Don't know your amyloid from your epithelium?
You need our...........

Basic Renal Electron Microscopy Training Day
Presented by Bart Wagner
Chief Biomedical Scientist
at Sheffield Northern General Hospital

Biomedical Imaging Unit
http://www.som.soton.ac.uk/research/sites/biu

Where: Southampton General Hospital, UK
When: Friday 30th September 2011
How much: £40.00 (£30.00 IBMS)
Contact: a.page@soton.ac.uk
IBMS accredited
Basic Renal EM workshop
Southampton
September 30th 2011

Renal Ultrastructure
Normal

Bart E Wagner
BSc CSc FIBMS Dip Ult Path
Chief Biomedical Scientist
Electron Microscopy Section
Histopathology Department
Northern General Hospital
Sheffield
South Yorkshire
UK
S5 7AU
bart.wagner@sth.nhs.uk
Tel+44(0)114-27 14154
Sheffield

• In the centre of England, in South Yorkshire.
• A post-industrial city.
• Population of half a million.
• Two large universities & hospitals.
• Built on 7 hills.
• Manchester, Leeds, Nottingham - all nearby.
• But immediately adjacent to the Peak District
Sheffield is very close to the Peak District National Park
Near where I live... The Peak District National Park
If you are visiting Sheffield

Chatsworth House. Near Sheffield - built circa 1560
Ultrastructure is an extension of histology and anatomy.
Fig. 74.8 Left kidney, oblique vertical hemisection: normal macroscopic appearance of the renal cortex and renal medulla and the major structures at the hilum of the kidney. In A, the fat body of the renal sinus and most of the major vessels at the hilum have been removed, and the renal pelvis has not been opened. In B, the renal pelvis has been opened to reveal the interlobar arteries. (B from Sobotta 2006.)
Fig. 16.30 Anatomy of adult kidney.

a Photograph of sectioned adult kidney, which has been fixed in formalin and the near natural colour restored in alcohol. Note the cortex (C), the medullary pyramid (M) culminating in the papillary tip (P), which protrudes into the lumen of a calyx (Ca). Interlobar arteries (IA) and arcuate arteries (ArcA) can also be seen. Little detail of cortical structure is visible with the naked eye, but the vertical linearity of the components of the medulla is highlighted by clusters of prominent blood vessels (vasa recta).

b In this H&E stained paraffin section prepared from the tissue block shown in a; the distinction between cortex (C) and medulla (M) can be easily seen. This section also shows the vertical linearity of the components of the medulla, both tubules and vessels.

At this low magnification, glomeruli can be seen as small dots in the cortex. Note that some areas of the cortex are free of glomeruli, but contain vertically running duct systems; these areas are known as medullary rays and represent the sites where cortical tubules drain into the collecting ducts.

c In this micrograph of cortex at a higher magnification than in b it can be seen that the medullary ray (MR) area is devoid of glomeruli and that the interlobular arteries (ILA) run in the glomeruli-rich area.
View of renal biopsy core as seen using stereo microscope
Resin blocks and their moulds

Picking up semi-thin sections

Cutting thin sections

Picking up thin sections onto copper grids
Thin section on 200 mesh hexagonal high transmission copper grid

Section should have as many glomeruli as possible
First chapter on renal anatomy and histology
Normal glomerulus – H&E

- Parietal epithelial cell/cell lining
- Bowman's capsule
- Bowman's space
- Visceral epithelial cell/podocyte
- Red Blood Cell/erythrocyte in capillary loop
- Endothelial cell
- Mesangial cell
Normal glomerulus – Toluidine Blue stained plastic section

- Efferent arteriole
- Distal convoluted tubule
- Afferent arteriole
- Urinary pole of glomerulus
- Proximal convoluted tubule
Thin-section electron micrograph of same block as previous slide

Vascular pole of glomerulus

Urinary pole of glomerulus
Mesangial cell surrounded by 5 capillary loops

Red blood cell in capillary lumen

Podocyte

Endothelial cell

Mesangial cell

Glomerular basement membrane
Can you label this slide?
Can you label this slide?
Mesangial cell
Endothelial cell
Podocyte
Erythrocyte within capillary lumen
Scanning electron microscopy of exterior of whole rat glomerulus
Scanning electron microscopy of rat glomerulus cut in half

Erythrocyte

Podocyte
Podocyte – light cell dark cell artefact caused by hypoxia

Neutrophil polymorphonuclear leukocyte
Higher magnification of previous image

Podocyte

Interdigitation of foot processes
Interdigitating foot processes
Slit diaphragm viewed *en face*
Rat glomerulus

