Recent Advances in Pathology: the 2022 Annual Review Issue of The Journal of Pathology

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Conflicts of interest: CSH is Editor-in-Chief of *The Journal of Pathology*, RP is an employee of The Pathological Society of Great Britain and Ireland and is the Senior Scientific Editor of *The Journal of Pathology*. PJC is a Senior Editor and NP is an Associate Editor of *The Journal of Pathology* and a co-author of two of the reviews in this issue. PB is author of one of the reviews in this issue.

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Abstract

The 2022 Annual Review Issue of *The Journal of Pathology*, Recent Advances in Pathology, contains 15 invited reviews on research areas of growing importance in pathology. This year, the articles include those that focus on digital pathology, employing modern imaging techniques and software to enable improved diagnostic and research applications to study human diseases. This subject area includes the ability to identify specific genetic alterations through the morphological changes they induce, as well as integrating digital and computational pathology with 'omics technologies. Other reviews in this issue include an updated evaluation of mutational patterns (mutation signatures) in cancer, the applications of lineage tracing in human tissues, and single cell sequencing technologies to uncover tumour evolution and tumour heterogeneity. The tissue microenvironment is covered in reviews specifically dealing with proteolytic control of epidermal differentiation, cancer associated fibroblasts, field cancerisation, and host factors that determine tumour immunity. All of the reviews contained in this issue are the work of invited experts selected to discuss the considerable recent progress in their respective fields and are freely available online (https://onlinelibrary.wiley.com/journal/10969896).

Key words:

3D reconstruction; adult stem cells; artificial intelligence; asthma; biomarkers; breast cancer; cancer; cancer-adjacent tissues; cancer-associated fibroblasts; chromothripsis; chronic obstructive pulmonary disease; clonal dynamics; clonality analysis; colon; computational pathology; convolutional neural networks; copy number aberrations; copy number signatures; cystic fibrosis; data repository; desquamation; digital pathology; DNA sequencing; epidermal inflammation; epidermis; epithelial transition states; extrachromosomal DNA; field cancerisation; filaggrin; functional pathology; genomics; haemopoietic stem cells; host; idiopathic pulmonary fibrosis; image analysis; image processing; immune checkpoint inhibitors; immune system; immunotherapy; *in situ* hybridisation; intestinal stem cells; intra-tumour heterogeneity; *in vivo*

models; lineage tracing; lung atlas; lung diseases; lung progenitors; lung stem cells; kallikrein-related peptidases; machine learning; metabolome; microbiome; mutational signatures; open science; PAR1; PAR2; patient stratification; prediction; prognosis; protease inhibitors; proteolytic cascades; quantitative methods; QuPath; single cell DNA sequencing; single cell RNA sequencing; single-cell transcriptomics; skin diseases; skin physiology; software; structural variants; subclone; tumour evolution; tumour phylogeny; virtual slide; whole-slide imaging; whole-slide scanning; whole genome sequencing.

Introduction

This Introductory Article accompanies publication of the 24th Annual Review Issue (ARI) of *The Journal of Pathology*. As has always been the case for our ARIs, the manuscripts contained herein are available online at no charge to the reader. This year's ARI encompasses a range of subjects for which the editors feel there is a particular need due to the rapid progress being made in these areas. In keeping with the aims of the journal to serve as a translational bridge between basic biomedical science and clinical medicine, our approach is to provide comprehensive and authoritative reviews of relevance to pathology. Thus, this ARI covers subjects that aid our understanding of disease through both theoretical and practical aspects, and that have direct clinical importance. In this way, we hope that the ARI will be a valuable resource for pathologists and scientists interested in understanding human disease processes and applying new information and new technologies for improved diagnosis and therapy.

