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Recent advances in the pathobiology of lymphoma

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Molecular genetic analysis of lymphoproliferative disorders

Array comparative genomic hybridisation (CGH) is a versatile and robust technique that can be applied to routine pathological specimens including formalin fixed paraffin embedded tissue biopsies for characterisation of genomic profile and identification of the genes targeted by genomic gains/amplification or deletion. The genomic gains and deletions identified by array CGH and their impact on clinicopathological presentations can then be readily investigated in large cohorts of cases by interphase fluorescence *in situ* hybridisation (FISH). Combination of these approaches continues to unravel novel genetic abnormalities in lymphomas, which provide new insights into the molecular pathogenesis and further markers potentially valuable in clinical applications. By study of ocular adnexal MALT lymphoma, Chanudet *et al.* demonstrated that A20, encoding an NF-κB inhibitor, is the target of 6q23 deletion and frequently inactivated by homozygous deletion [1]. Importantly, subsequent studies have shown that A20 is also inactivated by deletion and mutation in several other lymphoma subtypes characterised by constitutive NF-κB activation, including diffuse large B-cell lymphoma, primary mediastinal large B-cell lymphoma and Hodgkin lymphoma. The observation of such classical double genetic hits (Knudson's hypothesis) together with findings by *in vitro* functional investigations indicates that A20 is a new tumour suppressor in lymphoma. In another study of splenic marginal B-cell lymphoma (SMZL), Watkins *et al*. mapped the minimal common region of 7q deletion and demonstrated its utility in diagnosis and differential diagnosis of splenic B-cell lymphoma [2]. Lastly, Luan *et al.* revealed the genomic landscape of primary effusion lymphoma and highlighted the importance of SELPLG and CORO1C gene amplification /over expression in the lymphoma pathogenesis [3].

1. ***A20* deletion is associated with copy number gain at the *TNFA/B/C* locus and occurs preferentially in translocation-negative MALT lymphoma of the ocular adnexa and salivary glands**
   E Chanudet, H Ye, J Ferry, CM Bacon, P Adam, HK Müller-Hermelink, J Radford, SA Pileri, K Ichimura, VP Collins, RA Hamoudi, AG Nicholson, AC Wotherspoon, PG Isaacson and MQ Du
   The Journal of Pathology 2009; 217: 420-430. (Original Paper)

2. **Splenic marginal zone lymphoma: characterization of 7q deletion and its value in diagnosis**
   A James Watkins, Yuanxue Huang, Hongtao Ye, Estelle Chanudet, Nicola Johnson, Rifat Hamoudi, Hongxiang Liu, Gehong Dong, Ayoma Attygalle, Ellen D McPhail, Mark E Law, Peter G Isaacson, Laurence de Leval, Andrew Wotherspoon, Ming-Qing Du
   The Journal of Pathology 2010; 220: 461-474. (Original Paper)

3. **Primary effusion lymphoma: genomic profiling revealed amplification of *SELPLG* and *CORO1C* encoding for proteins important for cell migration**
   Shi-Lu Luan, Emmanuelle Boulanger, Hongtao Ye, Estelle Chanudet, Nicola Johnson, Rifat A Hamoudi, Chris M Bacon, Hongxiang Liu, Yuanxue Huang, Jonathan Said, Peiguo Chu, Christoph S Clemen, Ethel Cesarman, Amy Chadburn, Peter G Isaacson, Ming-Qing Du
   The Journal of Pathology 2010; 222: 166-79. (Original paper)

miRNA and lymphoma

miRNAs are small non-coding RNAs that can target mRNAs for degradation and suppress protein translation, and as such they could act as tumour suppressor genes or oncogenes [4]. This is a rapidly expanding research area in lymphoma. Leucci *et al.* investigated the expression pattern of miRNAs that are predicted to target c-Myc, in classical Burkitt lymphomas and found hsa-mir-34b to be preferentially and significantly reduced in those without the *MYC*-involved translocation, providing an alternative mechanism for *MYC* up-regulation [5]. Similarly, Wang *et al.* examined miRNA expression in chronic lymphocytic leukaemia / lymphoma and found distinct alterations associated with the proliferative centres [6], which are thought to be the source of the malignant cell expansion.

