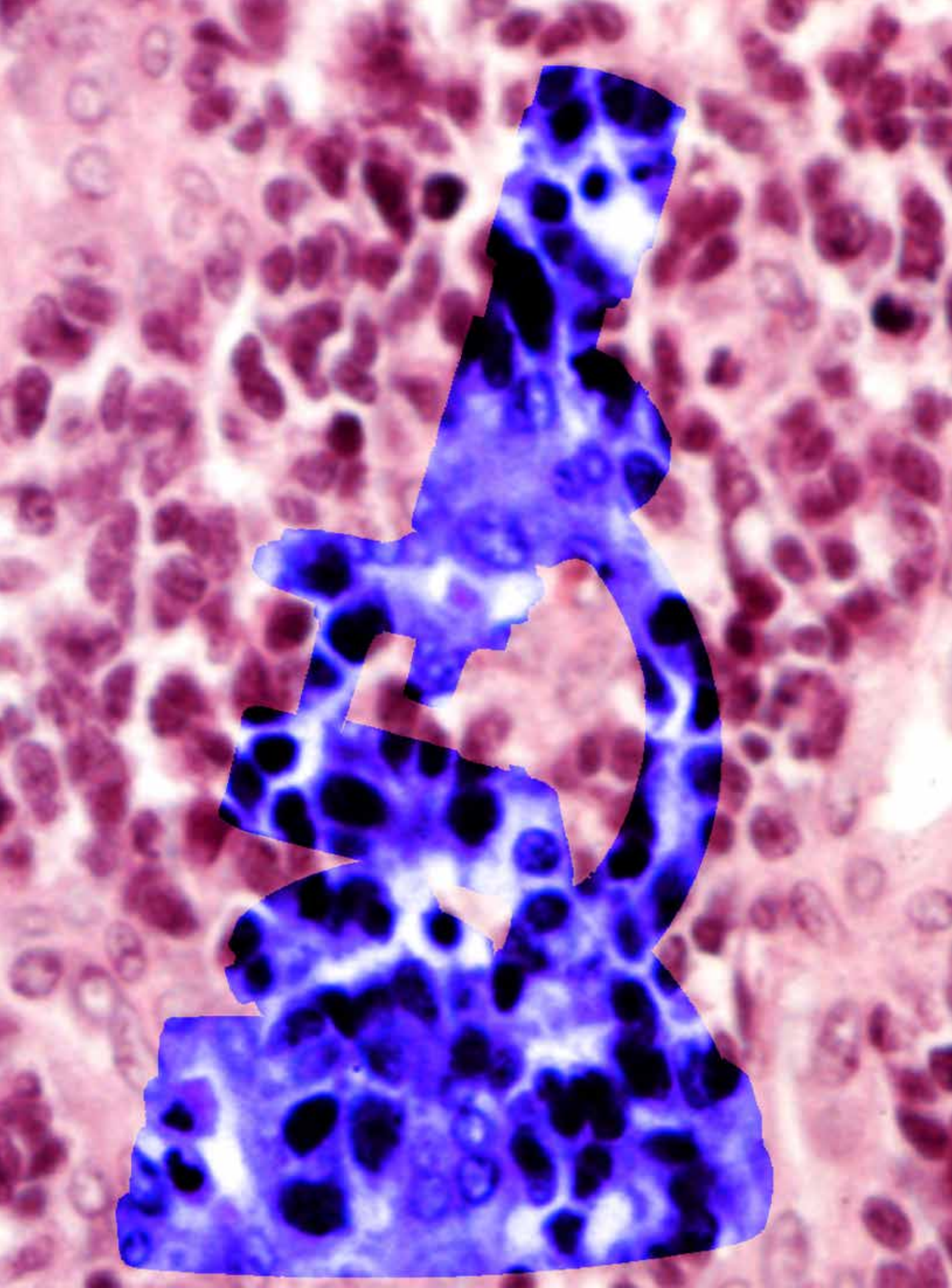

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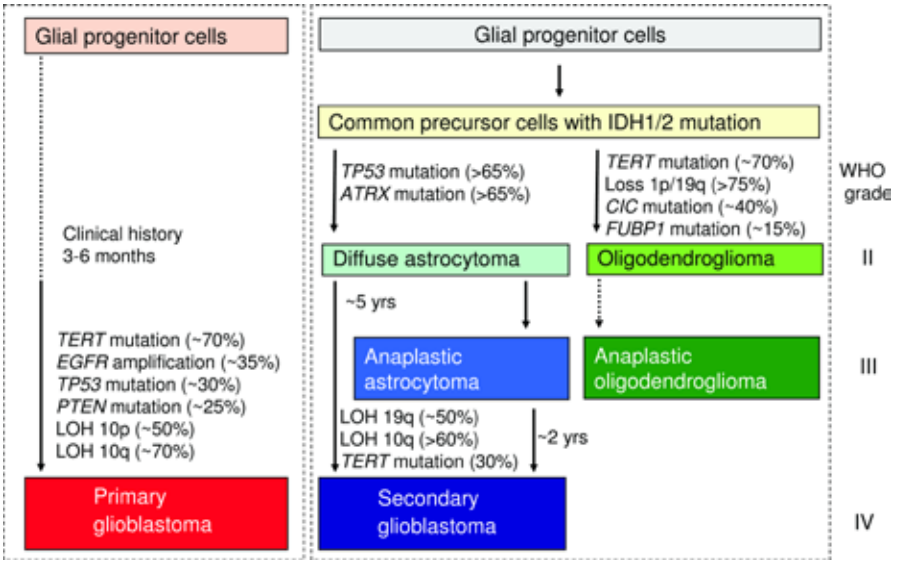
The Section of Molecular Pathology (MPA) studies the molecular basis of human neoplasms, in particular brain tumours, using tumour samples from patients with clinical and follow-up data. Histologically recognized phenotypes are correlated with genotypes and expression profiles in order to elucidate the 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, monitor 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 fourth edition of the World Health Organization (WHO) Classification of Tumours Series (WHO Blue Books). In 2012–2013, the fourth volume (Tumours of the Breast) and fifth volume (Tumours of Soft Tissue and Bone) were published. Currently, work on the sixth volume (Tumours of Female Reproductive Organs) and seventh volume (Tumours of Lung, Pleura, Thymus, and Heart) is in progress. The main projects of MPA during the 2012–2013 biennium are detailed below.

Genetic pathways to primary and secondary glioblastoma

Glioblastoma is the most common and aggressive malignant brain tumour. Most glioblastomas (~90%) develop rapidly *de novo* in elderly patients without clinical or histological evidence of a less malignant precursor lesion (primary glioblastomas). Secondary glioblastomas progress from low-grade diffuse astrocytoma or anaplastic astrocytoma. They manifest in younger patients, have a lesser degree of necrosis, are preferentially located in the frontal lobe, and carry a significantly better prognosis. Histologically, primary and secondary glioblastomas are largely indistinguishable, but they differ in their genetic and epigenetic profiles. Decisive genetic signposts of secondary glioblastoma are *IDH1* mutations, which are absent in primary glioblastomas and are associated with a hypermethylation phenotype. *IDH1* mutations are the earliest detectable genetic alteration in precursor low-grade diffuse astrocytomas and in oligodendrogliomas, indicating that these tumours are derived from neural precursor cells that differ from those of primary glioblastomas. There is increasing evidence that mutation of *IDH1* is a definitive diagnostic molecular marker of secondary glioblastomas and is more reliable and objective than clinical criteria. Despite a similar histological appearance, primary and secondary glioblastomas are distinct tumour entities that originate from different precursor cells and may require different therapeutic approaches. Genetic pathways to primary and

secondary glioblastoma are outlined in Figure 1.

