NEWSLETTER OF THE HONG KONG COLLEGE OF PATHOLOGISTS

PATHOLOGUE

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability

THE HONG KONG COLLEGE OF PATHOLOGISTS
SCIENTIA ILLUMINAT MEDICINAM
香港病理學專科學院

VOLUME 23, ISSUE 2 NOVEMBER 2014

INDEX

Message from the President

Both Hong Kong and our College have faced unprecedented challenges this year. Our College and our specialty have been in the spotlight of the media. There has been doubt on the standard of pathology training and qualification as well as pathology service in Hong Kong. While continuous self-reflection and review are constructive, we must not lose faith in our good practice and should continue our devotion to the community. With our sustained high quality of service, I am confident that the trust of the community can be maintained.

This is also a moment of celebration for our new Fellows and Members. On behalf of the College, I would like to extend my warmest welcome to all newly admitted Fellows and Members to the family of The Hong Kong College of Pathologists. You have also faced and defeated challenges bravely. After surpassing years of serious training and examinations, our Fellows are now qualified specialists in pathology. As consultants to bedside doctors, you will play pivotal role in diagnosis as well as guiding the plan of prevention and treatment of disease.

To let our community and even our medical profession understand our role and contribution, it is high time for us to reach out and explain our work. We have expressed our view on direct-to-consumer genetic tests earlier this year. Moreover, under the coordination of the Academy, our College has received extensive coverage in the media explaining our work and the contribution of our specialty to patients. In response to queries regarding the standard of pathology practice, the College has also explained to the public our stringent training and examination to secure their trust. Recently, in liaison with the international pathology community on International Pathology Day, a one-week exhibition in the Hong Kong Museum of Medical Sciences in November has been organized. We do not expect immediate or quick effects but our continuous sincere work should be able to break the barrier surrounding our profession.

The College has also been playing an active role in various local health planning issues in the field of genetics and genomics as well as screening and prevention of cancers.

I believe that the future of pathology profession is promising and we should all work together to make it better.

Prof. Cheung Nga Yin, Annie
The President
November 2014

THE EDITORIAL BOARD

Dr. LAI Sai Chak
(Chief Editor)
Dr. CHAN Chak Lam, Alexander
Dr. LO Cheuk Lam, Regina
Dr. LO Yee Chi, Janice
Dr. WONG Lap Gate, Michael
Dr. Cherry WU

Please send comments to:
Dr. LAI Sai Chak
(Chief Editor),
Address: *Forensic Pathologists' Office, 6/F Arsenal House West Wing, Police Headquarters, Arsenal Street, Wanchai, Hong Kong.*
E-mail: *sc_lai@dh.gov.hk*
Phone: *2860 2461*
Fax: *2804 1714*

Featured Article -

Should we be thinking about revalidation or recertification of pathologists?

Dr. Ma Shiu Kwan Edmond

In the wake of the alleged erroneous pathology reports from a new College fellow that hit the media, not only does the College need to defend the robustness of our training and assessment, professionalism and competence level of pathologists also come into question. While there are calls for a probationary period of practice for new fellows and discussion on reassessment of fellows by the College or other professional bodies, a pertinent issue to ponder upon is the revalidation and recertification of pathologists.

Revalidation in the United Kingdom

Revalidation is a measure used in the UK by the General Medical Council (GMC) to assure patients and the public, employers and other healthcare workers that licensed medical doctors are up to date and fit to practise. In order to maintain a licence to practise in the UK, the medical practitioner is expected to have at least one appraisal per year that is based on 'Good Medical Practice', the core guidance of the GMC for doctors. Revalidation has gone live from Monday, 3 December 2012. Licensed doctors need to maintain a portfolio of supporting information drawn from their practice which demonstrates how they are continuing to meet the requirements set out in the 'Good Medical Practice Framework for Appraisal and Revalidation'.

To help pathologists navigate the maze of documents, the Royal College of Pathologists provide a guidance document on supporting information for appraisal and revalidation [1]. The information can be divided into three main parts: 1. General information about the doctor and professional work. 2. Keeping up to date in terms of continuing professional development, review of practice, quality improvement activity and significant clinical incidents or untoward events. 3. Feedback on clinical practice by colleagues, patients and carers, and complaints and compliments. From a recent article in the Bulletin of the RCPath on early experience in revalidation [2], there are two points of note. The first is the recommendation of discussing the detailed content of individual performance in external quality assurance reports as an option with the appraiser. How many of us are willing to do that? The second is that although feedback from patients is considered important, direct patient contact is uncommon for pathologists (especially histopathologists) and poses obvious problems. Therefore in the long run it is advantageous for pathologists to go out of the laboratory to explore innovative ways of soliciting patient feedback.

Recertification in the United States

Across the Atlantic in the United States, certificates issued by the American Board of Pathology (ABP) are valid as long as the diplomate is meeting all reporting requirements for the Maintenance of Certification (MOC) program. The four parts of the program are: 1. Professionalism and professional standing. 2. Life-long learning and self-assessment. 3. Assessment of knowledge, judgement and skills. 4. Improvement in medical practice. The MOC cycle is 10 years and the key to recertification is to pass a secure examination during every cycle. The six areas of competency to be evaluated and outcome measured are: 1. Patient care. 2. Medical knowledge. 3. Practice-based learning and improvement. 4. Interpersonal and communication skills. 5. Professionalism. 6. System-based practice. From 1 January 2006, all certificates issued by the ABP are required to be maintained through MOC. Diplomates with non-time limited certificates are not required but

strongly encouraged to participate in the MOC. In fact, some employers may give incentives for doing so.

How do you actually evaluate the performance of a pathologist? The essential components can already be found in the revalidation program of the UK and the recertification program of the US. Detailed evaluation metrics are provided by the College of American Pathologists (CAP) [4]. For example, the professional practice evaluation of a haematopathologist includes: 1. Practice activity (volume of work performed), both administrative and clinical. 2. Timeliness (meeting turnaround time <TAT> goals), both administrative and clinical (e.g. TAT of bone marrow report). 3. Competence, both administrative (e.g. proficiency testing peer review) and clinical (e.g. peer review of blood smear reports). 4. General aspects, such as on-call reliability and continuing medical education. 5. Procedural, such as bone marrow aspiration and biopsy.

The road ahead -- some personal opinions

Our College is small and ranks 12 out of 15 among colleges of the Hong Kong Academy of Medicine in terms of numbers of fellows. Also pathology practice is subspecialty based and the distribution of fellows in each discipline is uneven. To run a revalidation or recertification program is an insurmountable task in terms of time commitment of fellows and administrative work for the College. There is also no guarantee that such a program ensures the competency of pathologists. Just imagine what would happen if a newly revalidated pathologist in the UK or a pathologist in the US recently passing the MOC examination commits serious error in diagnosis.

