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The Section of Molecular Pathology (MPA) studies the molecular basis of human neoplasms in particular brain tumours using tumour samples from patients with excellent clinical data and follow-up. We correlate histologically recognized phenotypes with genotypes and expression profiles to elucidate the molecular basis and genetic pathways that are operative in human neoplasms; identify molecular markers for improvement of tumour diagnoses and classification; identify genetic factors that predict sensitivity to treatment, tumour progression and patient outcome; and use genetic data to identify the etiology of human cancers. Since 2006, MPA has also been responsible for the 4th edition of the World Health Organization (WHO) Classification of Tumours Series (WHO Blue Books). The third volume, WHO Classification of Tumours of the Digestive System, was published in 2010; the fourth (WHO Classification of Tumours of the Breast) and fifth (WHO Classification of Tumours of Soft Tissues and Bone) volumes are in the editing stage.

A few of MPA's more important projects over the Biennium are detailed below.

Intratumoral patterns of genomic imbalance in glioblastoma

Glioblastomas are morphologically and genetically heterogeneous, but little is known about the regional patterns of genomic imbalance within glioblastomas. Using a reliable whole genome amplification (WGA) method, recently established in our laboratory to randomly amplify DNA from paraffin-embedded histological sections with minimum amplification bias, we assessed genome wide chromosomal imbalance by array CGH (Agilant 105K) in DNA from 2–5 separate tumour areas of 14 primary glioblastomas (total, 41 tumour areas). Chromosomal imbalances significantly differed among glioblastomas; the only alterations that were observed in ≥ 6 cases were loss of chromosome 10q, gain at 7p, and loss of 10p. Genetic alterations common to all areas analysed within a single tumour included gains at 1q32.1 *(PIK3C2B, MDM4)*, 4q11-q12 *(KIT, PDGFRA)*, 7p12.1–11.2 *(EGFR)*, 12q13.3–12q14.1 *(GLI1, CDK4)* and 12q15 *(MDM2)*, and loss at 9p21.1–24.3 *(p16INK4a/p14ARF)*, 10p15.3-q26.3 *(PTEN, etc)* and 13q12.11-q34 *(SPRY2, RB1)*. These are likely to be causative in the pathogenesis of glioblastomas (driver mutations). In addition, there were numerous tumour area-specific genomic imbalances which may be either non-functional (passenger mutations) or functional, but constitute secondary events reflecting progressive genomic instability, a hallmark of glioblastomas.

Genetic pathways to diffuse gliomas

Low-grade diffuse gliomas WHO grade II (diffuse astrocytoma, oligoastrocytoma, oligodendroglioma) are characterized by

frequent *IDH1/2* mutations (> 80%) that occur at a very early stage. In addition, the majority of diffuse astrocytomas (about 60%) carry *TP53* mutations, which constitute a prognostic marker for shorter survival. Oligodendrogliomas show frequent loss at 1p/19q (about 70% of cases), which is associated with longer survival. *IDH1/2* mutations are frequent (> 80%) in secondary glioblastomas that have progressed from low-grade or anaplastic astrocytomas. Primary *(de novo)* glioblastomas with *IDH1/2* mutations are very rare (< 5%); they show an age distribution and genetic profile similar to secondary glioblastomas and are probably misclassified. Using the presence of *IDH1/2* mutations as a diagnostic criterion, secondary glioblastomas account for approximately 10% of all glioblastomas. *IDH1/2* mutations are the most significant predictor of favourable outcome of glioblastoma patients. The high frequency of *IDH1/2* mutations in oligodendrogliomas, astrocytomas and in secondary glioblastomas derived thereof suggests that these tumours share a common progenitor cell population. The absence of this molecular marker in primary glioblastomas suggests a different cell of origin; both glioblastoma subtypes acquire a similar histological phenotype due to common genetic alterations, including the loss of tumour suppressor genes on chromosome 10q.