Figure 1. Genetic pathways to primary and secondary glioblastoma.



Frequent *BRAF* gain in low-grade diffuse gliomas with 1p/19q loss

Chromosomal 7q34 duplication and *BRAF-KIAA1549* fusion are characteristic genetic alterations in pilocytic astrocytomas. Focal gains at chromosome 7q34 appear to be common in diffuse astrocytomas, but their significance is unclear. We assessed *BRAF* gain and *BRAF* mutations in 123 low-grade diffuse gliomas. Quantitative polymerase chain reaction (PCR) revealed *BRAF* gain in 17/50 (34%) oligodendrogliomas, a significantly higher frequency than in diffuse astrocytomas (7/55; 13%; P = 0.011). *BRAF* gain was common in low-grade diffuse gliomas with 1p/19q loss (39%) and those lacking any of the genetic alterations analysed (31%), but was rare in those with *TP53* mutations (2%). Logistic regression analysis showed a significant positive association between 1p/19q loss and *BRAF* gain (P = 0.003) and a significant negative association between *TP53* mutations and *BRAF* gain (P = 0.004). Fluorescence in situ hybridization (FISH) analysis of 26 low-grade diffuse gliomas with *BRAF* gain additionally revealed *BRAF-KIAA1549* fusion in one oligodendroglioma. Sequencing of cDNA in 17 low-grade diffuse gliomas showed *BRAF-KIAA1549* fusion in another oligodendroglioma. These results suggest that low-grade diffuse gliomas with 1p/19q loss have frequent *BRAF* gains, and a small fraction of oligodendrogliomas may show *BRAF-KIAA1549* fusion.

Molecular mechanisms of mesenchymal differentiation in gliosarcomas

Gliosarcoma is a rare glioblastoma variant characterized by a biphasic tissue pattern with alternating areas that display either glial or mesenchymal differentiation. Previous analyses have shown identical genetic alterations in glial and mesenchymal tumour areas, suggesting that gliosarcomas are genetically monoclonal and that mesenchymal differentiation reflects the elevated genomic instability of glioblastomas. We compared genome-wide chromosomal imbalances using array comparative genomic hybridization in glial and mesenchymal tumour areas of 13 gliosarcomas. The patterns of gain and loss were similar, except for the gain at 13q13.3-q14.1 ($\log_2$ ratio > 3.0), containing the *STOML3*, *FREM2*, and *LHFP* genes, which was restricted to the mesenchymal tumour area of a gliosarcoma. Further analyses of 64 cases of gliosarcoma using quantitative PCR showed amplification of the *STOML3*, *FREM2*, and *LHFP* genes in 14 (22%), 10 (16%), and 7 (11%) mesenchymal tumour areas, respectively, but not in glial tumour areas. These results suggest that the mesenchymal components in a small fraction of gliosarcomas may be derived from glial cells with additional genetic alterations.

We also assessed 40 gliosarcomas for immunoreactivity of Slug, Twist, matrix metalloproteinase-2 (MMP-2), and MMP-9, which are involved in the epithelial–mesenchymal transition (EMT) in epithelial tumours. Nuclear Slug expression was observed in > 50% of neoplastic cells in mesenchymal tumour areas of 33 (83%) gliosarcomas, but not in glial areas ($P < 0.0001$). Nuclear Twist expression was observed in > 50% of neoplastic cells in mesenchymal tumour areas of 35 (88%) gliosarcomas, but glial tumour areas were largely negative except in four cases ($P < 0.0001$). Expression of MMP-2 and MMP-9 was also significantly more extensive in mesenchymal than in glial tumour areas. Of 20 ordinary glioblastomas, none showed Slug or Twist expression in > 10% neoplastic cells. Thus, expression of Slug, Twist, MMP-2, and MMP-9 was characteristic of mesenchymal tumour areas of gliosarcomas, suggesting that mechanisms involved in EMT in epithelial neoplasms may also play a role in mesenchymal differentiation in gliosarcomas.