There is no need at this moment for the College to initiate any revalidation or recertification process. There is however room for improving our CME/CPD program. Presently a fellow can sit through lectures that are CME accredited by the College and earn passive CME points even though the content may not be related to the specialty practised by the fellow, or even not related to pathology at all. While there is no intention to undermine the importance of a broad knowledge base in medicine, the CME/CPD program should at least be more focused to the specialty or scope of practice of pathologists. Also accruing a certain proportion of active CME points should be mandated, such as delivering or chairing lectures or presenting abstracts in conferences, in addition to CME points from passive participation or other activities. Valuable performance indicators of pathologists are found in quality assurance activities, peer review and clinical audit processes. Only through strengthening and improving these activities can the College be prepared to respond to potential pressure from the public or calls from the medical circle for our competence assessment.

Disclaimer: The opinion expressed is that of the author and does not represent the view of the HKCPath, the Credentials & Appeals Committee, or the Education Committee.

References

1. The Royal College of Pathologists. Guidance on supporting information for appraisal and revalidation for pathologists.
2. The Bulletin of the Royal College of Pathologists, July 2014; No. 167: 196 – 7.
3. American Board of Pathology. Maintenance of Certification Booklet of Information.
4. CAP White Paper. EvalumetricsTM for Pathologists' Ongoing Professional Practice Evaluation and Focused Professional Practice Evaluation. January 2013.

THE HONG KONG COLLEGE OF PATHOLOGISTS:

TOPICAL UPDATE

Volume 9, Issue 2 July 2014

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability

Editorial note: *Diffuse large B-cell lymphoma (DLBCL) is the commonest subtype of non-Hodgkin lymphoma, accounting for about 30% to 40% of newly diagnosed non-Hodgkin lymphoma worldwide and in Hong Kong. In this topical update, Dr. Choi Wai Lap reviews the genetic basis of DLBCL and its impact on classification, prognosis and even treatment. We welcome any feedback or suggestions. Please direct them to Dr. WS Wong (e-mail: sws_wong@yahoo.com.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.*

Molecular Classification and Genetic Alterations of Diffuse Large B-cell Lymphoma

Dr. CHOI Wai Lap
Department of Clinical Pathology
Tuen Mun Hospital

DLBCL	Diffuse Large B-cell Lymphoma
GEP	Gene expression profiling
COO	Cell of origin
IHC	Immunohistochemical
GCB	Germinal center B-cell like
ABC	Activated B-cell like
COO	Cell of origin
BCR	B-cell receptor
TLR	Toll-like receptor

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the commonest subtype of non-Hodgkin lymphoma, accounting for about 30% to 40% of newly diagnosed non-Hodgkin lymphoma worldwide and in Hong Kong.[1] DLBCL is heterogeneous in clinical presentation, morphology, immunophenotype, cytogenetics and prognosis. In the WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues published in 2008, several specific clinicopathological entities of DLBCL have been recognized, while leaving the rest to DLBCL, not otherwise specified, which is by far the most prevalent entity among the large B-cell lymphomas.[2] In the following discussion, the term DLBCL will be used interchangeably with DLBCL, not otherwise specified.

Gene expression profiling and molecular classification of DLBCL

Gene expression profiling (GEP) is the simultaneous measurement of the transcription levels of thousands of genes to their corresponding messenger RNAs (mRNAs). GEP can be achieved by various technologies including DNA microarray, serial analysis of gene expression (SAGE) and most recently next generation sequencing (RNA-Seq).

Using DNA microarray technology on DLBCL, two distinct molecular subgroups were discovered based on the similarity of their gene expression pattern with a possible cell of origin (COO): the germinal centre B-cell-like (GCB-cell-like, or abbreviated as GCB) and the activated B-cell-like (ABC-like, or abbreviated as ABC).[3] These molecular subgroups showed significantly different survival rates when treated with

conventional cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) chemotherapeutic regimen.

In order to facilitate the classification process by GEP, a statistical method based on Bayes' rule was then developed to estimate the probability of membership.[4] Cases that had less than 90% probability of belonging to either molecular subgroup would be regarded as 'unclassified' molecularly.[4] This statistical method laid the foundation of subsequent GEP studies using DNA microarray technology, which further confirmed the prognostication of the GCB and ABC molecular subgroups, even with the addition of rituximab to the CHOP (R-CHOP) regimen.[5,6]

DNA microarray technology showed that there are two distinct subgroups of diffuse large B-cell lymphoma (DLBCL) based on cell of origin (COO) classification: Germinal centre B-cell-like (GCB-cell-like) and Activated B-cell-like (ABC)

Immunohistochemical (IHC) algorithms to approximate the COO classification of DLBCL

Although the COO molecular classification of DLBCL is a powerful prognosticator, it is fairly difficult to apply in routine anatomical pathology practice. This can be attributed to the lack of expertise and facilities for DNA microarray in routine diagnostic laboratories, the relatively high costs of these DNA microarray chips, and the requirement of snap-frozen diagnostic materials.

Hans et al., therefore, developed an IHC algorithm based on GEP results using three IHC stains: CD10, BCL6 and MUM1/IRF4 in 2004.[7] While the Hans algorithm is relatively simple and has been used most extensively in clinical trials, it has been criticized for basing on treatment results of CHOP instead of R-CHOP, which have become the standard regimen for DLBCL.

Other IHC algorithms using different IHC stain combinations and different cutoff levels of the various markers were thus developed in patients treated with R-CHOP, including the Nyman, Choi, Tally and Visco-Young algorithms.[8-11] The first three of these and the Hans algorithm were compared head to head, and correlated with DNA microarray data of DLBCL samples from patients treated with R-CHOP.[10] In that comparison, the Tally, Choi and Hans algorithms all showed high sensitivity, specificity and predictive values. The three algorithms also showed high concordance with DNA microarray results at 93%, 87% and 86%, respectively. However, the Nyman algorithm had a low sensitivity and negative predictive value despite showing a fairly high concordance (81%).[10]

The most recent Visco-Young algorithm seemed to have a high concordance rate to DNA microarray data at 92.6%, but the sensitivity, specificity and predictive values were uncertain.[11] Besides, the hazard ratios of the COO molecular classification in univariate and multivariate analyses appeared to be less marked compared with the Nyman, Choi and Tally algorithms.[10,11]

The long list of IHC algorithms may seem puzzling and difficult for the anatomical pathologists to choose, but it seems that the Hans, Choi and Tally algorithms are among the most robust. Individual laboratory may choose among these three algorithms based on the availability of the IHC stains and familiarity to these algorithms.

The Hans, Choi and Tally algorithms are the most robust immunohistochemical algorithms to approximate the cell of origin classification of DLBCL.