Ladder shaped diaphragm with central band
Slit diaphragm human kidney

Glomerular basement membrane

Note: no central band
Glomerular capillary wall

- Foot processes
- Glomerular basement membrane
- Slit diaphragm
- Fenestrated endothelium
Figure 1.54  Molecular anatomy of the podocyte foot process (FP) and actin cytoskeleton. This schematic shows two adjacent podocyte FPs with the interposed slit diaphragm (SD) complex. The localization of NEPH-1 at the SD and its heterophilic interaction with nephrin remain to be established. The actin cytoskeleton is the common downstream pathway and receives input from three podocyte domains: the apical domain, the lateral SD-containing domain, and the basal domain of the FP sole plate, which links the podocyte to the GBM. Interference with any of the three domains will ultimately cause FP effacement and proteinuria/nephrotic syndrome. α-act-4, α-actinin-4; α3β1, α3β1 integrin; α-DG, α-dystroglycan, β-DG, β-dystroglycan; NHERF2, Na+/H+ exchanger regulatory factor 2; P, paxillin; P-cad, P-cadherin; synpo, synaptopodin; T, talin; V, vinculin. (From Mundel P, Shankland SJ. Podocyte biology and response to injury. J Am Soc Nephrol 2002;13:3005.)
Podocalyxin
Nephrin & p Cadherin
Actin
Dystroglycan
Alpha & Beta
Alpha3 Beta1 integrin
Collagen IV & Laminin
Zonula adherens (beltlike junction)
It consists of a dense plaque associated with catenins ($\alpha$, $\beta$, and $\gamma$). Actin filaments are attached to catenins. The intercellular space is bridged by cadherins connecting the opposite dense plaques.

Occluding junctions (tight junctions)
Consist of the transmembrane proteins occludin and claudin, associated with four major proteins (ZO-1, ZO-2, ZO-3, and AF-6) at the intracellular side. Occludin and claudin seal the intercellular space.

Macula adherens (spot desmosomes)
Desmosomes are symmetrical structures consisting of: 1. Plaques containing desmoplakin. 2. Linking cadherins (mainly desmocollins and desmogleins). 3. Keratin filaments attached to the plaques.

Immunoglobulin superfamily
Cell adhesion molecules belong to the immunoglobulin superfamily because they contain domains similar to immunoglobulins. CAMs do not require Ca$^{2+}$ to maintain homophilic adhesive interactions.

Selectin
Selectins are Ca$^{2+}$-dependent molecules with binding affinity for sugars. Selectins have an important role in the homing process.

Hemidesmosomes
Hemidesmosomes consist of specialized proteins (bullous pemphigoid antigen 1 and 2), integrin $\alpha 6\beta 4$, and anchoring filaments (laminin-5) extending into the basal lamina. Keratin filaments are anchored into the plate of the hemidesmosome.

Fibronectin
Proteoglycans
Proteoglycans (mainly heparan sulfate) interact directly with fibronectin and laminin.

Collagens
Type IV collagen
Entactin (nidogen)

Integrins
On the extracellular side, integrins interact directly with fibronectin and laminin. On the intracellular side, the $\beta$ subunits of integrin interact with actin through intermediate proteins ($\alpha$-actin, vinculin, and talin).

Laminin
Laminin consists of three polypeptide chains (A, B1, and B2) with binding sites for type IV collagen, proteoglycans, integrin, and entactin.
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Juxtaglomerular apparatus

Macula densa at end of thick ascending part of loop of Henle and beginning of distal convoluted tubule
Higher magnification of previous image

Macula densa part of distal convoluted tubule adjacent to extraglomerular mesangial/lacis cells
Macula densa cells

Higher magnification of previous image
Afferent arteriolar cells at point of juxtaglomerular apparatus contain renin and angiotensin II granules
Afferent arteriole

Higher magnification of previous image
Granular myoepithelial cells

Granules contain renin
Schwann cell partly wrapping around neuronal cell processes
Non-myelinated nerve in renal cortical interstitium

Innervation of smooth muscle cells of afferent and efferent arterioles
Cells lining Bowman’s space/parietal epithelial cell sitting on Bowman’s capsule

- Heterochromatin
- Nucleolus
- Euchromatin
Matrix attachment point/plaque

Cell cytoskeleton

Bowman’s capsule
Peritubular capillary

Pericyte and endothelial cells
Renal cortical interstitial fibroblast

Abundant rough endoplasmic reticulum

Fibrous collagen
Toluidine blue stained plastic section of renal cortical tubules

- Distal convoluted tubule (DCT)
- Proximal convoluted tubule (PCT)
- Peritubular capillaries (PTC)
Proximal convoluted epithelial cell
Microvilli to resorb water via aquaporins, apical vesicles to resorb and transport peptides, smooth endoplasmic reticulum to replace cell membrane.
Apex of tubular epithelial cell

Junctional complex

Tight, intermediate, desmosome junctions

Zonula occludens, zonula adherens, macula adherens
Junctional complex - endocervix
Zonula occludens

Zonula adherens

Gap junction

Macula adherens

Junctional complex - endocervix
Proximal convoluted tubular epithelial cell

Cell shrinkage demonstrating lateral border attachment points
Base of tubular epithelial cell, folding and interdigitating with neighbour

Light cell dark cell phenomenon
Base of tubular epithelial cell

Numerous mitochondria
Proximal convoluted tubular epithelial cell
Proximal tubular epithelial cell

- Mitochondrion
- Peroxisome
- Smooth endoplasmic reticulum
- Lysosome
Distal convoluted tubule
Distal convoluted tubule cell possibly having undergone apoptosis
Time for a quick break?

‘The mind cannot absorb what the backside cannot endure’