4. **Non-coding RNAs: regulators of disease**
   Ryan J Taft, Ken C Pang, Timothy R Mercer, Marcel Dinger, John S Mattick
   The Journal of Pathology 2010; 220: 126-139. (Invited Review)

5. ***MYC* translocation-negative classical Burkitt lymphoma cases: an alternative pathogenetic mechanism involving miRNA deregulation**
   E Leucci, M Cocco, A Onnis, G De Falco, P van Cleef, C Bellan, A van Rijk, J Nyagol, B Byakika, S Lazzi, P Tosi, H van Krieken and L Leoncini
   The Journal of Pathology 2008; 216: 440–450. (Original paper)

6. **miRNA analysis in B-cell chronic lymphocytic leukaemia: proliferation centres characterized by low miR-150 and high *BIC*/miR-155 expression**
   M Wang, LP Tan, MK Dijkstra, K van Lom, J-L Robertus, G Harms, T Blokzijl, K Kooistra, MB van t'Veer, S Rosati, L Visser, M Jongen-Lavrencic, PM Kluin and A van den Berg
   The Journal of Pathology 2008; 215: 13–20. (Original paper)

Tumour microenvironment

Mounting evidence indicates that the tumour microenvironment plays a critical role in the growth and survival of lymphoma cells. This is perhaps best illustrated in classical Hodgkin lymphoma and MALT lymphoma. The former is characterised by only a few malignant cells but an overwhelming background of various reactive cells, while the latter is featured by its causative relationship with microbial infection and response to *H. pylori* eradication in the case of the gastric form. How these lymphoma cells are stimulated and maintained by signals from their microenvironment is of great research interest. A recent review by Aldinucci *et al.* dissects the complex interactions between the neoplastic cells and their microenvironment in classical Hodgkin lymphoma, and discusses various reactive components as potential therapeutic targets [7]. The study by Deutsch *et al.* investigated the role of chemokine receptors in the development and progression of extragastric MALT lymphoma by a comprehensive survey of the expression of various chemokine receptors at multiple stages of the disease development [8].

7. **The classical Hodgkin's lymphoma microenvironment and its role in promoting tumour growth and immune escape**
   Donatella Aldinucci, Annunziata Gloghini, Antonio Pinto, Rosaria De Filippi and Antonino Carbone
   The Journal of Pathology 2010; 221: 248–263. (Review)

8. **Distinct signatures of B-cell homeostatic and activation-dependent chemokine receptors in the development and progression of extragastric MALT lymphomas**
   AJA Deutsch, A Aigelsreiter, E Steinbauer, M Frühwirth, H Kerl, C Beham-Schmid, H Schaider and P Neumeister
   The Journal of Pathology 2008; 215: 431–444. (Original paper)

Molecular pathogenesis of lymphoproliferative disorders

The development and differentiation of B and T-cells are tightly controlled by concert action of transcriptional factors. Many of the transcriptional factors are the target of genetic and /or epigenetic alterations in lymphoma. However, neither the genes regulated by these transcriptional factors, nor how these transcription factors are regulated are fully understood. Advances in characterisation of the role of these transcriptional factors in the biology of lymphocytes always shed light into the investigation of their pathogenic role in lymphoma, and vice versa.

PAX5, a B-cell specific transcriptional factor, is essential for B-cell development and function primarily by promoting the expression of the genes encoding proteins involved in B-cell receptor signalling. Interestingly, a recent study by Bougel *et al.* demonstrates that the human telomerase reverse transcriptase gene (*hTERT*) is the novel target of PAX5 in B-cells [9]. It remains to be investigated whether the PAX5 mediated hTERT expression plays a role in lymphoma.

BCL6 is another transcriptional factor / repressor, crucial for germinal centre formation and highly expressed in germinal centre B-cells and their derived B-cell lymphomas. BCL6 dictates germinal centre formation by

regulating the expression of a number of genes including the suppression of Blimp-1, an important transcription factor for plasma cell differentiation. By silencing BCL6 in diffuse large B-cell lymphoma cells and a series of *in vitro* experiments, Perez-Rosado *et al.* show that BCL6 also directly interacts with NF-κB and suppresses their transcriptional activities [**10**].

