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Recent advances in renal pathology

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The genetic basis of renal diseases

Understanding why individuals vary in their susceptibility to renal disease is the focus of a comprehensive review of the genetic basis of renal disease by McKnight *et al.* [1]. The authors note that single gene disorders account for less than 15% of end stage renal disease, even though over 300 inherited disorders have been described that affect the kidneys. In addition to outlining the analytical methods in use they explain how techniques that study DNA from affected and unaffected populations in different cohorts have progressed so that more complex multi-gene renal pathologies can now be tackled. They are cautious about the ability of certain genetic tests to predict risk of kidney disease.

1. **Unravelling the genetic basis of renal diseases; from single gene to multifactorial disorders**
   Amy J McKnight, Diane Currie and Alexander P Maxwell
   The Journal of Pathology 2010; 220: 198-216. (Invited Review)

Podocyte Biology

The interdigitating foot processes of podocytes are indispensible for maintaining the glomerular slit diaphragm filtration apparatus that is characteristic of these highly specialized post-mitotic cells. Welsh and Saleem [2] reviewed multiple functions of the protein nephrin. It spans the podocyte plasma membrane, forms bridges across the slit diaphragm, and signals intracellularly though several networks. Studies of nephrin-null podocytes supported the view that nephrin is the foundation for cytoskeletal patterning, cell polarity and membrane vesicle docking functions [2]. A newly recognised property of podocytes in a subset of human glomerular diseases is the *de novo* expression of ubiquitin C-terminal hydrolase-L1 (UCH-L1) [3, 4]. UCH-L1 is sometimes known by the name protein gene product 9.5 (PGP 9.5), and should not be confused with antibody UCHL1 (that recognises a 180 kDa low molecular weight isoform of the leukocyte common antigen). UCH-L1 is detectable in distal tubules, parietal cells and nerves of normal kidney biopsies, but the presence of ubiquitin and UCH-L1 in podocytes correlates with podocyte foot process effacement (or flattening) [3], and occurs with internalization of podocyte-specific proteins including nephrin and the actin binding protein alpha-actinin-4, both of which are needed to maintain the slit diaphragm [4]. Studies of cultured human podocytes revealed that suppression of UCH-L1 improved podocyte differentiation [3]. Analysis of co-cultures of rat podocytes with mesangial cells demonstrated that gain of UCH-L1 expression by podocytes is not a simple response to inflammatory cytokines, but instead results from activation by immune complexes formed against mesangial cells [4].

2. **Nephrin - signature molecule of the glomerular podocyte?**
   Gavin I Welsh, Moin A Saleem
   The Journal of Pathology 2010; 220: 328-337. (Invited Review)

3. **A new role for the neuronal ubiquitin C-terminal hydrolase-L1 (UCH-L1) in podocyte process formation and podocyte injury in human glomerulopathies**
   C Meyer-Schwesinger, TN Meyer, S Münster, P Klug, M Saleem, U Helmchen, RAK Stahl
   The Journal of Pathology 2009; 217: 452-464. (Original Paper)

4. **UCH-L1 expression of podocytes in diseased glomeruli and *in vitro***
   Yuan Liu, Jiajing Wu, Huijuan Wu, Tianzhan Wang, Hualei Gan, Xin Zhang, Ye Liu, Ruixi Li, Zhonghua Zhao, Qi Chen, Muyi Guo, Zhigang Zhang
   The Journal of Pathology 2009; 217: 642-653. (Original Paper)

Mechanisms of disease in mice and man

Nephrogenic systemic fibrosis (NSF) is a serious condition in which the skin shows focal 'woody' hardening, although many organs can be affected. First described around ten years ago and appearing confined to individuals with severe renal insufficiency, onset of this disease became linked to exposure to certain contrast agents. The paper by Edward *et al.* [**5**] was the first to show a direct relationship between gadolinium chelate and activation of fibroblasts that may be linked to the development of NSF in susceptible individuals. The authors also raised the question of whether the activated fibroblasts were derived from a resident renal population or from circulating fibrocyte precursors.