Particularly for oligoastrocytoma, the diagnostic criteria of low-grade diffuse gliomas are highly subjective. To establish genetic profiles for diffuse gliomas and to estimate their predictive impact, we screened 360 WHO grade II gliomas for mutations in the *IDH1, IDH2,* and *TP53* genes and for 1p/19q loss and correlated these with clinical outcome. Most tumours (86%) were characterized genetically by *TP53* mutation + *IDH1/2* mutation (32%), 1p/19q loss + *IDH1/2* mutation (37%), or *IDH1/2* mutation only (17%). *TP53* mutations only or 1p/19q loss only was rare (2% and 3%). The survival of patients with *TP53* mutation ± *IDH1/2* mutation was significantly shorter than that of patients with 1p/19q loss ± *IDH1/2* in both univariate and multivariate analyses. Thus, the molecular classification on the basis of *IDH1/2* mutation, *TP53* mutation and 1p/19q loss has power similar to histological classification and avoids the ambiguity inherent to the diagnosis of oligoastrocytoma.

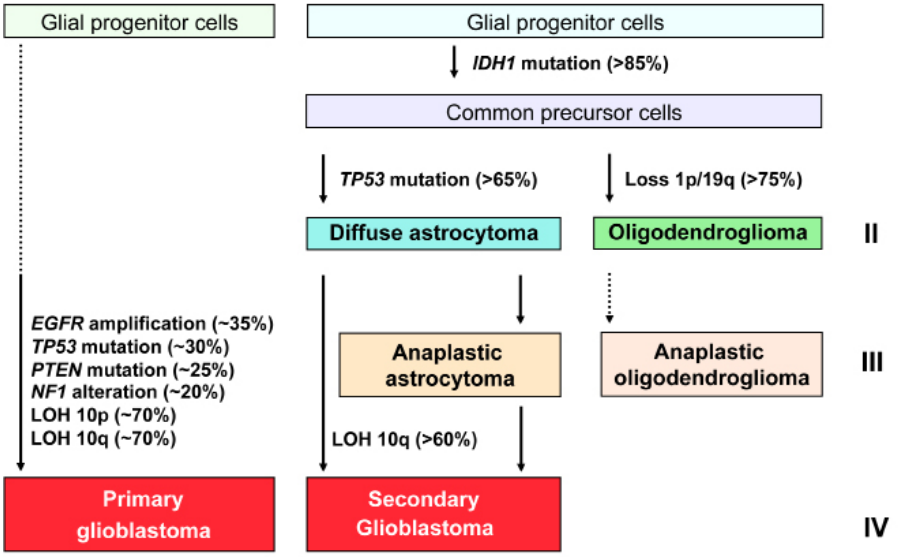

Figure 1. Genetic pathway to gliomas

Alterations in the RB1 pathway in low-grade diffuse gliomas lacking common genetic alterations

Most (> 90%) low-grade diffuse gliomas carry at least one of the following genetic alterations: *IDH1/2* mutation, *TP53* mutation or 1p/19q loss. Only 7% of cases were triple-negative (i.e. lacking any of these alterations). We carried out array CGH in 15 triple-negative WHO grade II gliomas (8 diffuse astrocytomas and 7 oligodendrogliomas), and showed loss at 9p21 ($p14^{ARF}$, $p15^{INK4b}$ and $p16^{INK4a}$ loci) and 13q14–13q32 (containing the *RB1* locus) in 3 cases and 2 cases, respectively. Further analyses in 31 triple-negative cases, as well as a total of 160 non-triple-negative cases, revealed that alterations in the RB1 pathway (homozygous deletion and promoter methylation of the $p15^{INK4b}$, $p16^{INK4a}$ and *RB1* genes) were significantly more frequent in triple-negative (26%) than in non-triple-negative cases (11%; P = 0.0371). These results suggest that a fraction of low-grade diffuse gliomas lacking common genetic alterations may develop through a distinct genetic pathway, which may include loss of cell-cycle control regulated by the RB1 pathway.