Digital Pathology

The last few years have seen two revolutions in pathology; the introduction of high throughput slide scanning to produce digitised images, and the introduction and continued development of user-friendly software to provide robust and sophisticated analyses of these images, variably termed digital pathology or computational pathology. In view of the ever increasing use and

developments in the field, we have six manuscripts that deal with different aspects of this area. Our first is from Zarella and Alvarez in the departments of Pathology and Biomedical Engineering at Johns Hopkins University (Baltimore, USA), who review the whole area of high throughput slide scanning to produce large-scale data repositories that in turn can be used to feed into artificial intelligence (AI) systems [1]. The second review, from Peter Bankhead in Edinburgh, UK, focuses on image analysis methods, highlighting the current disconnect between the existing possibilities for digital pathology and the reality of using sophisticated image analysis systems in a coherent, consistent manner. In addition to reviewing the basic approaches, the article discusses the challenges of developing a novel algorithm past proof-of-concept and the need for collaborative and multidisciplinary approaches, with openness and sharing as a key principle for the future [2]. The next review, from Kramer *et al*. in Leiden, Amsterdam and Utrecht (The Netherlands), discusses the massive advances in the medical speciality of pathology since its first description in the 15th century to the current day, emphasising the previous restrictions to the use of 2D sections and arguing that 3D analyses including spatial genomics technologies and functional pathology approaches are becoming possible with multimodal data integration and computational pathology [3]. Although discussed mainly in the context of cancer, these rapidly advancing technologies have exciting possibilities across all human diseases. The fourth article in this area comes from Anant Madabhushi and colleagues in Cleveland (Ohio, USA), who review the significant advances that digital pathology is providing for lung diseases [4]. These advances include the development of new AI tools for a wide spectrum of conditions such as cancer, idiopathic pulmonary fibrosis, tuberculosis and COVID-19. In addition, quantification of biomarkers for diagnosis, prognosis and prediction of response are discussed, along with the challenges facing the deployment of these technologies. Although concentrating on lung diseases, the same principles apply across all tissue pathologies. The fifth paper in the area of computational pathology comes from Cifci, Foersch and Kather (in Germany and the UK), who review the evidence that AI methods can predict the presence of specific genetic alterations in

conventional haematoxylin and eosin-stained tissue sections. They conclude that alterations in genes including *FGFR*, *IDH*, *PIK3CA*, *BRAF* and in DNA repair pathways can be predicted in multiple tumour types, and all of these are targetable with current therapies. The implementation of these approaches as screening tools for precision oncology is also discussed [5]. The final review in this section is from Dent and Diamandis (Toronto, Canada) and deals with the use of computational pathology to address the issue of intratumoural heterogeneity, in this case applied to guide objective proteomic analysis and support the design of personalised combination therapeutic combinations [6]. This notion of combining 'omics technologies with spatial information from image analysis is an exciting area for the future.

Genomics, transcriptomics and single cell tracing

The pace of advance in 'omics technologies is unrelenting and is providing ever more insights into normal and pathological tissues. The first review in this section from Steele, Pillay and Alexandrov in London, UK and San Diego, CA, USA provides an overview of mutational signatures, the "scars" present in damaged DNA that has been mis-repaired and that contain signs of the specific mutagenic agents involved. In addition to reviewing the utility of the more well-known signatures of single- or double-base substitutions and small insertions/deletions, the authors provide a summary of the emerging knowledge of the mutational signatures of copy number alterations and structural rearrangements. In the future, detailed analyses of these less well studied signatures will provide valuable information for basic science, cancer prevention and cancer treatment [7]. The next article, from Bowes *et al*. in the UK, Belgium and Texas (USA), discusses the recent developments in single cell DNA sequencing technologies that are providing new insights into the evolutionary dynamics of cancer from initiation to progression, metastasis and therapy resistance, including the use of *in situ* technologies to study spatial aspects of intratumoural heterogeneity and single cell genomics to perform lineage tracing [8]. These technologies will undoubtedly provide additional information leading to the development and