Other IHC markers useful for prognostication of DLBCL treated with R-CHOP

In recent years, additional markers for further refinement of the prognostication within the GCB and ABC DLBCL subgroups emerged. Meyer et al. found that any SPARC expression in DLBCL microenvironment was associated with a significantly longer overall survival, and patients with high SPARC positivity in the microenvironment also had a significantly longer event-free survival. These survival differences were mainly due to the prognostic effect of SPARC positive cells in the ABC subgroup, but not in the GCB subgroup.[12] Perry et al. further combined the COO classification, SPARC expression and microvessel density to formulate a new biologic prognostic model for all DLBCL, with the non-GCB phenotype, SPARC expression <5% and microvessel density quartile 4 being adverse factors for outcome.[13]

BCL2 expression predicted for inferior survival in patients with GCB DLBCL but not ABC DLBCL when R-CHOP was used.[14] Since the high BCL2 expression in GCB DLBCL is due to the t(14;18) translocation,[13] it is not surprising that patients with GCB DLBCL harbouring *BCL2* translocations would have poor outcomes.[15] Similar to the

double-hit B-cell non-Hodgkin lymphomas with both *MYC* and *BCL2* translocations, recent studies also found that MYC and BCL2 protein co-expression in DLBCL predicted for poor outcomes,[16-18] and it was observed that MYC/BCL2 co-expression was commoner in ABC DLBCL,[17, 18] which appeared to contribute significantly to their gene expression signature and adverse outcome of ABC DLBCL.[17] Another study also confirmed that high MYC expression, high BCL2 expression and low BCL6 expression were independent adverse factors for survival of DLBCL patients, and an IHC score based on the expression of these proteins and the *MYC* translocation status was also predictive for survival.[19]

The expression of CD30 was recently observed to predict for superior 5-year overall and progression-free survival, with this favourable outcome maintained in both the GCB and ABC subgroups.[20]

Among these prognostic markers discovered in the last few years, it seems that the combination of MYC and BCL2 expression is relatively easy to apply and has the potential of pinpointing about 30% of the DLBCL cases with incurable disease and poor survival outcomes when treated with the current standard R-CHOP regimen. Additionally, this segregation may be independent of the COO classification. Future clinical trials applying these newer markers or marker combinations are awaited.

Next generation sequencing (NGS) have rapidly broadened our understanding of the pathogenesis of the COO subgroup of DLBCL: certain genetic alterations are associated with individual molecular subgroups.

Genetic alterations of GCB DLBCL

In the recent few years, global screening of the genomes and transcriptomes of DLBCL using next generation sequencing (NGS) have rapidly broadened our understanding of the pathogenesis of the COO subgroup of DLBCL. These studies showed that certain genetic alterations are associated with individual molecular subgroups, shedding light on the oncogenic pathways that are essential for the GCB and ABC DLBCL.

As aforementioned, the t(14;18) is found in about 35% GCB DLBCL, leading to over-expression of BCL2, which is a key anti-apoptotic protein in GCB DLBCL.[21] The over-expression is due to the juxtaposition of *BCL2* to the potent regulatory elements of the immunoglobulin locus in the t(14;18), as well as by disrupting the suppression by BCL6.[22, 23] Other mechanisms of BCL2 upregulation found in GCB DLBCL without a t(14;18), include deregulation of Miz1, aberrant somatic hypermutation of BCL2 promoter sequences, and mutations in the BCL2 coding sequence.[22, 24]

About 15% of DLBCL harbour the t(8;14) which brings the *MYC* gene under the control of the immunoglobulin promoter, but this translocation is found in both COO subgroups.[25, 26] Amplifications of the microRNA (miR) 17-92 cluster on chromosome 13q are found in 12% of GCB DLBCL.[27] This cluster acts as a potential oncogene and accelerates MYC-induced lymphomagenesis and enhances oncogenesis by increasing proliferation and survival via inhibition of the tumor suppressor PTEN and thus activation of AKT.[27-30] Alternative mechanisms of inhibition of PTEN include deletions of *PTEN* on chromosome 10q, which are found in around 11% of DLBCL with a preference in GCB DLBCL, and are mutually exclusive to the miR-17-92 amplifications.[27, 30] Similarly, AKT can also be activated by phosphatidylinositol 3 kinase (PI3K) with activating mutations, which are found in about 8% of DLBCL, and are mutually exclusive to the loss of PTEN.[31] *GNA13* mutations are present in up to 25% of GCB DLBCL.[32, 33] GNA13 encodes Gα13, a G-protein that increases Rho-mediated motility, and Rho-mediated increased PTEN activity and potent AKT inhibition.[34, 35] *GNA13* mutations thus probably contribute to the spread of the neoplastic cells outside of the germinal centre niche and the deregulation of the AKT/mTOR pathway.

The chromatin modifying genes seem to play key roles in GCB DLBCL pathogenesis, as mutations of a number of these genes are preferentially found in GCB DLBCL. Recurrent activating mutations affecting residue Tyr641 of the polycomb-group histone methyltransferase EZH2 have been found in 21.7% of GCB DLBCL.[36] Mutated EZH2 trimethylates Lys27 of histone H3 and represses gene expression more vigorously by increased affinity to the substrate.[36-38] Another histone methyltransferase MLL2 is mutated in around 30% of DLBCL, with the majority of the mutations being inactivating ones.[32, 36] Myocyte enhancer factor 2B (MEF2B) is a DNA binding protein that cooperates with histone modifying enzymes to regulate gene expression, and is mutated in about 9% of DLBCL.[32, 36] In one study, monoallelic deletions and inactivating mutations of CREBBP and EP300 were found in nearly 39% of GCB DLBCL and only 17% of ABC DLBCL.[39] These two acetyltransferases are transcriptional co-activators in multiple signaling pathways. For instance, CREBBP and EP300 acetylate and inactivate BCL6 by disrupting the

recruitment of histone deacetylases and thus hindering the ability of BCL6 to repress transcription.[40, 41]

Mutations of p53 are more frequent in GCB DLBCL than in ABC DLBCL (38% vs 18%). Most of the mutations are missense or nonsense inactivating mutations, and mutated p53 confer worse survivals within both the GCB and ABC subgroups of DLBCL.[42]

Genetic alterations of ABC DLBCL

Constitutive activation of NF-κB plays an important role in ABC DLBCL survival,[43, 44] which can be due to several distinct genetic alterations affecting both positive and negative regulators of the pathway. These genetic alterations are thus predominantly seen in ABC DLBCL. *TNFAIP3* encodes for the negative NF-κB regulator A20 and is inactivated in about 30% of cases of DLBCL by biallelic mutations and/or deletions,[45] which are almost exclusively found in ABC DLBCL and rare in GCB DLBCL.[45-48]

The B-cell receptor (BCR) induced activation of NF-κB requires CARD11, a scaffold protein that mediates the activation of IκB kinase β.[49] Mutations of CARD11 are found in around 10% of ABC DLBCL and a smaller subset of GCB DLBCL.[45] These mutants probably activate the NF-κB pathway in the absence of BCR signaling and lead to constitutive NF-κB activation.[50] CD79A and CD79B are proximal BCR subunits and were found to be mutated in around 20% of ABC DLBCL.[43] The mutations increase surface BCR expression and abrogate a feedback inhibitor of BCR signaling,[43] leading to chronic active BCR signaling and consequent activation of the NF-κB pathway.

