Chapter 7

Summary and Discussion
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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common inherited disorder that predominantly manifests with progressive development of fluid-filled cysts in both kidneys. Cyst formation ultimately results in chronic renal failure. The disease is one of the major causes of chronic renal failure. Cysts arise from a defect in renal tubule epithelium function (Chapter 1, Figure 4). Due to an as yet unknown mechanism, a subset of epithelial cells undergoes a significant change that enables them to escape the regulatory mechanisms that normally regulate their function. These cells expand and grow out to form an isolated cyst. Cyst enlargement then occurs via fluid accumulation in the cyst lumen due to mislocalization of ion channels. Progressive development of cysts and accompanying fibrosis of the surrounding tissue disrupts renal function and ultimately results in chronic renal failure. In the majority of patients, the disease can be accounted for by a mutation in the either the \( PKD1 \) gene (1,2) or the \( PKD2 \) gene (3-5). The precise function of polycystin-1 and polycystin-2, the proteins encoded by the \( PKD1 \) and \( PKD2 \) gene respectively, remains to be elucidated. Therefore, it is still unclear how a mutation in polycystin-1 or polycystin-2 results in cyst formation. Data suggest that polycystins can exert multiple functions depending on the environment it is expressed in (cell type, differentiation status, etc.). Polycystin-1 and polycystin-2 have been shown to play a role in the cellular response upon extra-cellular stimuli, whether these are chemical (growth factors, stresses) or mechanical stimuli (fluid flow). Both polycystin-1 and polycystin-2 can regulate the cellular response by modulating signalling cascades, cell adhesion and/or cystoskeletal integrity, all of which are tightly coupled. Depending on the stimulus received, cell type, differentiation status, and other cellular and environmental factors, different functional aspects may be required to facilitate proper cellular responses. The molecular basis of polycystin-1 and polycystin-2 function in these signal transduction routes has not been elucidated yet. Polycystins may effect these signaling events via their ion channel activity or by modulating activity of interaction partners. It is clear however, that in the kidney, polycystins are required to maintain proper renal architecture and especially tubular integrity, since a defect in polycystin inevitably leads to polycystic kidney disease. Our work has focused on the renal pathology, because polycystic kidney disease and concomitant chronic renal failure is the major clinical threat for ADPKD patients. We set out to investigate the functions of polycystin-1 and polycystin-2 in order to gain further insight into the molecular and cellular processes that are disrupted in ADPKD cyst formation. Potential signaling pathways modulated by polycystin-1 were identified using a widely implemented approach based on reporter constructs. A C-terminal polycystin-1 construct was co-expressed in cells with luciferase reporter constructs to detect Wnt/\( \beta \)-catenin signaling or AP-1 activation (Chapter 2). In our hands, polycystin-1 did not modulate Wnt/\( \beta \)-catenin, whereas AP-1 activity was regulated by polycystin-1. Results were confirmed using immunofluorescence microscopy and western blot analysis to detect activated signaling components in normal and cystic cells and tissue. Using this approach we excluded Wnt/\( \beta \)-catenin signaling...
and identified AP-1 activation as a potential player in ADPKD cyst formation. The role of AP-1 in cyst formation was further analyzed in ADPKD cystic tissue using immunohistochemical and western blot analysis (Chapter 3). Since AP-1 is regulated by MAPK's, we explored the potential role of MAPK signaling by analyzing cystic epithelial cells derived from human ADPKD patients and from mouse renal cysts (Chapter 4). We report that ERK signalling activity is significantly impaired in human and mouse renal cystic epithelial cells and that polycystin-1 and polycystin-2 can modulate ERK activity. Our data clearly demonstrate that polycystins modulate intracellular signalling pathways, including MAPKs and AP-1 transcription factors. Moreover, both MAPK signalling and AP-1 activity are defective in cystic cells and tissue, indicating that these signalling events contribute to cyst formation or progression and may be crucial for maintaining renal epithelial cellular integrity and function. Direct immunoprecipitation studies will provide further insight into the mechanism by which polycystin exert their effect on MAPKs and AP-1. Other groups have reported that polycystins also modulate other intracellular signalling pathways including, NFAT and JAK-STAT transcription factors, and the mTOR pathway (7-9). These observations show that polycystins do not exclusively modulate MAPK signalling and AP-1 activity, but rather regulate a broad range of signalling routes within the cell. Since it is less likely that polycystins exert these effects via direct interaction with all of the corresponding intermediates, we hypothesize that polycystins may have selective molecular effects via which intracellular signalling events are achieved. One of the most likely candidates to relay the signal from polycystins is Ca\textsuperscript{2+} that is increased in the cell after activation of the polycystin channel. Other possible candidates are molecules up-stream of the signalling pathways and can be identified by direct immunoprecipitation experiments. Also, sub-cellular localization of polycystins can provide a relay mechanism: depending on the sub-cellular localization of the polycystin complex, in the plasma membrane at adhesion junctions, in the primary cilium, or in the cytosol, different cellular responses are achieved. Based on this, polycystins have been proposed to function as multi-purpose cellular tools that can act as a “master switch” between different signalling routes. In Chapter 5, we describe down-regulation of PKD1 and PKD2 after cellular stress as a potential factor in cyst formation. These data suggest that stress induced down-regulation of PKD1 and PKD2 during life may be an additional non-genetic modifying factor in cyst formation. Sub-lethal DNA damage is normally accumulated during life. In the case of ADPKD, germ-line inactivation of one allele in combination with down-regulation of PKD1 and PKD2, may just be sufficient to initiate cyst formation and progression. In this respect, it would be interesting to asses the effect of cellular damage on mouse models for Pkd1 and Pkd2. To test this hypothesis, we propose to induce cellular damage to heterozygous knock-out mice or to cross heterozygous mice with DNA-repair deficient mice. Based on our current data, we expect more severe and progressive polycystic kidney disease. Chapter 6 reviews potential therapeutic intervention strategies for cyst formation in ADPKD. Data on EGF/EGFR and AVP/cAMP signalling as potential targets for therapeutic intervention in ADPKD are outlined and discussed. Some of these reports present conflicting data. For instance, inhibition of EGFR activity
by pharmaceutical compounds such as EKI-785 and EKI-569, have been reported to improve disease progression in mouse models for polycystic kidney disease (10,11). In contrast, EGF supplemental treatment for polycystic kidney disease has been reported (12,13). Our data (Chapter 4) provide direct molecular evidence for impaired ERK signaling activity in human and mouse renal cystic cells at early and end-stage of disease and thereby establish a rationale for the EGF supplemental therapy proposed by Gattone II et al. and Ricker et al. (12,13).

In conclusion, much effort has been undertaken to unravel the molecular defects in polycystic kidneys disease. Our data contribute to this endeavour and identify MAPK signalling and AP-1 activity defects in both early and late polycystic kidneys disease. These results can be further confirmed by crossing mouse models for polycystic kidney disease with mouse models for these signalling components, for instance ERK knock-out mice. Comparison of single and compound heterozygous mice will provide further insight whether MAPK signalling and AP-1 activity are indeed mediators in polycystic kidney disease. These insights are crucial to develop directed therapeutic strategies for ADPKD.

References