application of novel therapies and rational design of combination therapies for cancer. The third article in this section, from Hamdan and Ewing (Edinburgh, UK) reviews the identification and application of structural variations in tumour genomes [9]. Structural variants are a major type of aberration and contribute to late stage tumour biology and intra-tumoural heterogeneity, and their evolutionary trajectories provide a window into clonal dynamics in tumours. Given their diversity in size and type (from simple insertion, deletion or inversion, to complex events of chromothripsis, chromoplexy and extrachromosomal DNA, among others), structural variants have been difficult to identify quantitatively from short-read sequencing. The increasing use of long-read whole genome sequencing will be critical for improving the accuracy of detection, paving the way for a fuller understanding of cancer biology and the impact of structural variants in clinical management. The use of single cell RNA sequencing is another rapidly expanding field with many applications. Carraro and Stripp at Cedars-Sinai (Los Angeles, CA, USA) review the use of single cell transcriptomics in lung development and a range of chronic lung pathologies, highlighting the advances in identifying disease-related changes in epithelial molecular phenotypes, with the emergence of novel epithelial transition states that characterise lung diseases [10]. These exciting advances bring the need for corroborative studies and will require a unified nomenclature in this rapidly maturing research field. Our final review in this section deals with lineage tracing in human tissues, where the genetic markers employed in mouse are not available. Gabbutt, Graham and colleagues in London, UK and Los Angeles, CA, USA review the history and current state of the art of lineage tracing, from the use of X-linked enzyme heterogeneity assays and mitochondrial DNA mutations to the ability to monitor DNA methylation and its fluctuation or the use of whole genome sequencing to identify somatic mutations to infer relatedness [11]. In particular, the authors highlight the use of mathematical modelling to measure clonal architecture of normal and diseased tissues to deduce the temporal dynamics of cell birth, death and replacement.

Tissue microenvironments

The role of the tissue microenvironment in maintaining tissue structure and homeostasis and the alterations that occur in disease has always been known and appreciated by pathologists. These features has been relatively overlooked by the focus on molecular categorisations of disease through genomics and transcriptomics that concentrate on the "diseased" cell population and not the "normal" surrounding cells. The error in this approach is especially highlighted in cancer, where changes in multiple cell types influence the ability of cancer cells to grow and prosper. In particular, the realisation that immunity is a common check on cancer growth has led to the development of immune checkpoint inhibitors as a primary therapeutic approach, with high success for some patients. However, responses are seen in only a minority of patients and are unpredictable, regardless of the availability of various recommended predictive markers. A review from Gunjur and colleagues in the UK and Australia introduces the emerging evidence for host factors as modifiers of tumour immunity and the mechanisms through which they influence therapeutic response. Importantly, many of these genetic, metabolic and host immune factors, as well the host's gut microbiota, are potentially modifiable themselves. Thus, host factors may provide avenues for co-therapies to enhance response to immune checkpoint inhibitors, as well as providing additional biomarkers to predict response [12]. The second article in this area deals with the increasing recognition that cancer-associated fibroblasts (CAFs) have important roles in cancer suppression and promotion, including effects on tumour immunity [13]. In their review, Cameron and colleagues from London summarise the evidence that CAFs are involved in therapy resistance and investigate why clinical targeting of CAFs has not been effective despite pre-clinical successes. Using pancreatic cancer as an exemplar, they review the recent identification of CAF subtypes with differential impacts on prognosis and therapy response, and provide a useful resource of current clinical trials targeting CAFs and stroma. They conclude that CAF subtypes may represent biomarkers for patient stratification and specific combination therapies to exploit the tumour microenvironment for therapeutic benefit. The next review, from

