P345

# P345 -Know your PLCE: A Role for PLCε in Controlling the Podocyte's Response to TGF-B1

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The podocyte is found on the urinary aspect of the glomerular basement membrane (GBM) and forms the final layer of the glomerular filtration barrier (GFB). Podocytes are thought to be the target cell in nephrotic syndrome pathogenesis. PLCE1 is one of the so-called nephrotic genes, yet unlike the others, encodes a lipid signalling enzyme rather than say component of the slit diaphragm or actin cytoskeleton regulator. The effects of mutations in PLCE1 in the mature podocyte are not clear.
A Conditionally immortalised human podocyte cell line from a DMS patient, with a SNP at nucleotide 321 that leads to a stop codon, was generated. This mutation severely truncates the protein such that no functional domains remain. This cell line was characterised in order to investigate the deleterious effect of this mutation on podocyte phenotype and function.
The PLCE1 mutant podocytes express lower levels of both epithelial and podocyte markers than the wild-type. Morphologically they appear more mesenchymal. They demonstrate a diminished response to TGF-B1 compared to the wild-type. Interestingly the PLCE1 mutant podocytes show no SMAD2 phosphorylation and robust SMAD3 phosphorylation. This is in stark contrast to the wild-type podocytes which show marked SMAD2 phosphorylation and only low level SMAD3 phosphorylation. It is thought that the SMAD2 pathway is anti-fibrotic while the SMAD3 pathway is pro-fibrotic. Upon closer inspection it was discovered that the PLCE1 mutant podocytes had equal levels of SMAD2 and SMAD3 while the wild-type podocytes had twice the amount of SMAD2 compared to SMAD3. This biases TGF-B1 signalling along the anti-fibrotic SMAD2 pathway in the wild type. Despite the 1:1 ratio between SMAD2 and SMAD3 the lack of SMAD2 phosphorylation shows that the PLCE1 mutant podocytes are biased towards the pro-fibrotic SMAD3 signalling.
A wild-type podocyte cell line had PLCE1 knocked out using CRISPR/Cas9 technology. This knock down influenced the SMAD2/3 ratio biasing it towards SMAD3 replicating the result seen in DMS patient derived podocyte cell line. This work evidences a mechanistic link between PLCε and the SMAD2/3 ratio.
This work suggests that by altering the SMAD2/3 ratio within the podocyte, PLCε enhances the pro-fibrotic response to TGF-B1. PLCE1 is the odd one out in the list of "nephrotic genes" since it does not encode a slit diaphragm component, ECM/adhesion complex component nor an actin cytoskeleton regulator. Hence it was not clear how mutations in this gene could lead to DMS or DDS. This work suggests that by altering the SMAD2/3 ratio within the podocyte, PLCε enhances the pro-fibrotic response to TGF-B1. This provides a novel therapeutic target to modulate the podocyte's response to TGF-B1 and may prove to be protective against the development of fibrosis.