Infiltration of renal glomeruli by circulating macrophages is thought to contribute to the progressive glomerular scarring seen in patients with Alport syndrome and also in mice that are unable to produce procollagen IV alpha 3 chains and thus model autosomal recessive Alport syndrome. One mechanism by which macrophages could be summoned is by glomerular cells releasing the cytokine Ccl2 that would be detected by its receptor Ccr2 on macrophages. Clauss *et al.* [**6**] hypothesised that blockade of the cytokine Ccl2 (by sequestration with synthetic binding RNA) would thereby reduce infiltration and ameliorate disease. Despite reducing macrophage infiltration this novel intervention did not reduce renal pathology scores or improve the shorter lifespan of these mice [**6**].

Interstitial fibrosis is a characteristic of chronic kidney disease of diverse origins. The source of these fibroblasts is debated: both resident renal populations and fibrocytes are likely to contribute, and many researchers favour the concept that some derive from the epithelium of damaged tubules by a process of epithelial – mesenchymal transition (EMT). Yang *et al.* [**7**] reported how Angiotensin II (which is pro-fibrotic in hypertensive and diabetic nephropathies) is EMT-inducing by acting specifically through the AT1 receptor. They investigated the signalling pathways involved by using small interfering RNA (siRNA) to suppress expression of specific Smad molecules *in vitro*, and by gene transfer of Smad7 in a remnant kidney model *in vivo*, and concluded that Smad3 is essential for mediating Ang II-driven EMT in rats. Hassane *et al.* [**8**] also investigated the role of SMAD signalling but in the context of autosomal polycystic kidney disease in cystic tissues from humans (*PKD1*-proven or linked) and mice that were either *Pkd1* null or in which *Pkd1* was disrupted postnatally using a kidney-specific inducible

mechanism. Disruption of *Pkd1* induced rapid cyst growth, but fibrosis in these mice was mild and EMT contributed only to a very low extent. They found that nuclear localisation of activated (phosphorylated) Smad2 was present in mature cyst epithelium and nearby interstitial fibroblasts consistent with TGFβ signalling playing a part in more advanced stages of cyst progression and fibrosis [8].

5. **Gadodiamide contrast agent 'activates' fibroblasts: a possible cause of nephrogenic systemic fibrosis**
   M Edward, JA Quinn, S Mukherjee, M-BV Jensen, AG Jardine, PB Mark, AD Burden
   The Journal of Pathology 2008; 214: 584-593. (Original Paper)

6. **Ccl2/Mcp-1 blockade reduces glomerular and interstitial macrophages but does not ameliorate renal pathology in *collagen4A3*-deficient mice with autosomal recessive Alport nephropathy**
   Sebastian Clauss, Oliver Gross, Onkar Kulkarni, Alejandro Avila-Ferrufino, Ewa Radomska, Stephan Segerer, Dirk Eulberg, Sven Klussmann, Hans-Joachim Anders
   The Journal of Pathology 2009; 218: 40-47. (Original Paper)

7. **Essential role for Smad3 in angiotensin II-induced tubular epithelial–mesenchymal transition**
   Fuye Yang, Xiao Ru Huang, Arthur CK Chung, Chun-Cheng Hou, Kar Neng Lai, Hui Yao Lan
   The Journal of Pathology 2010; 221: 390-401. (Original Paper)

8. **Elevated TGFβ–Smad signalling in experimental *Pkd1* models and human patients with polycystic kidney disease**
   Sabrine Hassane, Wouter N Leonhard, Annemieke van der Wal, Lukas JAC Hawinkels, Irma S Lantinga-van Leeuwen, Peter ten Dijke, Martijn H Breuning, Emile de Heer and Dorien JM Peters
   The Journal of Pathology 2010; 222: 21-31. (Original Paper)

