Recent Advances in Pathology: the 2020 Annual Review Issue of The Journal of Pathology

INTRODUCTORY ARTICLE FOR THE ANNUAL REVIEW ISSUE

Recent Advances in Pathology: the 2020 Annual Review Issue of The Journal of Pathology

C. Simon Herrington[1], Richard Poulsom[2] and Philip J Coates[3]*

[1] *Edinburgh Cancer Research Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK*

[2] *The Pathological Society of Great Britain and Ireland, London UK*

[3] *RECAMO, Masaryk Memorial Cancer Institute, Brno, Czech Republic*

**Correspondence to: P Coates, RECAMO, Masaryk Memorial Cancer Institute, zluty kopec 7, 656 53 Brno, Czech Republic. E-mail: coates_se@jpathol.org*

Abstract

This year's Annual Review Issue of The Journal of Pathology contains 18 invited reviews on current research areas in pathology. The subject areas reflect the broad range of topics covered by the journal and this year encompass the development and application of software in digital histopathology; implementation of biomarkers in pathology practice; genetics and epigenetics; and stromal influences in disease. The reviews are authored by experts in their field and provide comprehensive updates in the chosen areas, in which there has been considerable recent progress in our understanding of disease.

Keywords: Breast cancer; clear cell renal cell carcinoma; colorectal cancer; endometrial cancer; Mendelian randomisation; cancer predisposition; Lynch syndrome; DNA mismatch repair; apoptosis; gene variant interpretation; immunoediting; immunotherapy; PD-L1; TILs; biomarker risk-management; single-cell RNAseq; mass cytometry; scATACseq; glomerulonephritis; transplantation; chronic kidney disease; nephropathology; tuberculosis; host-directed therapy; long non-coding RNA; long intergenic non-coding RNA; anti-sense lncRNA; translation; stem cells; development; X chromosome inactivation; neurogenesis; neurodegenerative disease; diabetes; macrophage; receptor; inflammation; immunity; metabolic disorders; neoplasia; oxygen; hypoxia; hypoxia-inducible factor; pseudohypoxia; migration; invasion; metastasis; collective cell migration; RhoGTPase; Rac1; RhoA; Cdc42; macropinocytosis; chemotaxis; durotaxis; atherosclerosis; TREM2; foam cells; medulloblastoma; neuroblastoma; ependymoma; astrocytoma; glioblastoma; Ewing's tumour; DNA methylation profiling; diagnosis; algorithm; miRNA; multiple sclerosis; demyelinated lesions; white matter; grey matter; inflammatory lesions; chronic inactive lesions; Capicua; ATXN1L; SWI/SNF; HDAC; digital histology; computational pathology; image analysis; decision support; artificial intelligence; clonogen; ionising radiation; stem cell; oxidative stress; acute response; late response; senescence; autophagy; tissue regeneration; mesenchymal stem cells; mesenchymal stromal cells; Toll-like receptors; exosomes;

interleukin-6; COPD; ageing; ECM; genome-wide association study; CELSR1; sex differences; small airways; precision imaging; personalised treatment; biologics; monocyte; tumour microenvironment; clinical trial.

Conflict of interest statement: CSH is Editor-in-Chief, RP is Scientific Editor and PJC is a Senior Editor of The Journal of Pathology. RP is an employee of The Pathological Society of Great Britain and Ireland.

Running title: Recent advances in pathology

Introduction

This is the 22nd consecutive year that *The Journal of Pathology* has published an Annual Review Issue. As with all previous Issues, the manuscripts contained herein are freely available online (see https://onlinelibrary.wiley.com/journal/10969896). This 2020 Annual Review Issue covers a diverse range of subject areas in which we feel there has been considerable recent progress. Given the aims of the Journal to act as a translational bridge between basic biomedical science and clinical medicine, the subjects relate to recent advances in basic research that aid understanding of disease processes, as well as reviews of specific areas of more immediate clinical relevance. We hope that these articles will be of use equally for pathologists and scientists across the world.