NF-κB is also activated after stimulation of Tolllike receptors (TLR) and interleukin (IL)-1 and 8 receptors.[51, 52] MYD88 functions as a signaling adaptor protein and assembles a protein complex that activates NF-κB following TLRs, and IL-1 and IL-8 receptor stimulations.[51, 52] *MYD88* mutations are found in about 30% of ABC DLBCL. They all lead to the same amino acid substitution (L265P) in the TLR/IL-1 receptor domain.[53] The MYD88 L265P mutant may promote cell survival by activating NF-κB signaling.[53]

Normal GC B-cell development into plasma cells requires down-regulation of BCL6 by NF-κB, and expression of IRF4, BLIMP1 and XBP1.[54-56] In ABC DLBCL, several mechanisms that can interrupt this GC B-cell to plasma cell transition occur. Chromosomal translocations of *BCL6* on chromosome 3q27 are detected in about 35% of DLBCL cases, and are twice commoner in ABC DLBCL than in GCB DLBCL.[57-60] The commonest translocations involve the immunoglobulin heavy-chain promoter, resulting in constitutive expression of BCL6.[60, 61] Deregulated expression of BCL6 is thought to result in differentiation blockage, reduced p53mediated apoptotic response to DNA damage, and enhanced proliferation.[23, 54, 55, 62-64]

The *PRDM1* gene on chromosome 6q21 encodes for BLIMP1, a zinc finger transcriptional repressor that represses genes involved in BCR signaling and proliferation, and acts as a tumour suppressor.[65-67] Inactivating mutations and deletions of *PRDM1* are found in up to 30% of ABC DLBCL.[67-70] Additionally, *PRDM1* can be inactivated by transcriptional repression through constitutively active BCL6, as it is the case in patients carrying *BCL6* translocations. Indeed, chromosomal translocations of *BCL6* and genetic alterations affecting *PRDM1* are mutually exclusive.[68-70]

Amplifications of chromosome 18q21 and gain of *BCL2* gene are observed in 21% and 46% of ABC DLBCL, respectively. Together with NF-κB transactivation, they likely contribute to the BCL2 over-expression observed in 59% of ABC DLBCL.[71] Amplifications of the telomere of chromosome 19q are found in about 25% of ABC DLBCL.[27] SPIB, an ETS family transcription factor, may be a possible candidate gene on chromosome 19q essential for ABC DLBCL as down-regulation of SPIB was toxic to ABC DLBCL cell lines.[27]

Homozygous or heterozygous deletions of the INK4a/ARF locus are observed in about 30% of ABC DLBCL.[27] $P16^{INK4a}$ and $p14^{ARF}$ regulate the pRB and the p53 tumour suppressors, and inactivation of the p53 pathway via INK4a/ARF is found to inhibit apoptosis.[72, 73]

Conclusions

The emergence of technologies like DNA microarray and next generation sequencing has unraveled the molecular and genetic basis of DLBCL. The much better understanding of the pathogenetic pathways and the potential targets along these pathways has gradually opened the door for potential personalized medicine or tailored therapy for DLBCL patients in the future. Pathologists should be prepared to provide such information of molecular classification and genetic alterations of DLBCL when such a day comes.

The different genetic alterations found in GCB and ABC DLBCL may lead to tailored therapy utilizing different therapeutic agents in the future.

References

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. Blood 1997;89:3909-18.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW, ed. Pathology and Genetics of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
3. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503-11.
4. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America 2003;100:9991-6.
5. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. The New England Journal of Medicine 2002;346:1937-47.
6. Lenz G, Wright G, Dave SS, et al. Stromal gene signatures in large-B-cell lymphomas. The New England Journal of Medicine 2008; 359:231323.
7. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103:275-82.
8. Nyman H, Jerkeman M, Karjalainen-Lindsberg ML, Banham AH, Leppa S. Prognostic impact of activated B-cell focused classification in diffuse large B-cell lymphoma patients treated with R-CHOP. Mod Pathol 2009; 22:1094-101.
9. Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res 2009; 15:5494-502.
10. Meyer PN, Fu K, Greiner TC, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. J Clin Oncol 2011; 29:200-7.
11. Visco C, Li Y, Xu-Monette ZY, et al. Comprehensive gene expression profiling and immunohistochemical studies support application of immunophenotypic algorithm for molecular subtype classification in diffuse large B-cell lymphoma: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Leukemia 2012; 26:2103-13.
12. Meyer PN, Fu K, Greiner T, et al. The stromal cell marker SPARC predicts for survival in patients with diffuse large B-cell lymphoma treated with rituximab. American Journal of Clinical Pathology 2011; 135:54-61.
13. Perry AM, Cardesa-Salzmann TM, Meyer PN, et al. A new biologic prognostic model based on immunohistochemistry predicts survival in patients with diffuse large B-cell lymphoma. Blood 2012; 120:2290-6.
14. Iqbal J, Meyer PN, Smith LM, et al. BCL2 predicts survival in germinal center B-cell-like diffuse large B-cell lymphoma treated with CHOP-like therapy and rituximab. Clin Cancer Res 2011; 17:7785-95.
15. Visco C, Tzankov A, Xu-Monette ZY, et al. Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: a report from an International DLBCL rituximabCHOP Consortium Program Study. Haematologica 2013; 98:255-63.
16. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30:3460-7.
17. Hu S, Xu-Monette ZY, Tzankov A, et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. Blood 2013; 121:4021-31; quiz 250.
18. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol 2012; 30:3452-9.
19. Horn H, Ziepert M, Becher C, et al. MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood 2013; 121:2253-63.
20. Hu S, Xu-Monette ZY, Balasubramanyam A, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2013; 121:2715-24.
21. Weiss LM, Warnke RA, Sklar J, Cleary ML. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. The New England Journal of Medicine 1987; 317:1185-9.
22. Saito M, Novak U, Piovan E, et al. BCL6 suppression of BCL2 via Miz1 and its disruption in diffuse large B cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America 2009; 106:11294-9.
23. Ci W, Polo JM, Cerchietti L, et al. The BCL6 transcriptional program features repression of multiple oncogenes in primary B cells and is deregulated in DLBCL. Blood 2009; 113:5536-48.
24. Schuetz JM, Johnson NA, Morin RD, et al. BCL2 mutations in diffuse large B-cell lymphoma. Leukemia 2012; 26:1383-90.
25. Ladanyi M, Offit K, Jhanwar SC, Filippa DA, Chaganti RS. MYC rearrangement and translocations involving band 8q24 in diffuse large cell lymphomas. Blood 1991; 77:1057-63.
26. Morin RD, Gascoyne RD. Newly identified mechanisms in B-cell non-Hodgkin lymphomas uncovered by next-generation sequencing. Semin Hematol 2013; 50:303-13.
27. Lenz G, Wright GW, Emre NC, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proceedings of the National Academy of Sciences of the United States of America 2008; 105:13520-5.
28. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. Nature 2005; 435:828-33.
29. Xiao C, Srinivasan L, Calado DP, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol 2008; 9:405-14.
30. Chan WJ. Pathogenesis of diffuse large B cell lymphoma. Int J Hematol 2010; 92:219-30.
31. Abubaker J, Bavi PP, Al-Harbi S, et al. PIK3CA mutations are mutually exclusive with PTEN loss in diffuse large B-cell lymphoma. Leukemia 2007; 21:2368-70.
32. Morin RD, Mendez-Lago M, Mungall AJ, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 2011; 476:298-303.
33. Morin RD, Mungall K, Pleasance E, et al. Mutational and structural analysis of diffuse large B-cell lymphoma using whole-genome sequencing. Blood 2013; 122:1256-65.
34. Green JA, Suzuki K, Cho B, et al. The sphingosine 1-phosphate receptor S1P(2) maintains the homeostasis of germinal center B cells and promotes niche confinement. Nat Immunol 2011; 12:672-80.
35. Li Z, Dong X, Wang Z, et al. Regulation of PTEN by Rho small GTPases. Nat Cell Biol 2005; 7:399-404.
36. Morin RD, Johnson NA, Severson TM, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 2010; 42:181-5.
37. Sneeringer CJ, Scott MP, Kuntz KW, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human Bcell lymphomas. Proceedings of the National Academy of Sciences of the United States of America 2010; 107:20980-5.
38. Yap DB, Chu J, Berg T, et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood 2011; 117:2451-9.
39. Pasqualucci L, Dominguez-Sola D, Chiarenza A, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature 2011; 471:189-95.
40. Bereshchenko OR, Gu W, Dalla-Favera R. Acetylation inactivates the transcriptional repressor BCL6. Nat Genet 2002; 32:606-13.
41. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 1997; 90:595-606.
42. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated

with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. Blood 2012; 120:3986-96.
43. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. The Journal of Experimental Medicine 2001; 194:1861-74.
44. Jost PJ, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. Blood 2007; 109:2700-7.
45. Compagno M, Lim WK, Grunn A, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 2009; 459:717-21.
46. Kato M, Sanada M, Kato I, et al. Frequent inactivation of A20 in B-cell lymphomas. Nature 2009; 459:712-6.
47. Novak U, Rinaldi A, Kwee I, et al. The NF-{kappa}B negative regulator TNFAIP3 (A20) is inactivated by somatic mutations and genomic deletions in marginal zone lymphomas. Blood 2009; 113:4918-21.
48. Schmitz R, Hansmann ML, Bohle V, et al. TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. The Journal of experimental medicine 2009; 206:981-9.
49. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. Nat Rev Immunol 2006; 6:799-812.
50. Lenz G, Davis RE, Ngo VN, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 2008; 319:1676-9.
51. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. Nature 2010; 465:885-90.
52. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science 2010; 327:291-5.
53. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 2011; 470:115-9.
54. Tunyaplin C, Shaffer AL, Angelin-Duclos CD, Yu X, Staudt LM, Calame KL. Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. J Immunol 2004; 173:1158-65.
55. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity 2000; 13:199-212.
56. Klein U, Casola S, Cattoretti G, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. Nat Immunol 2006; 7:773-82.
57. Offit K, Jhanwar S, Ebrahim SA, Filippa D, Clarkson BD, Chaganti RS. t(3;22)(q27;q11): a novel translocation associated with diffuse nonHodgkin's lymphoma. Blood 1989; 74:1876-9.
58. Iqbal J, Greiner TC, Patel K, et al. Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B-cell lymphoma. Leukemia 2007; 21:2332-43.
59. Lo Coco F, Ye BH, Lista F, et al. Rearrangements of the BCL6 gene in diffuse large cell non-Hodgkin's lymphoma. Blood 1994; 83:1757-9.
60. Ye BH, Lista F, Lo Coco F, et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. Science 1993; 262:747-50.
61. Ye BH, Rao PH, Chaganti RS, DallaFavera R. Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. Cancer research 1993; 53:2732-5.
62. Basso K, Saito M, Sumazin P, et al. Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. Blood 2010; 115:975-84.
63. Phan RT, Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. Nature 2004; 432:635-9.
64. Phan RT, Saito M, Basso K, Niu H, DallaFavera R. BCL6 interacts with the transcription factor Miz-1 to suppress the cyclin-dependent kinase inhibitor p21 and cell cycle arrest in germinal center B cells. Nat Immunol 2005; 6:1054-60.
65. Lin Y, Wong K, Calame K. Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. Science 1997; 276:596-9.
66. Shaffer AL, Lin KI, Kuo TC, et al. Blimp1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity 2002; 17:51-62.
67. Mandelbaum J, Bhagat G, Tang H, et al. BLIMP1 is a tumor suppressor gene frequently disrupted in activated B cell-like diffuse large B cell lymphoma. Cancer Cell 2010; 18:568-79.
68. Calado DP, Zhang B, Srinivasan L, et al. Constitutive canonical NF-kappaB activation cooperates with disruption of BLIMP1 in the pathogenesis of activated B cell-like diffuse large cell lymphoma. Cancer Cell 2010; 18:580-9.
69. Pasqualucci L, Compagno M, Houldsworth J, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. The Journal of experimental medicine 2006; 203:311-7.
70. Tam W, Gomez M, Chadburn A, Lee JW, Chan WC, Knowles DM. Mutational analysis of PRDM1 indicates a tumor-suppressor role in diffuse large B-cell lymphomas. Blood 2006; 107:4090-100.
71. Iqbal J, Neppalli VT, Wright G, et al. BCL2 expression is a prognostic marker for the activated B-cell-like type of diffuse large B-cell lymphoma. J Clin Oncol 2006; 24:961-8.
72. Pinyol M, Cobo F, Bea S, et al. p16(INK4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of nonHodgkin's lymphomas. Blood 1998; 91:2977-84.
73. Pinyol M, Hernandez L, Martinez A, et al. INK4a/ARF locus alterations in human nonHodgkin's lymphomas mainly occur in tumors with wild-type p53 gene. The American Journal of Pathology 2000; 156:1987-96.

Report on Research Project:

Editorial Note: This is a report by Dr Choi Wai Lap, receiver of Chan Woon Cheung Education and Research Fund in Pathology in 2011, on his funded research project.

The Effects of DNA Methylation Patterns and C-kit Gene Amplifications in Core Binding Factor (CBF) Acute Myeloid Leukaemia on Patient Outcomes

Dr. Choi Wai Lap

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease harbouring different cytogenetic and molecular aberrations in individual patients. One of the most common chromosomal aberrations of AML is the t(8;21)(q22;q22) translocation which leads to the AML1-ETO fusion protein. The AML1-ETO fusion protein recruits the N-CoR/mSin3A/HDAC complex, thereby transcriptionally repressing its target genes. Although AML with t(8;21)(q22;122) is regarded as intermediate risk AML with a long term overall survival of around 40-50%, there seems to be some patients who relapse frequently and have less favourable outcomes. In Western series, it has been noted that those t(8;21) AML patients with their leukaemic cells harbouring c-kit mutations fare poorly. However, in local t(8;21) AML patients, it seems that most of them fare poorly and so further risk factors not previously known may be present. Recently, DNA methylation arrays have been used to classify AML into different subgroups including the t(8;21) one. We therefore aim to investigate the epigenetic features of the leukaemic cells of these patients to look for possible epigenetic abnormalities that may provide clues to the relatively less favourable prognosis of these local patients.

Materials and Methods

The diagnostic bone marrow specimens of 22 t(8;21) AML patients were retrieved from the archives of the Department of Pathology, the University of Hong Kong, after approval by the Institution Review Board.