Acute kidney injury in rodents and man

Tubular injury following periods of ischaemia then reperfusion may be followed by rapid regeneration, although interstitial fibrosis and permanent vascular damage may develop following severe ischaemia reperfusion injury (I/RI). Mulder *et al.* [9] hypothesized that heparin-binding EGF (HB-EGF), one of the lesser-studied growth factor ligands of EGFR, could modulate I/RI severity and carried out a series of investigations in human tissues and cell cultures, and rodents following I/RI. Increased expression of *HBEGF* mRNA occurred in renal graft biopsies following reperfusion whether the graft was from a living or deceased donor, and *Hbegf* mRNA was found induced within 90 minutes of reperfusion in rats, but was this a beneficial response or part of the process inducing damage? Blockade of EGFR function *in vivo*, by blocking its phosphorylation with a small molecule inhibitor, reduced interstitial macrophage infiltration and the appearance of myofibroblasts 4 days after I/RI in rats, which might indicate an early beneficial effect of blocking HB-EGF action. This was endorsed by their observation that *Hbegf* null mice were more resistant to I/RI, and had little interstitial macrophage infiltration. Yagihashi *et al*. [10] studied acute kidney injury induced by hindlimb ischaemia that serves as a model of peripheral vascular disease and traumatic crush injury. As with direct renal I/RI, hypoxia plays a part and here it was considered to cause activation of the polyol pathway (that converts glucose to sorbitol and then fructose, but also acts on several aldehydes) even though tissues were euglycaemic. The authors studied mice that were either aldose

reductase (AR) null mutants or normal mice pre-treated with an orally-effective AR inhibitor to establish that an active polyol pathway was needed for renal damage to result from remote ischaemia reperfusion; renal damage was attenuated when accumulation of sorbitol and fructose in non-perfused muscles was reduced, concomitant with reduction of their levels in the kidney itself.

9. **Heparin binding epidermal growth factor in renal ischaemia/reperfusion injury**
   Gemma M Mulder, Willemijn N Nijboer, Marc A Seelen, Maria Sandovici, Eelke M Bos, Wynand BWH Melenhorst, Monika Trzpis, Niels J Kloosterhuis, Lydia Visser, Rob H Henning, Henri GD Leuvenink, Rutger J Ploeg, Susan W Sunnarborg, Harry van Goor
The Journal of Pathology 2010; 221: 183-192. (Original Paper)

10. **The role of the polyol pathway in acute kidney injury caused by hindlimb ischaemia in mice**
    Soroku Yagihashi, Hiroki Mizukami, Saori Ogasawara, Shin-Ichiro Yamagishi, Hitoshi Nukada, Noriaki Kato, Chihiro Hibi, Sookja Chung, Stephen Chung
    The Journal of Pathology 2010; 220: 530-541. (Original Paper)

Indicators of disease

A recent review of the aptly named Kidney injury molecule-1 (KIM-1) by Waanders *et al.* [**11**] explains that this membrane-anchored glycoprotein is virtually undetectable in normal kidney but is induced rapidly in proximal tubules following damage. The expression of KIM-1, known also as hepatitis A virus cellular receptor–1 (HAVCR-1), on damaged tubular epithelium confers phagocytic capabilities that might be beneficial in clearing cellular debris and resolving casts. Urinary levels of cleaved (shed) KIM-1 appear to offer a useful non-invasive indicator of the current status of proximal tubular epithelium in humans and rodents and has been used to identify patient sub-groups likely to fare poorly, e.g. after transplantation.

11. **Kidney injury molecule-1 in renal disease**
    Femke Waanders, Mirjan M van Timmeren, Coen A Stegeman, Stephan JL Bakker, Harry van Goor
    The Journal of Pathology 2010; 220: 7-16. (Review)