9. **PAX5 activates the transcription of the human telomerase reverse transcriptase gene in B cells**
   Stéphanie Bougel, Stéphanie Renaud, Richard Braunschweig, Dmitri Loukinov, Herbert C Morse III, Fred T. Bosman, Victor Lobanenkov and Jean Benhattar
   Journal of Pathology 2010; 220: 87–96. (Original paper)

10. **BCL6 represses NF-κB activity in diffuse large B-cell lymphomas**
    A Perez-Rosado, MJ Artiga, P Vargiu, A Sanchez-Aguilera, A Alvarez-Barrientos and MA Piris
    The Journal of Pathology 2008; 214: 498–507. (Original paper)

Aberrant protein expression is a characteristic feature of many lymphoma subtypes and serves as a critical immunophenotypic marker for lymphoma diagnosis. However, the molecular mechanism underlying the aberrant protein expression in many lymphoma subtypes is unknown. The neoplastic cells (Hodgkin and Reed-Sternberg cells) of classical Hodgkin lymphoma are derived from germinal centre B-cells, but often do not express surface B-cell antigens. By investigating how the EBV associated latent membrane protein 1 (LMP1) contributes to the lymphoma pathogenesis, Vockerodt *et al.* found that LMP1 induced transcriptional profile changes characteristic of Hodgkin lymphoma cell lines, including down regulation of many surface B-cell markers [**11**]. CD30 is another marker highly expressed in several lymphoma subtypes. Franchina *et al.* investigated the transcriptional control of CD30 expression and identified the transcription factor Yin-Yang-I as a novel regulator of CD30 [**12**].

11. **Epstein–Barr virus oncoprotein, latent membrane protein-1, reprograms germinal centre B cells towards a Hodgkin's Reed–Sternberg-like phenotype**
    M Vockerodt, SL Morgan, M Kuo, W Wei, MB Chukwuma, JR Arrand, D Kube, J Gordon, LS Young, CB Woodman and PG Murray
    The Journal of Pathology 2008; 216: 83–92. (Original paper)

12. **The *CD30* gene promoter microsatellite binds transcription factor Yin Yang 1 (YY1) and shows genetic instability in anaplastic large cell lymphoma**
    M Franchina, AJ Woo, J Dods, M Karimi, D Ho, T Watanabe, DV Spagnolo and LJ Abraham
    The Journal of Pathology 2008; 214: 65–74. (Original paper)

Mouse models of lymphoma

In vivo studies have hugely advanced our knowledge of lymphoma pathogenesis. Mouse models of lymphoma provide a critical tool not only for addressing basic biological questions but also for validating therapeutic agents. There are many mouse models of lymphoma described in the literature, but few of them have been comprehensively characterised at clinical, morphological, immunophenotypic and genetic levels, as has been

done in the study of human lymphoma. Thus, the research utility of mouse models of lymphoma is not yet fully explored. In their paper in the *Journal of Pathology*, Qi *et al.* report their findings on comprehensive characterisation of mouse anaplastic plasmacytomas [**13**]. The commentary by de Jong and Janz provides critical thoughts on how a faithful mouse model of lymphoma can help advance research into human lymphoma [**14**].

13. **Anaplastic plasmacytomas: relationships to normal memory B cells and plasma cell neoplasms of immunodeficient and autoimmune mice**
    Chen-Feng Qi, Dong-Mi Shin, Zhaoyang Li, Hongsheng Wang, Jianxum Feng, Janet W Hartley, Torgny N Fredrickson, Alexander L Kovalchuk and Herbert C Morse
    The Journal of Pathology 2010; 221: 106–116. (Original paper)

14. **Anaplastic plasmacytoma of mouse—establishing parallels between subtypes of mouse and human plasma cell neoplasia**
    Daphne de Jong and Siegfried Janz
    The Journal of Pathology 2010; 221: 242–247. (Invited Commentary)