Recent advances in epigenetics and non-coding RNAs in health and disease

The first article in this section discusses the current thinking on long non-coding RNAs (lncRNAs) and their roles in development and disease. Julie Aspden and colleagues firstly provide a definition for lncRNAs and discuss the different categories of lncRNAs that have been identified. The conservation of lncRNAs and its value for inferring function are critically reviewed, followed by a discussion of the potential functions of lncRNAs according to their intracellular location. The review concludes with a comprehensive analysis of the evidence that some lncRNAs play important roles in normal development, in neurodegenerative disease and in human malignancies, suggesting their potential as biomarkers and/or therapeutic targets. Strikingly, although there are more than 16,000 lncRNAs in our genome, only about 50 of these are understood at a functional level [1]. Our second review in this area deals with the recent data on microRNAs (miRNAs) in multiple sclerosis (MS), indicating that each type of MS lesion in the white and grey matter shows a distinct miRNA profile. The functional effects of these different miRNAs are discussed, paying particular attention to the cell types involved and the possibility of exosomal delivery of miRNAs. In addition, certain miRNAs are shared, whilst other miRNAs show opposite regulation in different lesions. Since

miRNAs can be detected in blood or cerebrospinal fluid (CSF), they may act as biomarkers for disease. Thus, the investigation of miRNAs has inspired novel concepts for the pathogenesis and pathophysiology of MS, with potential diagnostic and monitoring applications [2]. The third review in this area examines DNA methylation in disease, concentrating on stratification of brain tumours for appropriate treatments matched to the underlying biology of the tumour. The review covers the role of molecular profiling, highlighting that DNA methylation has had the largest impact of these approaches for brain tumour classification. The authors also assess how machine learning-based algorithms are being developed and applied for clinical application in these diseases. This field can be regarded as an example of how molecular profiling influences the understanding of disease processes, as well as providing unanticipated benefits for pathology practice, along with certain risks [3].

Genetics of cancer

This Annual Review Issue contains three reviews that deal with very different aspects of cancer genetics. The first of these reviews current understanding of the molecular genetic causes of Lynch syndrome and the emergence of associated tumours. In particular, the authors discuss the methods and their interpretation involved in accurate diagnosis, together with providing sources of appropriate databases and current information on this subject. These approaches include genetic analysis, but also the application of immunohistochemical approaches based on the underlying defects [4]. The second review provides an update on the role of capicua (CIC) in human malignancies. Recurrent mutations in the *CIC* gene were first identified in oligodendroglioma, but CIC is now known to be dysregulated in multiple tumour types including sarcomas, adenocarcinomas of stomach, lung and breast, melanoma and multiple myeloma, mainly through translocation, point mutation or homozygous or heterozygous deletion. The review discusses the role of CIC in normal neurogenesis and development of other organs and the effects of its dysregulation in malignant diseases,

where it is now clear that CIC acts as a tumour suppressor and its loss associates with poor clinical features. The new and emerging information on this previously poorly studied gene suggests multiple actions, including cell cycle regulation, metabolism, genome stability and epigenetic regulation that contribute to its normal functions and to its effects in malignancy [5]. The final contribution in this section discusses how Mendelian randomisation can be used to identify causal links between an exposure and a specific outcome. In this approach, genetic variants are used as a proxy for exposure and are subsequently analysed for outcome in the population with the variant; this can be considered as a "natural" randomised clinical trial. As such, Mendelian randomisation can theoretically identify new risk factors as well as serving to validate or refute current views. The review from Harvinder Gala and Ian Tomlinson discusses the history, basic principles and methodologies of Mendelian randomisation approaches, together with the current limitations and potential improvements. The article concludes with examples of how Mendelian randomisation has been employed in studies of cancer and in identifying cancer predisposition traits [6].

Stromal cells and cancer microenvironments

The effects of tumour microenvironments have perhaps been neglected in the recent past due to the emphasis placed on the identification of gene mutations in the tumour cells themselves and their roles in promoting growth, survival, *etc*. However, pathologists have long known that tumours are heterogeneous and that the non-malignant cells admixed with the tumour are important determinants of malignancies and their progression. In particular, Sabine Galland and Ivan Stamenkovic discuss the current views on mesenchymal stromal cells (MSCs, also referred to as mesenchymal stem cells), which represent a key population of pluripotent cells involved in normal organogenesis and tissue homeostasis, regeneration and repair, as well as tumour formation and progression. Due to their currently loose definition, MSCs probably exist as several subpopulations with diverse functional properties. In cancer, MSCs may directly promote or inhibit growth and progression through cell-cell

