Section of Molecular Pathology (MPA)

Section Head
Dr Hiroko Ohgaki

The Section of Molecular Pathology 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 recognised phenotypes with genotypes and expression profiles, with the objectives of elucidating the molecular basis and genetic pathways that are operative in human neoplasms; identifying molecular markers for improvement of tumour diagnoses and classification; identifying genetic factors that predict sensitivity to treatment, tumour progression and patient outcome; and using genetic data to identify the etiology of human cancers. Since 2006, the Section of Molecular Pathology has also been responsible for the 4th edition of the World Health Organization (WHO) Classification of Tumours Series (WHO Blue Books). The second volume, WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues, was published in 2008, and the third volume (WHO Classification of Tumours of the Digestive System) is in the editing stages.

In its current configuration the Section consists of a single Group, the Molecular Pathology Group (MPA), with the objectives stated above. A few of its more important projects over the Biennium are detailed below.

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IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas and as early events in the development of astrocytomas and oligodendrogliomas

IDH1 encodes isocitrate dehydrogenase 1, which participates in the citric acid cycle and was first reported to be mutated in a study of sequencing >20 000 protein coding genes (Parsons *et al.* Science. 321:1807-1812 2008). We assessed *IDH1* mutations in 321 gliomas of various histological types and biological behavior. A total of 130 *IDH1* mutations were detected, all located at codon 132; 91% of these were G->A mutations (R132H). *IDH1* mutations were frequent in low-grade diffuse astrocytomas (88%) and in secondary glioblastomas that developed through progression from low-grade diffuse or anaplastic astrocytoma (82%). Similarly high frequencies of *IDH1* mutations were found in oligodendrogliomas (79%) and oligoastrocytomas (94%). Analysis of multiple biopsies from the same patient (51 cases) showed that there was no case in which an *IDH1* mutation occurred after acquisition of a *TP53* mutation or loss of 1p/19q, suggesting that *IDH1* mutations are very early events in gliomagenesis and may affect a common glial precursor cell population. *IDH1* mutations were co-present with TP53 mutations in 63% of low-grade diffuse astrocytomas, and with LOH 1p/19q in 64% of oligodendrogliomas. They were rare in pilocytic astrocytomas (10%) and primary glioblastomas (5%), and absent in ependymomas.

Our analyses of *IDH1* mutations in glioblastomas from a population-based study (407 cases) showed that approx. 9% of all glioblastomas in a population contain *IDH1* mutations, and that glioblastoma patients with *IDH1* mutations are significantly younger (mean 47.9 years) and show longer survival than those without *IDH1* mutations. *IDH1* mutations were frequent in glioblastomas diagnosed as secondary (22/30; 73%), but rare in primary glioblastomas (14/377; 3.7%: P<0.0001). *IDH1* mutations as genetic marker of secondary glioblastoma corresponded to the respective clinical diagnosis in 95% of cases. *IDH1* mutations are the therefore most reliable molecular marker of secondary glioblastomas available and should be used to complement clinical criteria to distinguish them from primary glioblastoma. The frequent presence of *IDH1* mutations in secondary glioblastomas and their almost complete absence in primary glioblastomas reinforces the concept that despite their histological similarity, these subtypes are genetically and clinically distinct entities.

We assessed *IDH1* mutations in brain tumors diagnosed in patients from 3 families with Li-Fraumeni syndrome. We identified *IDH1* mutations in 5 astrocytomas that developed in carriers of a *TP53* germline mutation. Without exception, all were R132C, which in sporadic astrocytomas accounts for <5% of *IDH1* mutations. This remarkably selective occurrence of R132C mutations may reflect differences in the sequence of genetic events, with a preference for R132C mutations in astrocytes or precursor cells that already carry a germline *TP53* mutation.

Role of mutations in the Nijmegen breakage syndrome gene *(NBS1)* in brain tumours

Nijmegen breakage syndrome, caused by *NBS1* germline mutations, is a rare autosomal recessive disease with clinical features that include microcephaly, increased radiosensitivity and predisposition to cancer. *NBS1* plays a key role in DNA double-strand break repair and the maintenance of genomic stability. There may be functional interactions between *NBS1* and the *TP53* pathways.

We assessed whether *NBS1* mutations play a role in the pathogenesis of sporadic medulloblastomas. Screening for mutations in the *NBS1* gene (all 16 exons) and the *TP53* gene (exons 5-8) revealed that 7 of 42 (17%) medulloblastomas carried a total of 15 *NBS1* mutations (10 missense point mutations and 5 intronic splicing mutations). Of five medulloblastomas with *TP53* mutations, four (80%) contained *NBS1* mutations, and there was a significant association between *TP53* mutations and *NBS1* mutations (P=0.001), suggesting that medulloblastomas characterised by *NBS1* mutations typically associated with mutational inactivation of the *TP53* gene.

We also screened 87 glioblastomas for *NBS1* mutations, and showed 12 *NBS1* mutations (8 missense and 4 intronic mutations) in 9 of 28 (32%) primary (de novo) glioblastomas carrying two or more *TP53* mutations. In contrast, *NBS1* mutations were not detected in 19 primary glioblastomas with one *TP53* mutation, nor in 21 primary glioblastomas without *TP53* mutations. These results suggest that multiple *TP53* mutations in some glioblastomas are due to deficient repair of DNA double-strand breaks caused by mutational inactivation of the *NBS1* gene.

