patients may be clonally related to the respective tumours as indicated by identical patterns of LOH analysis and chromosome-9 deletions in FISH as well as duplicate p53 mutation sequence in hyperplasia-papilloma-recurrent papilloma or dysplasia carcinoma in situ in the same patient. Oligoclonality or monoclonality of multifocal carcinoma was also discovered using similar methods in support of an intraluminal dissemination of multifocal cancers. Professor Hofstädter also reported the role of MSI and chromosome 8p deletions with several candidate genes (e.g. *sfrp1* and *TRAIL-R2*). Loss of *sfrp1* expression was found in 30% of urothelial carcinomas and associated with papillary growth pattern and lower survival rate of patients with invasive papillary tumours despite the fact that no mutation in the coding sequence and promoter region was identified in 20 urothelial carcinomas with 8p loss and five cell lines as well as rare promoter methylation (<10%). cDNA-array expression analysis might be beneficial for sharper tumour classification of urothelial tumours in a manner of hierarchical clusters. A gene expression analysis was found to contribute to discrimination of non-invasive from invasive papillary tumours via 40 differentially expressed genes.

Central nervous system tumours

Professor Otmar D. Wiestler (German Cancer Research Center, Heidelberg, Germany) addressed major changes in the new WHO Classification of Brain Tumours, including new tumour entities and variants, detailed criteria for diagnosis and tumour grading and molecular and genetic alterations. As listed in astrocytic neoplasms, pilocytic astrocytoma, diffuse low-grade astrocytoma, anaplastic astrocytoma and glioblastoma multiforme (GBM) are regarded as

grades I, II, III and IV, respectively. Tanycytic ependymoma, pineal parenchymal tumour of intermediate differentiation, large cell medulloblastoma, rhabdoid meningioma and teratoma with malignant transformation were novel tumour variants in the new WHO fascicle; whereas, polar spongioblastoma, cysts and tumour-like lesions (Rathke cyst, nasal glioma) and locally invading cancers are not mentioned, while pituitary adenomas are put into another WHO fascicle. He also reviewed recent molecular advances in brain tumours. There are two different genetic pathways leading to GBM, type I (primary) *de novo* from precursor cells and type II (secondary) as progression from low-grade astrocytoma, with the exception of pilocytic astrocytoma, which is associated with indolent biological behaviour and rare progression. Amplification of EGFR and chromosome 10 LOH were most common in the tumorigenesis of type-I GBM, while p53 or 17p LOH as well as 19q LOH were most common in type-II GBM. DNA aberrant methylation might also play an important role in the initiation and development of gliomas. In a study on GBM, oligodendroglioma and pilocytic astrocytoma using DMH CpG chips, tumour methylation-specific patterns were obtained, which not only helped the histopathological diagnosis but also guided the identification of multiple novel candidate genes. 1p and 19q LOH is frequently involved in oligodendroglioma tumours. The LOH incidence at both loci was found higher in the oligodendroglioma (WHO II) than in the anaplastic oligodendroglioma (WHO III), implicating prognostic significance.

Classical and desmoplastic medulloblastoma had differences in tumorigenesis, as the classical was associated with 17p LOH and *Myc* amplification, while the desmoid was associated with 9p22 LOH and *PTCH* inactivation. Overexpression of c-Myc may be related to unfavourable prognosis in medulloblastoma patients. A recent study on 34 tumours also demonstrated that expression profilings via cDNA array chip can make desmoplastic and classic medulloblastoma distinguishable.

Lung tumours

Professor Iver Petersen (Charité, Berlin, Germany) reported on changes in the new WHO lung cancer classification, which will incorporate more clinical data (epidemiology, signs and symptoms, imaging, prognosis) and particular genetic characteristics on the still morphologically defined subtypes. Predictive factors being subdivided into clinical, histopathological and genetic parameters, however, opened the window for new subentities. Generally speaking, the morphological subtypes have remained essentially unchanged. The issue of morphological heterogeneity and phenotypical transitions is still contentious; on practical grounds, a minimal component of 10% is generally required to diagnose mixed tumour entities such as adeno-squamous carcinoma, pleomorphic carcinoma or combined small carcinoma with a large cell component. A few variants of squamous cell carcinoma (SCC) are additionally mentioned, i.e. SCC resembling transitional cell carcinoma