contact interactions or secreted factors, or indirectly through modifying innate and adaptive immune responses. This review provides a detailed summary of MSC subtypes and their regulation and dysregulation in normal situations and in tumour growth and suppression, including the potential role of MSC-derived exosomes and MSCs as targets for therapeutic intervention. Remaining issues in this field include the identification and definition of MSC subtypes, the differences between MSCs and other or more differentiated stromal cells, and their respective contributions to disease [7]. Macrophages are a second cell type increasingly recognised as playing important roles in tumour pathology. Tumour-associated macrophages (TAMs) have been intensively investigated in the past decade for their phenotypes and their functions, reviewed here by Tim Beltraminelli and Michele De Palma [8]. In addition to summarising the recent advances in our knowledge of TAMs and their functions in promoting angiogenesis, suppressing anti-tumour immunity, facilitating metastasis and imparting drug resistance, the authors highlight the results of recent pre-clinical trials and the clinical development of novel therapies that target TAMs [8]. The third review article in this section comes from Christopher Pugh and colleagues, who provide an updated view of tumour hypoxia, concentrating on the roles of diagnostic pathology and tissue-based research rather than cell culture based research. Their article discusses how mammalian cells sense oxygen (including a recently recognised system originally identified in plants) and how gradients of oxygen availability produce microenvironments in cancers. The concept of pseudohypoxia is introduced, where oncogenic stimulation produces constitutive activation of hypoxia responses, which has direct relevance for tumour evaluation. The article also includes an overview of the different methodologies available to quantify tumour hypoxia and recent approaches to targeting hypoxia-related pathways that depend on knowledge of the signalling components being utilised by the individual tumour [9]. Finally in this section, we include a review of the effects of tumour environments in driving plasticity of cell migration programmes that lead to metastatic spread. Savvas Nikolaou and Laura Machesky discuss the two major mechanisms of cancer cell invasion, achieved by single or by collective cell invasion pathways. In the former, migration of individual cells is controlled by the Rho

GTPase Rac1, interacting with the Scar/WAVE complex and causing nucleation of branched actin filaments that enables the formation of lamellipodia, filopodia and invadopodia. Collective cell adhesion additionally requires coordination of cell-cell adhesion and/or repulsion and involves the activities of both leader cells and follower cells that show differences in morphology, gene expression and signalling pathway activation. The review discusses these processes in terms of the stressful environment in which tumour cells exist. The data indicate that both normal and tumour cells adapt migration and invasion according to the various stresses and demands of their environment and that a better understanding of the range of responses may reveal weaknesses that can be exploited therapeutically [10].

Inflammation, damage and repair

Chronic obstructive pulmonary disease (COPD) is caused by exposure to damaging particles and gases. Current treatments aim to improve symptoms and prevent exacerbations, but there are no treatments that modify the disease itself, nor are there any that are based on the individual's pathology. Wim Timens and colleagues review the recent progress in defining and understanding the underlying processes that are responsible for abnormal inflammatory responses and extracellular matrix changes that lead to altered small airways in this disease, including gender-associated features and the effects of age. The improved ability for disease endotyping and the ensuing development of strategies using biological agents to target specific pathological features will allow personalised treatments in the future [11]. *M. tuberculosis*, the causative agent of tuberculosis, has evolved multiple strategies to persist within infected cells and hijack immune mechanisms, leading to tissue damage in the host. Persistence also inhibits the effects of antibiotic therapies and allows the development of drug-resistant strains. These factors have led to the development of a radically different approach to therapy, the targeting of host immune responses rather than targeting of the infectious agent. This host-directed therapy (HDT) approach can enhance the effect of antibiotics to shorten treatment times, promote immunological memory to protect against

relapse, and reduce the pathology associated with long-term immune response including matrix destruction and fibrosis. Liana Tsenova and Amit Singhal review the pathology of tuberculosis including the heterogeneity of pathologies caused by infection and the host reactions that are induced. They emphasise recent data on HDT with already available drugs such as metformin, statins, corticosteroids, imatinib, inhibitors of phosphodiesterase or indolamine dioxygenase, and the gastric proton-pump inhibitor, lansoprazole. They conclude that HDT is a promising line of approach, tailored to the individual's lesions and their immune status [12].