Promoter methylation and polymorphisms of the *MGMT* gene in glioblastomas: A population-based study

O^6-Methylguanine-DNA methyltransferase (*MGMT*) is a repair enzyme that removes promutagenic O^6-methylguanine adducts in DNA in order to protect cells from acquisition of G:C->A:T mutations. *MGMT* promoter methylation and polymorphisms may affect *MGMT* expression and activity. We assessed *MGMT* promoter methylation and polymorphisms (Leu84Phe, Ile143Val, c.-56C>T) in 371 glioblastomas diagnosed at the population level. *MGMT* methylation was observed in 165 (44%) glioblastomas, with a higher frequency in females than males (53% vs. 39%; *P*=0.0106), and in secondary than primary glioblastomas (73% vs. 43%, *P*=0.0074). The frequency of *TP53* G:C->A:T mutations in glioblastomas with *MGMT* methylation was 25%, which was significantly higher than that in glioblastomas without *MGMT* methylation (16%; *P*=0.0385). The *MGMT* 143 Val allele was significantly less frequent in glioblastomas than in a healthy European Caucasian population, and was associated with longer survival than those with the *MGMT* 143 Ile allele (hazard ratio 0.70; 95% CI=0.48-1.01). These results suggest that *MGMT* methylation may be associated with susceptibility to acquire *TP53* G:C->A:T mutations, and that *MGMT* polymorphisms may affect the risk and prognosis of glioblastomas.

Common polymorphisms in the *MDM2* and *TP53* genes and the relationship between *TP53* mutations and patient outcomes in glioblastomas

MDM2 SNP309 is associated with a younger age of tumour onset in patients with Li-Fraumeni syndrome, and *TP53* codon 72 polymorphism decreases its apoptotic potential. Glioblastomas frequently show genetic alterations in the *TP53* pathway. We assessed *MDM2* SNP309 in 360 glioblastomas, and correlated these with patient age and survival, as well as other alterations in the TP53 pathway. Frequencies of the *MDM2* SNP309 T/T, T/G and G/G genotypes in glioblastomas were 40%, 46% and 14%, respectively. Multivariate analysis showed that the *MDM2* SNP309 G/G allele was significantly associated with favourable outcome in female glioblastoma patients (hazard ratio 0.54; 95% CI=0.32–0.92). There was a significant association between *MDM2* SNP309 G alleles and *TP53* codon 72 Pro/Pro in glioblastomas. Glioblastoma patients with *TP53* codon 72 Pro/Pro genotype were significantly younger than Arg/Arg carriers (mean 50.2 vs. 56.1 years; *P*=0.018). Multivariate analysis showed that those with *TP53* codon 72 Arg/Pro allele had significantly shorter survival than those with the Arg/Arg allele (hazard ratio 1.35; 95% CI=1.07–1.71). Detailed analyses revealed that the *TP53* codon 72 Pro allele was significantly associated with shorter survival among patients with glioblastomas carrying a *TP53* mutation, and among those treated with surgery plus radiotherapy.

Whole genome amplification for array CGH using DNA extracted from formalin-fixed paraffin-embedded histological sections

Array comparative genomic hybridization (CGH) is useful to assess genome-wide chromosomal imbalance, but the requirement for relatively large amounts of DNA can be a limitation, in particular for samples extracted from small tumour areas on paraffin sections. Whole genome amplification (WGA) can be carried out before array CGH to obtain sufficient DNA, but the possibility of artefacts due to biased amplification cannot be excluded. We optimized the WGA protocol to generate sufficient DNA with minimum amplification bias. Using formalin-fixed paraffin-embedded histological sections of tumours carrying known *TP53* mutations, LOH 1p, LOH 10q, LOH 19q, and *EGFR* amplification, we first optimised the protocol so that these genetic alterations are detected after WGA. We found that a ligation step before WGA is important, as it allows a short reaction time with Phi29 to generate WGA-DNA with greatly decreased amplification bias. Using template >150 ng of DNA, a ligation step before WGA, and a short reaction time with Phi29 DNA polymerase (<1.5 h), we obtained WGA-DNA (>4 _g) with minimum amplification bias (<3-fold). Using this protocol, we carried out array CGH (Agilent 105K) before and after WGA. Pearson correlation analysis indicated a significant positive correlation in array CGH results between DNA before and after WGA (*P*<0.0001). These results suggest that genetic analyses are possible using WGA-DNA extracted from

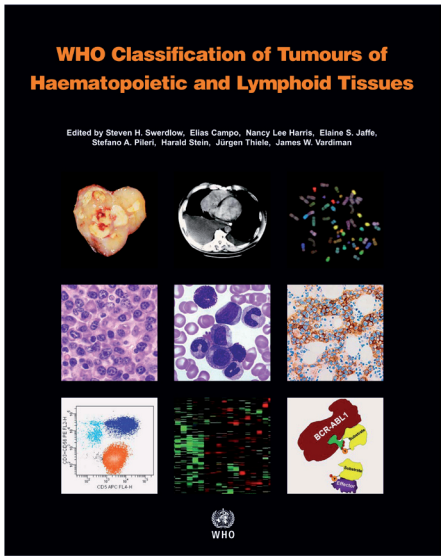
paraffin sections, but that they should be carried out with a carefully optimised and controlled protocol.

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. This project therefore has a substantial impact in not only pathology communities, but also cancer registration, epidemiology studies, clinical trials, and cancer research in general.

IARC has been responsible for this book project 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 over 30 000 copies have already been printed and distributed worldwide. We are currently preparing the 3rd volume, *Tumours of the Digestive System*, with 4 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) and with >110 contributors. The consensus and editorial conference is scheduled for December 2009, and the book is scheduled to be published in 2010.



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Publications

Original articles

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