Renal cancer

New insight into the way clear cell renal cell carcinoma (ccRCC) cells can escape from the primary tumour to form distant metastases was provided by the study by Kats-Ugurlu *et al.* [**12**]; discussed in an accompanying Invited Commentary by Hart [**13**]. Clusters of tumour cells with basement membrane or vascular fragments attached were detectable within the outflow from the renal vein of kidneys undergoing resection, but only if the primary was a VEGFA-expressing ccRCC. VEGFA would encourage the formation of dilated peripheral vessels that could accommodate expanding nests of tumour cells. The authors raise the possibility that early intervention in VEGF:Receptor signalling could offer a way to prevent this mode of spread. Profiling gene expression at the mRNA level is a well-established approach to understanding which pathways are altered in specific cancer types, and for seeking markers of tumour behaviour. The microRNA profile of tissues similarly merit investigation but this is a relatively undeveloped area. The study by Weng *et al.* [**14**] describes how a ‘next generation’ deep

sequencing platform together with miRNA expression arrays and RT-PCR validation steps were used on paired frozen and formalin-fixed paraffin-embedded (FFPE) samples of ccRCC and benign kidney. Sample numbers were small yet the authors found deep sequencing detected more miRNA than did microarrays and that results from FFPE tissue correlated well with results from frozen tissue. Traditional FFPE tissue archives could offer a valuable resource for similar studies of patient cohorts.

12. **Circulating tumour tissue fragments in patients with pulmonary metastasis of clear cell renal cell carcinoma**
    Gursah Kats-Ugurlu, Ilse Roodink, Mirjam de Weijert, Dorien Tiemessen, Cathy Maass, Kiek Verrijp, Jeroen van der Laak, Rob de Waal, Peter Mulders, Egbert Oosterwijk, William Leenders
    The Journal of Pathology 2009; 219: 287-293. (Original Paper)

13. **New evidence for tumour embolism as a mode of metastasis**
    Ian R Hart
    The Journal of Pathology 2009; 219: 275-276. (Invited Commentary)

14. **MicroRNA profiling of clear cell renal cell carcinoma by whole-genome small RNA deep sequencing of paired frozen and formalin-fixed, paraffin-embedded tissue specimens**
    Lihong Weng, Xiwei Wu, Hanlin Gao, Bing Mu, Xuejun Li, Jin-Hui Wang, Chao Guo, Jennifer M Jin, Zhuo Chen, Maricela Covarrubias, Yate-Ching Yuan, Lawrence M Weiss and Huiqing Wu
    The Journal of Pathology 2010; 222: 41-51. (Original Paper)

Potential therapies?

The potential to exploit the properties of stem cells for treating genetic and other diseases is very much *en vogue*. Hopkins *et al.* [**15**] explored comprehensively the options for kidney disease, including an overview of the problems resulting from the way mammalian kidneys develop. Addressing the possibility that some genetic defects might be treatable by introduction of cells into the pre-immune embryo, Guillot *et al*. [**16**] describe the fate and functioning of human mesenchymal stem cells administered prenatally to genetically modified mice that model human osteogenesis imperfecta (OI). A low level of chimaerism was established in the glomeruli of post-natal mice and these cells contributed a human α2(I) chain complementing that missing from OI mice, and reducing the accumulation of abnormal α1(I) homotrimers seen in untreated OI mice.

15. **Stem cell options for kidney disease**
    C Hopkins, J Li, F Rae, MH Little
    The Journal of Pathology 2009; 217: 265-281. (Invited Review)

16. **Transplantation of human fetal mesenchymal stem cells improves glomerulopathy in a collagen type Iα2-deficient mouse**
    PV Guillot, HT Cook, CD Pusey, NM Fisk, S Harten, J Moss, I Shore, G Bou-Gharios
    The Journal of Pathology 2008; 214: 627-636. (Original Paper)

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 Why is Nephrin a signature molecule for podocytes?

Question 2 Why is KIM-1 potentially such a useful marker of renal tubular injury?

Question 3 What is a *Spiegelmer*, and what can they be used for?

Question 4 How might a *protease-independent mechanism of metastasis* potentially be inhibited?

Question 5 What methods can be used to determine the *donor origin* of cells after stem cell grafting?

Question 6 In cell cultures, how might the expression of a specific protein be up-regulated or down-regulated artificially?

Question 7 Where do the *myofibroblasts* of interstitial fibrosis or nephrogenic systemic fibrosis come from?

Question 8 What are *miRNA* and how can deep sequencing reveal their relative abundance?