The third review in this section comes from Bill McBride and Dörthe Schaue on the damaging effects of ionising radiation and the subsequent tissue responses. They introduce the historical aspects of radiation effects that have guided radiation oncologists and review recent data using, for example, cell lineage tracing to investigate heterogeneity of effects according to cell and tissue types. This structural diversity and plasticity within tissue compartments is the major theme of the review. In general, tissues with rapid turnover and highly proliferative stem/progenitor cells show acute responses, whereas slow turnover tissues show late responses and rely more on proliferation and re-programming events in mature cells. In addition, they highlight that inflammation may play an important role in causing tissue failure, as well as potentially enhancing anti-cancer immune responses for immunotherapy. The authors conclude that recent advances in radiotherapy may change our views, including that hypofractionation may have adverse effects on rapid turnover tissues and inflammatory responses, whilst the introduction of charged particles such as protons and carbon ions provides uncertainties particularly for late responding tissues [13]. Finally in this section, Siamon Gordon and colleagues review the key roles that macrophages play in coordinating the response to a wide variety of insults, as well as their roles in maintaining tissue homeostasis and development. Macrophages are heterogeneous cell populations and display a wide variety of surface receptor molecules that bind and respond to host and exogenous ligands including pathogenic organisms. These receptor-ligand interactions are

the main theme of the review, including a detailed discussion of the receptor molecules and their roles in recognising and removing pathogens and effete and dying host cells. The presence of these surface molecules provides information about macrophage function and phenotypes and the review argues that their use as antigen markers has lagged in clinical pathology, as has their potential as drug targets in immunotherapy [14].

Modern approaches and new technologies in research and clinical practice

Immunotherapy is an increasingly attractive therapy for a variety of cancer patients. The most common approach is immune checkpoint inhibition based on PD-L1, which is now a standard of care for several haematological and solid tumour types in many countries. The assessment of PD-L1 is therefore a key biomarker for patient selection. The whole area of immune checkpoint inhibition is reviewed by a worldwide set of experts, coming from a total of 79 groups of clinical and scientific researchers, led by Paula Gonzalez-Ericsson and Melinda Sanders. The authors review current practice relating to PD-L1 assessment in triple negative breast cancer using the different antibodies and systems available, including variability between platforms and inter-reader reproducibility. They emphasise that PD-L1 is only one component of the immunological spectrum, leading to the proposal that tumour-infiltrating lymphocytes (TILs) should also be assessed. They therefore suggest a standardised approach combining immunohistochemistry for PD-L1 with TIL assessment in haematoxylin and eosin-stained sections as an improved biomarker for PD-1/PDL-1 inhibition therapy. The path to implementation in clinical trials and daily practice through a risk management framework is also presented. Although the review is specifically related to triple negative breast cancer, the approach has obvious value for other solid tumour types [15]. On a related theme, the accurate and reproducible quantification of target molecules is a prerequisite for the type of study in the previous review. This area is rapidly advancing in pathology research and is reviewed by Ralf Huss and Sarah Coupland, who discuss the application of multiplex staining methods, high resolution imaging and image analysis tools to

aid tissue diagnostics and research. They discuss that, together with high end computer hardware, machine- and deep-learning solutions, there is a huge opportunity to improve the potential of personalised medicine to enable the administration of modern and emerging therapeutics. They emphasise especially that these digitalised and computerised approaches and the resulting software-driven clinical decision-making process will require significant changes in education and training for their introduction into clinical routines [16].

The final two articles in the 2020 Annual Review Issue concern the use of newly developed single cell methodologies and their impact on understanding both normal physiology and the pathophysiology of selected conditions. Single cell methodologies have increased massively in scale and scope, including the ability to perform genome-wide RNA or DNA sequencing in individual cells. The epigenetic landscape of single cells can also be identified, as can sites of active gene transcription. Such methodologies have a vast number of potential applications. Benjamin Stewart and Menna Clatworthy review single cell technologies in general, and focus on the new information provided in renal diseases. For example, they point out that single cell transcriptional profiling has identified the specific cell types affected by renal disease susceptibility genes, indicating that many nephrotic syndrome genes are expressed by podocytes, genes associated with hypertension are expressed in nephron tubular cells, and lupus susceptibility genes are expressed in B-cells infiltrating the kidney in lupus nephritis. In particular they review how these technologies will bridge the gap between molecular characterisation and classic morphological approaches to nephropathology [17]. The other review on the use of single cell technologies comes from Lisa Willemsen and Menno PJ de Winther on the subject of immune cells in atherosclerotic plaques. In their review they provide a detailed consideration of how single cell methodologies, including CyTOF (cytometry by time of flight; which allows high dimensional protein analysis of single cells) and single cell RNAseq approaches, have combined to define three main macrophage subsets in atherosclerotic plaques. These three subtypes, resident-like, pro-inflammatory and anti-inflammatory TREM2^{hi} foamy macrophages show specific locations in the plaque and

have specific phenotypes and functional properties. Their review also discusses some of the limitations of current single cell studies, including high technical noise and variability of single cell RNAseq data with false-negative read counts, which are being improved by analytical as well as methodological advances [18].