TET2 promoter methylation in low-grade diffuse gliomas lacking *IDH1/2* mutations

The *TET2* gene encodes the α-KG-dependent enzyme that catalyses the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, thus producing DNA demethylation. Miscoding mutations of the *TET2* gene have been detected in 10–25% of acute myeloid leukemias lacking *IDH1/2* mutations. Most low-grade diffuse gliomas carry *IDH1/2* mutations (> 85%), but molecular mechanisms of pathogenesis in those lacking *IDH1/2* mutations remain to be elucidated. We screened for miscoding mutations and promoter methylation of the *TET2* gene in 29 low-grade diffuse gliomas lacking *IDH1/2* mutations. Single-strand conformational polymorphism followed by direct sequencing showed the absence of miscoding mutations in *TET2*. Methylation-specific PCR revealed methylation of the *TET2* promoter in five of 35 cases (14%). In contrast, none of 38 low-grade diffuse gliomas with *IDH1/2* mutations had *TET2* promoter methylation. These results suggest that *TET2* promoter methylation, but not *TET2* mutation, may be an alternative mechanism of pathogenesis in a small fraction of low-grade diffuse gliomas lacking *IDH1/2* mutations.

Genetic alterations in microRNAs in medulloblastomas

MicroRNAs (miRNAs) control a variety of cellular processes via the regulation

of multiple target genes. We screened 48 medulloblastomas for mutation, deletion and amplification of nine miRNA genes, which were selected on the basis of the presence of potential target sequences within the 3′-untranslated region of the MYCC mRNA. Differential PCR revealed deletions in miR-186 (15%), miR-135a-1 (33%), miR-548d-1 (42%), miR-548d-2 (21%) and miR-512–2 (33%) genes, whereas deletion or amplification was detected in miR-135b (23%) and miR-135a-2 (15%). In miR-33b, deletion, amplification or a mutation at the precursor miRNA were detected in 10% of medulloblastomas. Overall, 35 out of 48 (73%) medulloblastomas had at least one alteration. Real-time PCR revealed MYCC overexpression in 11 of 37 (30%) medulloblastomas, and there was a correlation between MYCC overexpression and miR-512–2 gene deletion (P = 0.0084). Antisense-based knockdown of miR-512–5p (mature sequence of miR-512–2) resulted in significant upregulation of MYCC expression in HeLa and A549 cells, while forced overexpression of miR-512–2 in medulloblastoma/PNET cell lines DAOY, UW-228–2 and PFSK resulted in downregulation of MYCC protein. Furthermore, the results of luciferase reporter assays suggested that miR-512–2 targets the *MYCC* gene. These results suggest that alterations in the miRNA genes may be an alternative mechanism leading to MYCC overexpression in medulloblastomas.