Prognostic molecular markers in diffuse astrocytomas

Diffuse astrocytomas (WHO grade II) tend to advance to secondary glioblastomas, but the time until progression and the clinical outcome vary significantly. Despite having distinct genetic profiles, primary and secondary glioblastomas have similar histological features. We hypothesized that the highly malignant phenotype of glioblastoma may be attributable to genetic alterations that are common to both glioblastoma subtypes.

Since loss of heterozygosity (LOH) at 10q has been known to be frequent (> 60%) in both primary and secondary glioblastomas, we first searched for commonly deleted genes at 10q in glioblastomas with *IDH1* mutations (a hallmark of secondary glioblastoma) and those without *IDH1* mutations (typical for primary glioblastoma) in data from The Cancer Genome Atlas (TCGA). With log-ratio thresholds of –1.0, 10 genes were identified; with log-ratio thresholds of –2.0, only the

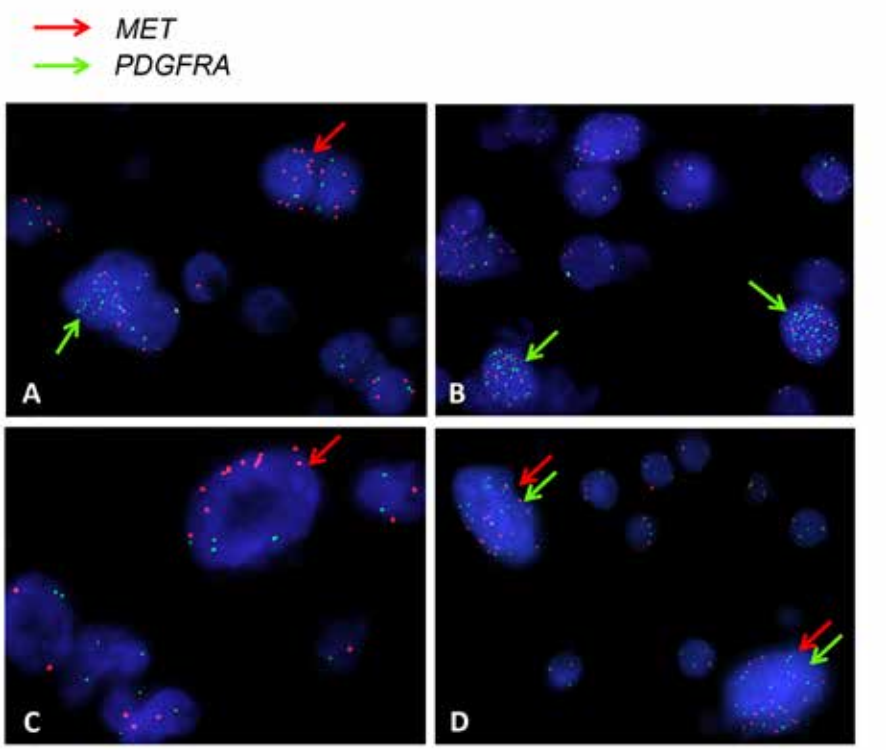
DMBT1 (deleted in malignant brain tumour 1) gene at 10q26.13 remained as a deleted gene in glioblastomas with or without *IDH1* mutations (12.5% vs 8.0%). We then analysed a total of 404 gliomas by differential PCR and found a *DMBT1* homozygous deletion at a similar frequency in primary and secondary glioblastomas (20% vs 21%). A fraction (11%) of diffuse astrocytomas showed a *DMBT1* homozygous deletion that was significantly associated with shorter overall survival (52.8 vs 84.0 months; $P = 0.003$). These results indicate that a *DMBT1* homozygous deletion is present in a small fraction of diffuse astrocytomas and is associated with an unfavourable clinical outcome.