Conclusion

This collection of invited review articles illustrates the pace of change in the technology that can be applied to research focused on understanding disease mechanisms, and also shows how such research can have a rapid impact on disease diagnosis and patient management. We hope that these reviews will be of value to the readership of the Journal.

Author contributions: All authors were involved in writing and editing the manuscript. All authors approved the final manuscript for publication.

References

1. Tsagakis I, Douka K, Birds I *et al*. Long non-coding RNAs in development and disease: conservation to mechanisms. *J Pathol* 2020; https://doi.org/10.1002/path.5405
2. Teuber-Hanselmann S, Meinl E, Junker A. MicroRNAs in grey and white matter Multiple Sclerosis lesions: Impact on pathophysiology. *J Pathol* 2020; https://doi.org/10.1002/path.5399
3. Pickles JC, Stone TJ, Jacques TS. Methylation-based algorithms for diagnosis: experience from neuro-oncology. *J Pathol* 2020; https://doi.org/10.1002/path.5397
4. Cerretelli G, Ager A, Arends MJ, *et al*. Molecular pathology of Lynch syndrome. https://doi.org/10.1002/path.5422

5. Wong D, Yip S. Making heads or tails - The emergence of capicua (CIC) as an important multifunctional tumour suppressor. *J Pathol* 2020; https://doi.org/10.1002/path.5422
6. Gala H, Tomlinson I. The use of Mendelian randomisation to identify causal cancer risk factors: promise and limitations. *J Pathol* 2020; https://doi.org/10.1002/path.5421
7. Galland S, Stamenkovic I. Mesenchymal stromal cells in cancer: A review of their immunomodulatory functions and dual effects on tumor progression. *J Pathol* 2020; https://doi.org/10.1002/path.5357
8. Beltraminelli T, De Palma M. Biology and therapeutic targeting of tumour-associated macrophages. *J Pathol* 2020; https://doi.org/10.1002/path.5403
9. Macklin PS, Yamamoto A, Browning L, *et al*. Recent advances in the biology of tumour hypoxia with relevance to diagnostic practice and tissue-based research. *J Pathol* 2020; https://doi.org/10.1002/path.5402
10. Nikolaou S, Machesky LM. The stressful tumour environment drives plasticity of cell migration programmes, contributing to metastasis. *J Pathol* 2020; https://doi.org/10.1002/path.5395
11. Brandsma CA, Van den Berge M, Hackett TL, *et al*. Recent advances in chronic obstructive pulmonary disease pathogenesis: from disease mechanisms to precision medicine. *J Pathol* 2020; https://doi.org/10.1002/path.5364
12. Tsenova L, Singhal A. Effects of host-directed therapies on the pathology of tuberculosis. *J Pathol* 2020; https://doi.org/10.1002/path.5407
13. McBride WH, Schaue D. Radiation-induced tissue damage and response. *J Pathol* 2020; https://doi.org/10.1002/path.5389
14. Gordon S, Plüddemann A, Mukhopadhyay S. Plasma membrane receptors of tissue macrophages: functions and role in pathology. *J Pathol* 2020; https://doi.org/10.1002/path.5404
15. Gonzalez-Ericsson PI, Stovgaard ES, Sua LF, *et al*. The path to a better biomarker: application of a risk management framework for the implementation of PD-L1 and TILs as

immuno-oncology biomarkers into breast cancer clinical trials and daily practice. *J Pathol* 2020; https://doi.org/10.1002/path.5406

16. Huss R, Coupland SE. Software-assisted decision support in digital histopathology. *J Pathol* 2020; https://doi.org/10.1002/path.5388
17. Stewart BJ, Clatworthy M. Applying single-cell technologies to clinical pathology: progress in nephropathology. *J Pathol* 2020; https://doi.org/10.1002/path.5417
18. Willemsen L, de Winther MPJ. Macrophage subsets in atherosclerosis as defined by single-cell technologies. *J Pathol* 2020; https://doi.org/10.1002/path.5392