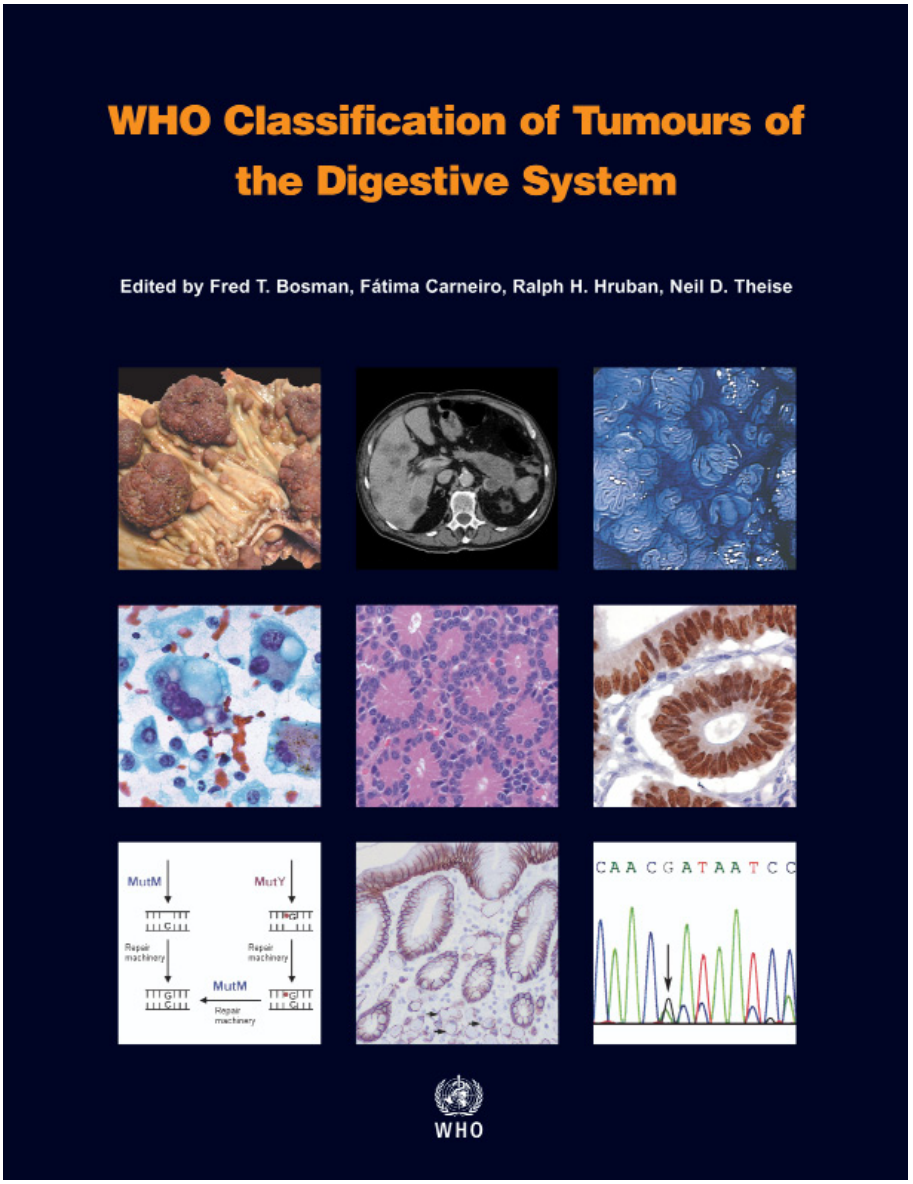

Figure 2. Cover of the book "WHO Classification of Tumours of the Digestive System"

WHO classification of tumours series (WHO Blue Books)

The objective of this project is to establish a pathological and genetic classification and grading of human tumours that is accepted and used worldwide. Without clearly defined clinical and histopathological diagnostic criteria and, more recently, genetic and expression profiles, epidemiological studies and clinical trials are difficult to conduct. Therefore, this project has high impact in not only pathology communities, but also cancer registration, epidemiology studies, clinical trials and cancer research in general.

IARC has been responsible for the WHO Blue Books since the 3rd edition (2000–2005), which covered all organ sites in 10 volumes. Diagnostic criteria, pathological features and associated genetic alterations were described in a strictly disease-oriented manner. For each volume, 10 000–35 000 copies were printed and distributed worldwide.

The current edition (4th edition) was initiated in 2006, with four new series editors (Dr Fred Bosman, University of Lausanne, Switzerland; Dr Elaine Jaffe, National Institutes of Health, Bethesda, USA; Dr Sunil Lakhani, University of Queensland, Brisbane, Australia; and Dr Hiroko Ohgaki, IARC). The first volume of the 4th edition, Tumours of the Nervous System, was published in June 2007. The second volume, Tumours of the Haematopoietic and Lymphoid Tissues, was published in September 2008, and > 35 000 copies have already been printed and distributed worldwide. The third volume, Tumours of the Digestive System, with four volume editors (Dr F. Bosman, Lausanne, Switzerland; Dr F. Carneiro, Porto, Portugal; Dr R.H. Hruban, Baltimore, USA; and Dr N.D. Theise, New York, USA) was published in 2010, and > 8000 copies have been distributed. The fourth volume, Tumour of the Breast, is in preparation with five volume editors (Dr Sunil R. Lakhani, University of Queensland, Brisbane, Australia; Dr Ian Ellis, University of Nottingham, United Kingdom; Dr Stuart Schnitt, Beth Israel Deaconess Medical Center, Boston, USA; Dr Puay Hoon Tan, Singapore General Hospital, Singapore; and Dr Marc J. van de Vijver, Academic Medical Center, Amsterdam, the Netherlands). The consensus and editorial meeting was held at IARC