We selected 16 genes that have been implicated in previous methylation and gene expression profiling studies of AML, either the methylation status of the gene being clustered for the classification of t(8;21) AML or are being significantly upregulated or the expression of the gene being up- or down-regulated between t(8;21) AML cells and CD34+ haematopoietic cells. These 16 genes were also chosen based on one or more of the following characteristics:

1) Involvement in normal haematopoiesis and leukaemogenesis.
2) Oncogenesis in other cancers.
3) Regulation of signaling pathways for cell survival and cell proliferation.
4) Regulation of cell cycle control.
5) Regulation of epigenetic modification.

We extracted the DNA from these AML samples, performed bisulphite conversion followed by PCR of the individual target gene for pyrosequencing of their gene promoters to determine the methylation status. The promoter CpG methylation status of these genes was compared between the clinical cases and normal CD34+ haematopoietic cells.

We also performed PCR amplification of exons 8 and 17 of the *c-kit* gene and Sanger sequencing of the *c-kit* gene to determine the mutational status of the leukaemic cells in a parallel fashion.
The results of c-kit mutation status, and the promoter CpG island methylation status were correlated with event-free and overall survival of the patients.

Results

Among the 23 diagnostic specimens of t(8;21) AML, 52.2% (12/23) had a *c-kit* gene mutation in the tested exons. Seven cases harboured only a single *c-kit* gene mutation: 4.4% (1/23) in exon 8, 26.1% (6/23) in exon 17 codon 816 and 13.0% (3/23) in exon 17 codon 822. There were 8.7% (2/23) of cases showing both insertion or deletion in exon 8 and point mutations in exon 17 codon 816. The percentage of *c-kit* mutation in this study mirrored what have been studied in other published series. Patients with *c-kit* mutations had worse event-free survival than patients without *c-kit* mutation (p=0.026), and a trend towards less favourable overall survival (p=0.072).

The 16 genes selected for promoter methylation study were as follows: *FSTL1, BCL7A, CSAP2, CDK8, THBS4, PIAS2, HOXA9, LIFR, BHLHB3, TSC22D1, PAWR, NT5E, ID2, MARCKS, SPARC, MEL18.*

Pyrosequencing was first performed on the Kasumi-1 cell line, which is a *c-kit* mutated t(8;21) AML cell line. DNA of CD34+ cell was used as the normal control. Among the 16 genes, *PAWR, THBS4* and *MEL18* were hypermethylated, while all the others have a similar methylation percentage between Kasumi-1 cell line and CD34+ cells.

Pyrosequencing *PAWR, THBS4* and *MEL18* genes were then performed on diagnostic samples of t(8;21) AML. It was found that 59.1%, 86.4% and 6.7% of t(8;21) AML patients had promoter hypermethylation of *PAWR, THBS4* and *MEL18.*

The promoter methylation status of both the *THBS4* gene and the *PAWR* genes did not correlate with *c-kit*¬ gene mutation status within t(8;21) AML (p=0.65 and p=0.069) respectively.

By using the methylation status of *THBS4* and *PAWR* genes to dichotomize the patients, there were no significant difference in OS (*THBS4 p=0.332, PAWR p=0.383*) and EFS (*THBS4 p=0.642, PAWR p=0.337*).

Conclusions

Our work showed that slightly over half of the t(8;21) AML cases harboured *c-kit* exon 8 and/or 17 mutations, with *c-kit* mutated t(8;21) AML has a tendency of poor OS and significant poor EFS when compared to patients without *c-kit* mutation. *THBS4* showed a hypermethylation status in almost all t(8;21) AML cases and that *PAWR* showed a hypermethylation status in approximately half of the t(8;21) AML cases. The hypermethylation status of *PAWR* or *THBS4* gene did not have significant correlations with t(8;21) AML with *c-kit* mutation, and the methylation status of these two genes did not have significant effects on survivals. Further studies are required to show the possible functional roles of these two genes in the pathogenesis of t(8;21) AML.

Out of the Whitecoat:

My Sake Journey

Dr. HUI Yin, Melody

I have never been an alcohol connoisseur, but in the past few years I got acquainted with sake due to my husband's business. And then I fell hopelessly in love with this part of Japanese culture.

Although the ingredients include only rice, water, koji and yeast, sake could express a diversity of tastes and aromas. Depending on the styles and skills of the brewers, sake could taste flavourful, light and smooth, rich, or aged. It could smell rice-like, fruity, floral, nutty or even chocolate-like; there could be a hundred possible fragrances.

▲ *Some of the author's favourite sakes*

Sake's ability to pair with food adds to its charisma. Made from rice, sake especially harmonizes with Chinese cuisine. The right sake brings out the best flavours in food, while the right dishes enhance the character of the drink. It is an amazing chemistry.

I attend sake dinners frequently, featuring varied brands of sake matching with Chinese, Japanese, French and even Italian cooking styles. Each dining experience gives me lots of surprises and stimulations.

▲ *Tasting numerous sakes from the brewery Nabeshima (鍋島) at the Dynasty Club*

▲ *Sake dinner at Imasa, Peninsula, featuring the brewery Tenryo (天領)*

The Journey Begins

Visiting sake breweries has soon become my favourite holiday activity. I have travelled with my husband to breweries in Gifu, Toyoma, Saga, Niigata and Nagasaki. As good water and rice are essential for brewing sake, these breweries are often located in rural areas with abundant natural resources, far away from the pollution of cities.

Each rural village or countryside in Japan has its unique scenery, weather, culture, buildings, and delicacies. Whether one enjoys sake or not, the journey could never be disappointing. The Japanese are well-known for their hospitality. During our brewery visits, we are often invited to dinners by the enthusiastic hosts. They make sure that we thoroughly appreciate their local dishes and food resources, and how the cuisine could flawlessly pair with the sake they are so proud of.

Sake Brewery Tenryo

The first brewery I visited was Tenryo (天領) in Gero, Gifu prefecture. Situated in the beautiful Hida-Hagiwara region, with rich soil and distinct weather for growing sake rice, as well as high quality water flowing down from the Northern Alps of Japan, the brewery has every advantage to produce outstanding sake. Some of their products have won National Gold Awards in five consecutive years, and some have been chosen for the Japan Airlines first class menu.

▲ *The author holding a heavy rod for mixing the fermentation mash*

We were toured around the sake factory and introduced to various brewing processes. The complicated yet carefully-monitored craftsmanship was most impressive. We were shown the rice milling machine, the site where rice steaming occurred, the huge fermentation and saccharification tanks, the utensils used for the mixing and pressing, and the tobintori, suspended cotton bags from which sake was allowed to drip out naturally and filtered. It was memorable and eye-opening.

▲ *The author with huge fermentation tanks behind*

We were generously treated with manifold sakes, the most important one being the Namazake (生酒), which was not heated for pasteurization after the final mash was pressed. It was best consumed fresh within the brewery itself, as the slightest imperfection in temperature control during transportation to other provinces could alter its delicate condition. The sake tasted especially intriguing after we learnt how it was made.