Sotiropoulou, Zingkou and Pampalakis in Greece, re-examines our understanding of the proteolytic cascades that regulate epidermal homeostasis and are altered in skin diseases [14]. They especially note the recent evidence for disparities between human and mouse model systems that have changed our views on the importance of individual proteolytic pathways. Recent research has also provided new knowledge on the mechanisms and functional interaction of these cascades and their previously unknown spatial restrictions, and there is new evidence for links with lipid metabolism in the epidermis. By combining established concepts with recent advances, the article provides an up-to-date view of the complexity of proteolysis regulation and signalling pathways in epidermal health and disease. The final article in this issue deals with the concept of field cancerisation in breast as an explanation for tumour recurrence, which occurs in up to 15% of patients within the first 10 years after surgery. This concept implicates the creation of a field of molecularly altered cells from which the tumour originates, or the presence of a tissue environment that predisposes to cancer initiation and malignant progression. The review from the groups of Jones and Chelala and colleagues in London, UK discusses the current evidence for such concepts in human breast cancers, reviewing the histopathological and mammographic changes already recognised, together with evidence that histologically normal tissues adjacent to and even distant from a tumour show molecular aberrations and genomic instability. Integrating molecular pathology and epidemiology to identify relevant factors such as environmental exposures, host genetics and lifestyle will be vital to examine the complex relationships with breast cancer risk, requiring comprehensive sets of appropriately annotated patient material within biobank collections. It is hoped that the current progress in studies of cancerisation fields will improve our understanding of breast cancer processes, and lead to improved screening strategies and treatments [15].

Author contributions statement

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

References

1. Zarella MD, Alvarez KR. High throughput whole-slide scanning to enable large-scale data repository building. *J Pathol* 2022; **https://doi.org/10.1002/path.5923.**
2. Bankhead P. Developing image analysis methods for digital pathology. *J Pathol* 2022; **https://doi.org/10.1002/path.5921.**
3. Kramer CJH, Vreeswijk MPG, Thijssen B, *et al.* Beyond the snapshot: Optimizing prognostication and prediction by moving from fixed to functional multidimensional cancer pathology. *J Pathol* 2022; **https://doi.org/10.1002/path.5915**.
4. Viswanthan VS, Toro P, Corredor G, *et al*. The state of the art in artificial intelligence in lung digital pathology. *J Pathol* 2022; **https://doi.org/10.1002/path.5966**.
5. Cifci D, Foersch S, Kather JN. Artificial intelligence to identify genetic alterations in conventional histopathology. *J Pathol* 2022; **https://doi.org/10.1002/path.5898.**
6. Dent A, Diamandis P. Integrating computational pathology and proteomics to address tumor heterogeneity. *J Pathol* 2022; **https://doi.org/10.1002/path.5905.**
7. Steele CD, Pillay N, Alexandrov LB. An overview of mutational and copy number signatures in human cancer. *J Pathol* 2022; **https://doi.org/10.1002/path.5912**.
8. Bowes A, Tarabichi M, Pillay N, *et al*. Leveraging single cell sequencing to unravel intra-tumour heterogeneity and tumour evolution in human cancers. *J Pathol* 2022; **https://doi.org/10.1002/path.5914**.
9. Hamdan A, Ewing A. Unravelling the tumour genome: the evolutionary and clinical impacts of structural variants in tumourigenesis. *J Pathol* 2022; **https://doi.org/10.1002/path.5901**

10. Carraro G, Stripp BR. Insights gained in the pathology of lung disease through single cell transcriptomics. *J Pathol* 2022; **https://doi.org/10.1002/path.5971**
11. Gabbutt C, Wright NA, Baker A-M, *et al*. Lineage tracing in human tissues. *J Pathol* 2022; **https://doi.org/10.1002/path.5911**.
12. Gunjur A, Manrique-Rincon AJ, Klein O, *et al*. 'Know thyself'- host factors influencing cancer response to immune checkpoint inhibitors. *J Pathol* 2022; **https://doi.org/10.1002/path.5907**.
13. Menezes S, Okail MH, Jalil SMA, *et al*. Cancer-associated fibroblasts in pancreatic cancer: new subtypes, new markers, new targets. *J Pathol* 2022; **https://doi.org/10.1002/path.5926**.
14. Sotiropoulou G, Zingkou E, Pampalakis G. Reconstructing the epidermal proteolytic cascades in health and disease. *J Pathol* 2022; **https://doi.org/10.1002/path.5888**.
15. Gadaleta E, Thorn GJ, Ross-Adams H, *et al*. Field cancerization in breast cancer. *J Pathol* 2022; **https://doi.org/10.1002/path.5902**