A similar approach was used to identify commonly (> 35%) amplified genes in glioblastomas with and without *IDH1* mutations in data from TCGA. A total of 25 genes were identified, of which 21 were located at 7q31-34. We then screened 264 gliomas for gain of the *MET* gene at 7q31.2 with quantitative PCR. *MET* gain was detected in primary glioblastomas (47%) and secondary glioblastomas (44%), suggesting that this genetic alteration plays a role in the pathogenesis of both glioblastoma subtypes. It was also common in diffuse astrocytomas (38%), but less frequent in oligodendrogliomas (16%). *MET* gain in diffuse astrocytomas was associated with shorter survival (median, 43.0 vs 70.7 months; $P = 0.004$), suggesting that *MET* gain is a useful prognostic marker for diffuse astrocytomas.

PDGFRA gain in low-grade diffuse gliomas

Glioblastomas with a proneural expression signature are characterized by frequent *IDH1* mutations (i.e. a genetic hallmark of secondary glioblastomas) and *PDGFRA* (platelet-derived growth factor receptor-α) amplification. Mutations in *IDH1/2* are frequent and early genetic events in diffuse astrocytoma, a precursor to secondary glioblastomas, but little is known about the role and timing of *PDGFRA* amplification in these tumours. We assessed *PDGFRA* gain in 342 low-grade diffuse gliomas by quantitative PCR. Gain in *PDGFRA* was detected in 27 (16%) of 166 diffuse astrocytomas, significantly more frequently than in oligodendrogliomas (3%; $P < 0.0001$). Analyses using previously published data from our laboratory showed an inverse correlation between *PDGFRA* gain and *IDH1/2* mutations ($P = 0.018$) or 1p/19q loss ($P < 0.0001$). Most diffuse astrocytomas showed *IDH1/2* mutations and/or *PDGFRA* gain (154 [93%] of 166). Mean survival of diffuse astrocytoma patients with *PDGFRA* gain was 8.8 ± 1.6 years, similar to that of patients with *IDH1/2* mutations (7.8 ± 0.5 years) or *TP53* mutations (7.6 ± 0.6 years), but significantly longer than that of those with *MET* gain (4.4 ± 0.7 years). Dual-colour FISH in 6 diffuse astrocytomas with *PDGFRA*/*MET* co-gain identified by quantitative PCR revealed that *PDGFRA* and *MET* were typically amplified in different tumour cell populations. Tumour cells with co-amplification were also focally observed, suggesting intratumour heterogeneity even in diffuse astrocytomas.

Figure 2. Dual-colour fluorescence in situ hybridization (FISH) analysis in diffuse astrocytomas shows intratumoral heterogeneity of amplification of *PDGFRA* (green) and *MET* (red). *PDGFRA* amplification and *MET* amplification were observed in separate cells in the same tumour (A). *PDGFRA* amplification (B) or *MET* amplification (C) were seen in individual diffuse astrocytoma cells within the same specimen. Rare tumour cells displayed co-amplification of *PDGFRA* and *MET* (D). Source: Motomura *et al.* (2013); reproduced with the permission of the publisher.



Genetic alterations in microRNAs in medulloblastomas

MicroRNAs (miRNAs) regulate a variety of cellular processes via the regulation of multiple target genes. We screened 48 medulloblastomas for mutation, deletion, and amplification of 9 miRNA genes that 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 the *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-135*b (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, 35/48 (73%) medulloblastomas had at least one alteration. Real-time RT-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 the medulloblastoma/primitive neuroectodermal tumour 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.

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 is of great importance not only in pathology communities, but also to cancer registration, epidemiology studies, clinical trials, and cancer research in general.

Figure 3. Cover of *WHO Classification of Tumours of the Breast*, fourth edition.