Sake Brewery Maboroshi No Taki

Recently we visited the sake brewery Maboroshi No Taki (幻之瀧) at Kurobe, Toyama prefecture. This was the only brewery in Toyama that owned natural springs within the factory complex. There were two springs, one from 90 meters underground and the other from 120 meters. The cool, glistening, pristine water was free to all visitors and passer-by to enjoy. Though both types of water were of supreme quality, it was not difficult to tell the differences between them.

In Japan, water connoisseurship is highly respected. The Kurobe water has been honoured one of the best 100 types of water (日本名水百選), famous for its extreme softness and richness in minerals. As a result, the sake produced by Maboroshi No Taki shows an exceptionally smooth and soft quality, described as "so smooth that couldn't be retained within the mouth".

Apart from visiting the brewery, we managed to run into the buri season at Toyama. Located at the coast of Sea of Japan, this area produces enormous amount of buri and kanburi in late winter and spring. We had multiple sumptuous meals solely comprised of sashimi of buri, firefly squids, white shrimps, salmon roe and many other seafood, freshly caught in the same day, at an unbelievably low price! Matched with the elegant aroma and mellow taste of sake, our appetite became unsatisfiable. Toyama is a must-revisit place.

▲ *The author tasting top quality water from the springs at the brewery Maboroshi No Taki (幻之瀧)*

▲ *Fresh delicious kanburi sashimi at Toyama, costing only HK$70!*

The Drinking Vessels: more than meets the eyes

▲ *Sake cups of different shapes, enhancing tastes and aromas*

My love towards sake extends to its drinking utensils.

Just like serving green tea in distinctive teapots and cups, the Japanese are very particular about serving sake in specific containers. Cups of different shapes and materials should be used for tasting GinJoshu (吟釀酒), Dai-ginjoshu (大吟釀酒), Junmaishu (純米酒), Honjozoshu (本釀造酒), Genshu (原酒), Koshu (古酒) and sparkling sake etc.. The practice enriches the sake aroma and highlights the subtle changes as each second passes, making the most out of the flavours.

In addition to traditional drinking vessels made of ceramics, porcelain and lacquer, sake can be served in glassware. My husband and I visited the factory of Toyo-Sasaki Glass at Chiba prefecture, the oldest and largest glassware company in Japan. Besides appreciating how the skilful workers manufactured glass sake cups in attractive colours and patterns, we had the opportunity to blow and create our own glasses! It was quite an experience to brag about.

▲ *Artistic glass cup from Toyo-Sasaki Glass Company*

I start to collect assorted sake cups and bottles. O-choshi (vessel used for pouring sake), gui-nomi (cup holding two to three sips), masu (square wooden cup), and kiki-choko (white cup with two blue concentric circles in the bottom, used for taste-testing varied types of sake) from distinguished companies or special sake events have become my targets. When I visited an antique shop in Hida, I was thrilled to see sets of sakazuki, small shallow cups made of red lacquer with gold-painted art, of over 100 years of history. With the help of the owner of Tenryo brewery, I managed to purchase them at a good bargain. Like any other things else, sake could be an excuse for the expression of a woman's shopaholic genes.

▲ *The author's collection of masu (square wooden cups)*

In the coming years, I look forward to visiting many more sake breweries in various parts of Japan, learning about this interesting art, and sharing the enjoyment with other sake aficionados.

OBITUARY:
DR. RINA WONG KWAI YING (黃桂英醫生)

Dr. Rina Wong Kwai Ying (黃桂英醫生), *also known as Daw Aye Yi, was born on 2-June-1929. She passed away on 19-May-2014, aged 84 years.*

Dr. Rina Wong's ancestral home was in Guangdong, Tai Shan. She was married to Mr. Kenneth Chiu (also known as U Kyaw Win). She is survived by her three children Vincent, George, and Victor, three granddaughters Allison, Ashely and Angela, and one grandson Nathan. All her children and grandchildren reside in the USA.

Dr. Rina Wong studied medicine at University of Yangon and received her Bachelor of Medicine and Bachelor of Surgery in 1954. Right after her graduation, she began her career at the pathology department of Yangon Institute of Medicine, which was part of Yangon General Hospital until 1956. She earned increasing responsibilities and was appointed Professor of Pathology by 1976. During her early medical career, she attended University of Lucknow on UN scholarship, completed her Advanced Degree in Clinical Pathology in 1960, and obtained MRCPath in 1965 at Queen Charlotte Hospital in London, UK.

As early as 1964, Dr. Wong founded a general medicine and clinical pathology clinic in order to serve a broader community in Yangon, Mayanmar. Her clinic was known to have a high level of care and exacting standards. It was designated as the first clinic to perform immigration medical examination by the US Embassy during that time.

Dr. Wong emigrated from Yangon to Hong Kong in 1977 and assumed the position of Chief of Service in Pathology in Alice Ho Miu Ling Nethersole Hospital until 1988. Afterwards, she joined Ruttonjee Hospital and Pamela Youde Nethersole Eastern Hospital from 1989 to 1994. During vacations, Dr. Wong worked as a visiting pathologist consultant in Thailand and Australia and obtained licence to practice medicine in Taiwan.

She retired at the age of 65. After retirement, she provided consultation to multiple pathology clinics and continued to serve the community. She served as Chairwoman of Hong Kong Myanmar Doctor's Association from 1994 till 2004 consecutively.

To her sons and closed family members, Dr. Wong was a teacher, a doctor, a mentor, a friend, a sister and a mother to everyone who crossed her path. She worked hard, provided and cared for her siblings. She would passionately help without any expectation. She was always positive and enthusiastic. She was generous and forgiving; caring and compassionate; competitive and inspirational, a perfect role model for them. There is no word to describe her love for them.

Elaine Gwi

OBITUARY:
DR. JOANNA HO CHOR YING (何楚盈醫生)

Dr. Joanna Ho Chor Ying (何楚盈醫生), *who was born on May 14th 1939 in Macau, passed away at home in Hong Kong on the evening of June 8th, 2014, aged 75 years.*

Joanna studied at Macao Sacred Heart Elementary School and Puiching Middle School, then in 1956 she went to Geneva, Switzerland, for further education. In 1960 she was admitted to Beijing Medical University and after graduation in 1965, returned to Hong Kong. In 1967, Joanna went to McGill University, in Montreal, Canada and where she attained a Master's Degree in Pathology.

In 1971, Joanna joined The Department of Pathology, University of Hong Kong, first as a Clinical Pathologist and was later promoted to Senior Clinical Pathologist, working at Queen Mary Hospital until her retirement in 1999. In 1985 and 1993, she was nominated as a Fellow of the Royal College of Pathologists of Australasia and a Fellow of the Hong Kong College of Pathologists, respectively.

During her professional years of involvement with the HKU Department of Pathology and Queen Mary Hospital, Dr. Ho epitomized and demonstrated the fine art and science of Anatomical Pathology. With her participation in routine pathology service, in all its aspects, as well as her dedication and commitment to research and teaching, she became a role model for both medical students and trainees in pathology. Her enthusiasm for research in her chosen areas of interest, especially in GIT pathology, attracted collaborative involvement with many colleagues from other medical disciplines, resulting in papers in journals of international stature.