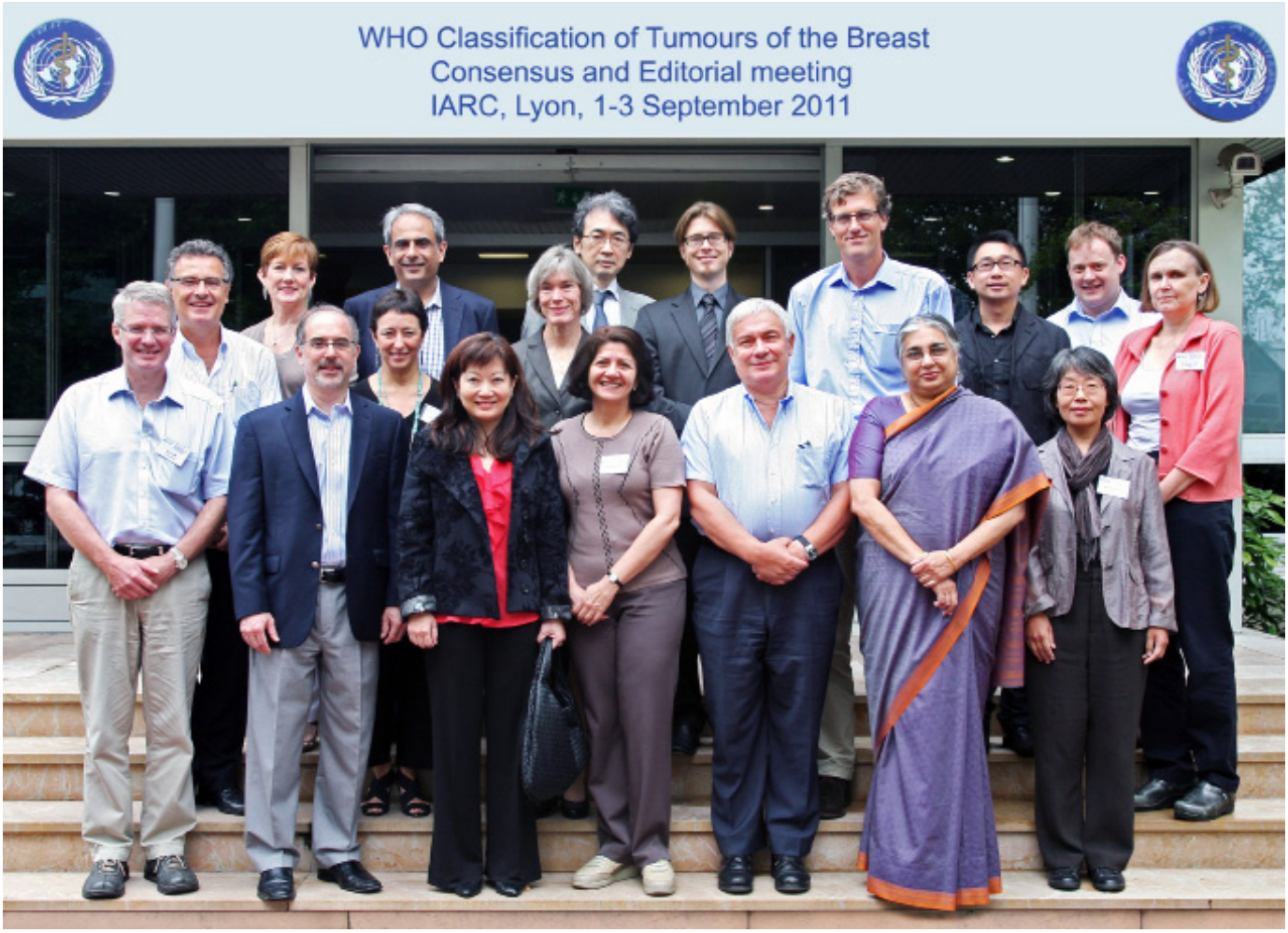

Figure 3. Photo of Working Group of Consensus and Editorial meeting of WHO Classification of Tumours of the Breast