and alveolar space-filling type of peripheral SCC, which is growing without destruction of alveolar framework and represents about 5% of peripheral SCC. The latter is contrasting with the conventional expanding type of SCC, showing infiltration and destruction of the normal lung architecture. Spindle cell SCC has been grouped with sarcomatoid carcinoma. For adenocarcinoma, the Noguchi subtypes being defined by a mixture of gross and histological features were mentioned. These include type A (localised bronchioloalveolar carcinoma), type B (localised bronchioloalveolar carcinoma with central alveolar collapse), type C (localised bronchioloalveolar carcinoma with fibroblastic proliferation), type D (poorly differentiated adenocarcinoma), type E (tubular adenocarcinoma) and type F (papillary adenocarcinoma with a compressive growth pattern). This typing is of great significance in the assessment of clinical treatment and prognosis. Of the six types, types A and B, thought to be in situ peripheral adenocarcinoma, showed no lymph-node metastasis and the most favourable prognosis (100% 5-year survival). Thus, both cancers may be potentially cured by limited resection (e.g. wedge resection); however, the diagnosis needs a good computed tomography correlation and require entire sectioning for histology. Conversely, type C appeared to be an advanced stage of types A and B; whereas, types D, E and F are small but advanced adenocarcinomas with less favourable prognoses.

Professor Petersen also documented that molecular pattern analysis on the DNA, RNA or protein level using CGH, array-CGH, cDNA/oligo-microarray and mass spectrometry expression analysis may be able to refine the morphological tumour classification. In a cDNA array study on lung cancers and normal lungs, gene expression patterns not only segregated the four major morphological subtypes of lung tumours but also provided gene clusters characteristic of adenocarcinoma subgroups with morphological and survival difference. Furthermore, expression profile of large cell lung cancer was suggestive of epithelial-mesenchymal transition. Protein analysis using matrix-assisted laser desorption/ionisation time-of-flight revealed that the proteomic pattern of lung cancers could not only classify correctly 34 primary non-small cell lung cancers (NSCLC) and 8 normal lung samples according to 91 MS peaks but also discriminated 34 primary NSCLCs from 7 metastases or recurrent lung tumours as indicated by 23 distinct MS peaks. Six groups of lung cancers with prognostic differences were generated by analysing the proteomic patterns of 15 MS peaks.

UICC TNM staging

Professor Christian Wittekind (Leipzig University, Germany) summarised the improvements in the 6th edition of the International Union Against Cancer (UICC) tumour node metastasis (TNM) Classification of malignant tumours. He gave an overview on the changes of the different tumour entities as listed in the classification and made comments on the importance and clinical usefulness of them. R classification was introduced to describe the absence or presence of residual tumour after treatment. Three descriptors were designated R0 (no residual tumour), R1 (microscopic residual tumour), and R2 (macroscopic residual tumour). In the example of gastrectomy specimen, circumferential resection margin (CRM) has to be considered as margins in minor ligament, gastrocolic and mesocolic ligament, hepatoduodenal ligament and subserosal soft tissue in non-peritonealised regions. According to data of residual tumour in gastric carcinoma resection specimen from Erlangen Cancer Center, in R1-tumour patients, CRM was most frequently involved (67%, 79 of 118). As the sentinel lymph node is the first lymph node to receive lymphatic drainage from a primary tumour, it is a good indicator for lymph-node metastasis. Occasionally, there is more than one sentinel lymph node. The following designations are applicable when sentinel lymph node assessment is attempted: pNX(sn) (sentinel lymph node could not be assessed), pN0(sn) (no sentinel lymph node metastasis) and pN1(sn) (sentinel lymph node metastasis). Isolated tumour cells (ITCs) are single tumour cells or small clusters of cells not more than 0.2 mm in greatest dimension (micrometastasis ≤ 0.2 cm), which are usually detected using immunohistochemistry or molecular methods. However, ITCs do not typically show evidence of metastatic activity (e.g. proliferation or stromal reaction) or penetration of vascular or lymphatic sinus walls. In explaining further developments of the TNM system, the example colorectal carcinoma was mentioned: stage III is separated into stages IIIA, IIIB and IIIC. The 5-year survival rate was 59.8%, 42.0% and 27.3%, respectively. Finally, he addressed that the TNM system and its variables were essential important prognostic factors. In addition to these well-established factors, the UICC also considered the spectrum of prognostic factors in a new publication, including new promising molecular markers.

Acknowledgements The authors thank the contributors Lirong Chen, Joachim Diebold, Weigang Fang, Heinz Höfler, Ferdinand Hofstädter, Thomas Kirchner, Gandi Li, Weiping Liu, Iver Petersen, Mingpeng She, Harald Stein, Otmar D. Wiestler, Christian Wittekind, Bingquan Wu, Jie Zheng and all participants for the excellent lectures and interesting discussions. In particular, the great hospitality of the Chinese colleagues is very much acknowledged.