Joanna was involved in the functioning of the HK Pathology Society which evolved into the Hong Kong College of Pathologists. She was one of the original 25 subscribers and later a founding fellow of the College.

As their seniority increased in HKU/QMH, Joanna, Dr Lily Ma and Dr Sophia Chih Hsu became established as the trio affectionately referred to as the 'aunties' to whom junior members of the combined department could approach with their troubles, knowing that they could find solace and guidance from their counsel.

After Joanna's retirement in 1999 she bravely dealt with the challenge of Parkinson's disease and despite her mobility being severely compromised she was as determined as ever to pursue life enthusiastically. She managed to continue with social engagements and was always eager for reunions with old colleagues, even attending an annual College dinner.

Joanna was married to Mr. Ai (倪琢明先生) in 1965, and is survived by her son, Dr Victor Ai, and her daughter, Dr Dawn Ai, of whom she was immensely proud while she held a very special place in her heart for her grand-daughter Gemma Ho.

Joanna's characteristic charm, ready smile and sincerity will be fondly remembered. She was a wonderful and caring woman who both aided and nurtured the professional development of a generation of anatomical pathologists at HKU/QMH as well as being held in great affection by her contemporaries and seniors. We are all privileged to have known her and had her company.

Drs Robert Collins, Lily Ma and ST Yuen

CONGRATULATIONS!!

We are pleased to announce that the following candidates have passed the Membership Examination or Fellowship Assessment this year.
Congratulations!!

Dr. HO Siu Lun
(Fellowship Assessment – Anatomical Pathology)

Dr. LAU Wing Sze
(Fellowship Assessment – Anatomical Pathology)

Dr. LEUNG Ying Kit
(Fellowship Assessment – Anatomical Pathology)

Dr. LO Yan Fai
(Fellowship Assessment – Anatomical Pathology)

Dr. TING Shun Hin
(Fellowship Assessment – Anatomical Pathology)

Dr. LUI Leo
(Fellowship Assessment – Clinical Microbiology and Infection)

Dr. WANG Kin Fong, Teresa
(Fellowship Assessment – Clinical Microbiology and Infection)

Dr. CHONG Yeow Kuan
(Membership Examination – Chemical Pathology)

Dr. KWOK Ka Ki
(Membership Examination – Forensic Pathology)

Dr. TSANG Chin Yeung
(Membership Examination – Forensic Pathology)

▲ *Forensic Pathologists having lunch on the day of Membership Practical. Front row (left to right): Prof. Roger Byard (External Examiner), Dr. Philip Beh (Chief Examiner). Back row (left to right): Dr. Lai Sai Chak, Dr. Wong Hon Man, Dr. Foo Ka Chung.*

▲ *Examiners for Anatomical Pathology at viva examination. Front row (left to right): Dr. LAM Wing Yin, Prof. KHOO Ui Soon (Chief Examiner), Dr. Sanjiv MANEK (External Examiner), Dr. LEE Kam Cheong. Back row (left to right): Dr. CHAN Chak Lam Alexander, Dr. IP Pun Ching Philip, Dr. LAU Lin Kiu, Dr. LUI Yun Hoi.*

▲ *Examiners in Chemical Pathology. Front row (left to right): Prof Eric Lam, Dr. Tony Mak, Dr. Penelope Coates (External Examiner), Dr. Anthony Shek (Chief Examiner), Dr. Sidney Tam, Dr. Michael Chan. Back row (left to right): Dr. Morris Tai, Dr. Chloe Mak, Dr. Liz Yuen, Prof Rossa Chiu, Dr. Angel Chan, Dr. WT Poon.*

Programme of the 23rd Annual General Meeting

29 November 2014, Saturday

Hong Kong Academy of Medicine Jockey Club Building,
99 Wong Chuk Hang Road, Aberdeen, Hong Kong

1:00 p.m. – 5:00 p.m.	The 10th Trainee Presentation Session
5:00 p.m. – 5:30 p.m.	The 23rd Annual General Meeting
5:30 p.m. – 6:00 p.m.	Reception
6:00 p.m. – 6:50 p.m.	Conferment Ceremony Admission of New Fellows and Members and Presentation of Fellowship and Membership Certificates Conclusion of Conferment Ceremony
6:50 p.m. – 7:00 p.m.	Group Photo of Stage Party
7:00 p.m. – 8:00 p.m.	The 23rd T. B. Teoh Foundation Lecture: "The Multiple Faces of Papillomavirus" Prof. CHAN Kay Sheung, Paul Chairman Department of Microbiology The Chinese University of Hong Kong Hong Kong
8:00 p.m. – 10:00 p.m.	Chinese Banquet Dinner

THE HONG KONG COLLEGE OF PATHOLOGISTS

SCIENTIA ILLUMINAT MEDICINAM
香港病理學專科學院

International Pathology Day Exhibition
The Science Behind Medicine

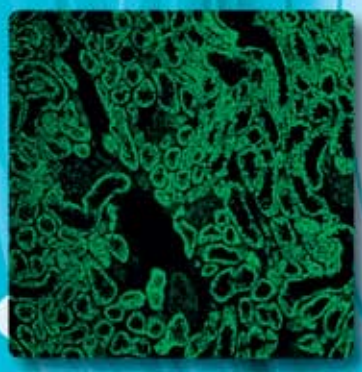
「國際病理學日」展覽
醫學背後的科學

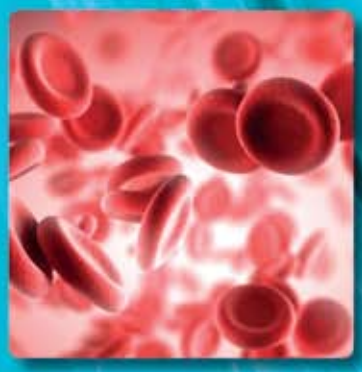
日期: 5/11/2014 - 11/11/2014

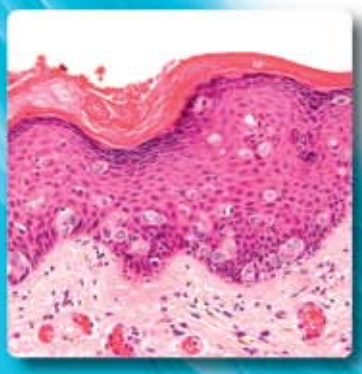
Date: 5-11 November 2014

地址: 香港醫學博物館 香港半山區堅巷2號

Venue: Hong Kong Museum of Medical Sciences
No.2, Caine Lane, Mid-Levels, Hong Kong







交通路線 Transportation Direction

開放時間 Opening Hours

星期一 Mon	休 館 Closed
星期二至星期六 Tue - Sat	10am - 5pm
星期日 Sunday	1pm - 5pm

入場費用 Admission Fee

全免 Free

鳴謝贊助 Sponsors

janssen · ASP · Roche · BECKMAN COULTER · QIAGEN · NOVARTIS ONCOLOGY · BIO-RAD · gsk