in September 2011, and the book is scheduled to be published in summer 2012. The fifth volume of the 4th edition, Tumours of Soft Tissue and Bone, has been initiated with four volume editors (Dr Christopher D. Fletcher, Brigham and Women's Hospital, Boston, USA; Dr Pancras C.W. Hogendoorn, Leiden University Medical Center, Leiden, the Netherlands; Dr Julia A. Bridge, University of Nebraska Medical Center, Omaha, USA; and Dr Fredrik Mertens, Lund University, Sweden), and the consensus and editorial meeting is scheduled for April 2012.

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Publications

Antonelli M, Buttarelli F, Arcella A *et al.* (2010). Prognostic significance of histological grading, p53 status, YKL-40 expression, and IDH1 mutations in pediatric high-grade gliomas. *J Neurooncol,* 99:209–215. doi:10.1007/s11060-010-0129-5 PMID:20174854

Fiaschetti G, Castelletti D, Zoller S *et al.* (2011). Bone morphogenetic protein-7 is a MYC target with prosurvival functions in childhood medulloblastoma. *Oncogene,* 30:2823–2835.doi:10.1038/onc.2011.10 PMID:21317922

Grunder E, D'Ambrosio R, Fiaschetti G *et al.* (2011). MicroRNA-21 suppression impedes medulloblastoma cell migration. *Eur J Cancer,* 47:2479–2490.doi:10.1016/j.ejca.2011.06.041 PMID:21775132

Kim YH, Lachuer J, Mittelbronn M *et al.* (2011). Alterations in the RB1 pathway in low-grade diffuse gliomas lacking common genetic alterations. *Brain Pathol,* 21:645–651.doi:10.1111/j.1750-3639.2011.00492.x PMID:21470325

Kim YH, Nobusawa S, Mittelbronn M *et al.* (2010). Molecular classification of low-grade diffuse gliomas. *Am J Pathol,* 177:2708–2714.doi:10.2353/ajpath.2010.100680 PMID:21075857

Kim YH, Pierscianek D, Mittelbronn M *et al.* (2011). TET2 promoter methylation in low-grade diffuse gliomas lacking IDH1/2 mutations. *J Clin Pathol,* 64:850–852.doi:10.1136/jclinpath-2011-200133 PMID:21690245

Lv S-Q, Kim YH, Giulio F *et al.* (2011). Genetic alterations in microRNAs in medulloblastomas. Brain Pathol. In press. PMID:21793975

Nobusawa S, Lachuer J, Wierinckx A *et al.* (2010). Intratumoral patterns of genomic imbalance in glioblastomas. *Brain Pathol,* 20:936–944. PMID:20406234

Nobusawa S, Stawski R, Kim YH *et al.* (2011). Amplification of the PDGFRA, KIT and KDR genes in glioblastoma: a population-based study. *Neuropathology,* 31:583–588.doi:10.1111/j.1440-1789.2011.01204.x PMID:21382095

Ohgaki H, Kim YH, Steinbach JP (2010). Nervous system tumors associated with familial tumor syndromes. *Curr Opin Neurol,* 23:583–591.doi:10.1097/WCO.0b013e3283405b5f PMID:21042217

Ohgaki H, Kleihues P (2011). Genetic profile of astrocytic and oligodendroglial gliomas. *Brain Tumor Pathol,* 28:177–183.doi:10.1007/s10014-011-0029-1 PMID:21442241

Toedt G, Barbus S, Wolter M *et al.* (2011). Molecular signatures classify astrocytic gliomas by IDH1 mutation status. *Int J Cancer,* 128:1095–1103. doi:10.1002/ijc.25448 PMID:20473936