Advances in prognosis and therapy of haematological malignancies

Over the last few decades, significant advances have been made in characterisation of the genetics /molecular pathways that underpin the pathogenesis of many haematological malignancies. These advances together with the rapid development of specific small-molecule inhibitors lead to an ever growing research interest and expansion of clinical trials in targeted therapy. Several papers recently published in the *Journal of Pathology* report advances in this field. Hussain *et al.* demonstrated that over-expression of XIAP was significantly associated with poor overall survival in diffuse large B-cell lymphoma (DLBCL) and pharmacological inhibition of XIAP, particularly in the presence of AKT inhibitor, induced marked apoptosis of DLBCL cells *in vitro* [**15**]. In an early study, the same group showed that a subset of DLBCL expressed S-phase kinase protein 2 (SKP2), a crucial component of the ubiquitin ligase complex that mediates the degradation of p27kip1, and that the proteasome inhibitor mediated killing of DLBCL cells may involve inhibition of SKP2 mediated P27kip1 degradation [**16**]. In mantle cell lymphoma (MCL), Wang *et al.* demonstrated that JNK was constitutively activated and its inhibition resulted in growth arrest of the lymphoma cells *in vitro* [**17**]. Lastly, the reviews by Cillessen *et al.* and Hamilton *et al.* provide a timely update on this progressing research area [**18**, **19**].

15. **Prognostic significance of XIAP expression in DLBCL and effect of its inhibition on AKT signalling**
    Azhar R Hussain, Shahab Uddin, Maqbool Ahmed, Rong Bu, Saeeda O Ahmed, Jehad Abubaker, Mehar Sultana, Dahish Ajarim, Fouad Al-Dayel, Prashant P Bavi and Khawla S Al-Kuraya
    The Journal of Pathology 2009; 222: 180-190. (Original Paper)

16. **S-phase kinase protein 2 is an attractive therapeutic target in a subset of diffuse large B-cell lymphoma**
    S Uddin, A Hussain, M Ahmed, A Belgaumi, F Al-Dayel, D Ajarim, P Bavi and KS Al-Kuraya
    The Journal of Pathology 2008; 216: 483–494. (Original paper)

17. **JNK is constitutively active in mantle cell lymphoma: cell cycle deregulation and polyploidy by JNK inhibitor SP600125**
    Miao Wang, Çiğdem Atayar, Stefano Rosati, Anneke Bosga-Bouwer, Philip Kluin and Lydia Visser
    The Journal of Pathology 2009; 218: 95–103. (Original paper)

18. **Molecular targeted therapies for diffuse large B-cell lymphoma based on apoptosis profiles**
    Saskia AGM Cillessen, Chris JLM Meijer, Michitaka Notoya, Gert J Ossenkoppele and Joost J Oudejans
    The Journal of Pathology 2010; 220: 509–520. (Invited review)

19. **Targeted therapy in haematological malignancies**
    Ashley Hamilton, Paolo Gallipoli, Emma Nicholson and Tessa L Holyoake
    Journal of Pathology 2010; 220: 404–418. (Invited review)

Questions

The following questions can be answered by reading and reflecting upon the above annotation and the papers that are cited within it. Within the Royal College of Pathologists **Continuing Professional Development (CPD) scheme**, CPD points may be earned by writing reflective notes on the papers in this Virtual Issue and the questions are designed to act as a focus for this activity. To do this, you may wish to use the Royal College of Pathologists' **reflective notes form**.

Question 1 What is the principle of array comparative genomic hybridisation (array CGH), its advantages and limitations? Can array CGH detect chromosome translocation?

Question 2 How may a tumour suppressor gene be identified and validated?

Question 3 What are miRNA and how may miRNA play an important role in lymphoma development?

Question 4 In the context of Hodgkin lymphoma, how could the tumour microenvironment help the malignant cells to proliferate and survive?

Question 5 How may the deregulation of the expression of transcriptional factors such as PAX5 and BCL6 contribute to lymphoma pathogenesis?

Question 6 What are the requirements for an ideal mouse model of lymphoma, and how could a good mouse model help our research in human lymphoma?

Question 7 In the context of diffuse large B-cell lymphoma, why there is a need for target therapy and which targets and pathways may be potentially exploited in therapy?