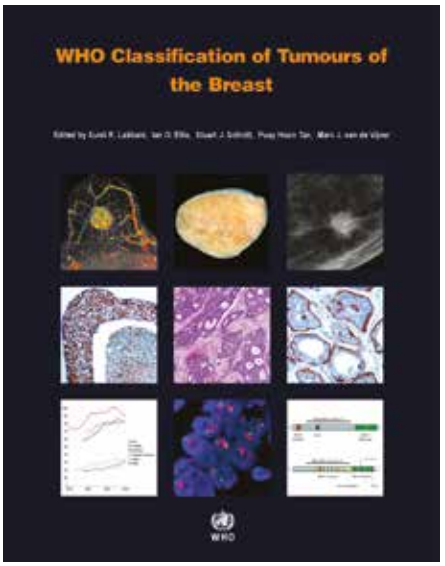


Figure 4. Working Group members at the consensus and editorial meeting of *WHO Classification of Tumours of Soft Tissue and Bone*. The meeting was held at the University of Zurich, Switzerland, on 18–20 April 2012. Photograph courtesy of Norbert Wey.

IARC has been responsible for this project since the third 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 latest edition (fourth) of the WHO Classification of Tumours Series 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).

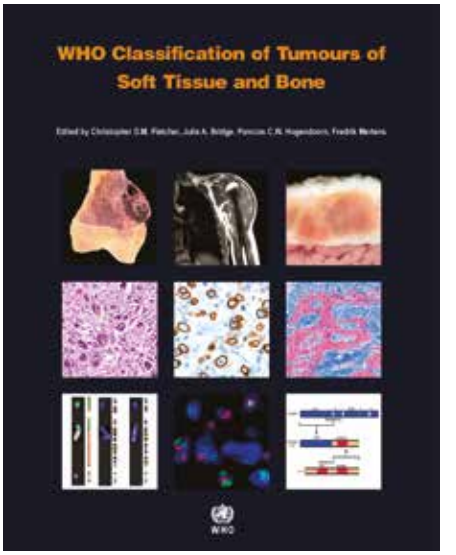
• The first volume of the fourth edition, Tumours of the Central Nervous System, was published in June 2007.

• The second volume, Tumours of Haematopoietic and Lymphoid Tissues, was published in September 2008 and > 35 000 copies have been distributed worldwide.

• The third volume, Tumours of the Digestive System, with four 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.

• The fourth volume, Tumours of the Breast, with five editors (Dr Sunil R. Lakhani, University of Queensland, Brisbane, Australia; Dr Ian Ellis, University of Nottingham, United Kingdom; Dr Stuart Schnitt, Beth Israel

Figure 5. Cover of *WHO Classification of Tumours of Soft Tissue and Bone*, fourth edition.



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), was published in July 2012.

• The fifth volume, Tumours of Soft Tissue and Bone, with four 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), was published in January 2013.

• The sixth volume, Tumours of Female Reproductive Organs, with four editors (Dr Robert J. Kurman, Johns Hopkins University, Baltimore, USA; Dr Maria Luisa Carcangiu, Fondazione IRCCS, Institute Nazionale dei Tumori, Milano, Italy; Dr Simon Herrington, Centre for Oncology and Molecular Medicine, Ninewells Hospital and Medical School, Dundee, United Kingdom; and Dr Robert H. Young, Massachusetts General Hospital, Harvard Medical School, Boston, USA), is in preparation. The consensus and editorial meeting was held on 13–15 June 2013, and the book is scheduled to be published in the spring of 2014.

• The seventh volume, Tumours of the Lung, Pleura, Thymus, and Heart, with five editors (Dr William D. Travis, Memorial Sloan Kettering Cancer Center, New York, USA; Dr Alexander Marx, University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany; Dr Elisabeth Brambilla, Centre Hospitalier Universitaire de Grenoble, France; Dr Andrew Nicholson, Royal Brompton Hospital, London, United Kingdom; and Dr Allen Burke, University of Maryland, Baltimore, USA), is in preparation and is scheduled to be published in the spring of 2015.

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