Chapter 10

c-Myc Signalling in the Genetic Mechanism of Polycystic Kidney Disease

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Abstract

The Myc family of transcription factors regulates major biological processes such as proliferation, stem/progenitor cell pluripotency, metabolism, apoptosis, cell growth and differentiation. The most-studied member c-Myc is essential in embryonic development and cellular homeostasis. Dysregulation of c-Myc protein function is not only associated with malignant transformation and human tumors but is also implicated in autosomal dominant polycystic kidney disease (ADPKD), a human genetic disorder, considered a neoplasia in disguise. Studies from human ADPKD kidneys, caused by mutation in the

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PKD1 or PKD2 genes, revealed high expression of c-Myc with strong signal detected over cystic tubular epithelium. Consistent with human ADPKD pathogenesis, mouse models produced by dysregulation of Pkd1 and Pkd2 gene dosage show stimulation of renal c-Myc expression. Induced renal c-Myc expression is also observed in several non-orthologous animal models of PKD. Significantly, c-Myc overexpression specifically targeted to renal epithelial cells in transgenic mice closely reproduces human ADPKD. The specific causal effect of c-Myc in PKD was demonstrated by targeting different oncogenes which could not mimic the PKD phenotype. In fact, c-Myc was shown to be a major mediator of renal cystogenesis through various mechanisms and signalling pathways. Most importantly, inhibition of c-Myc *in vivo*, directly by repressing translation or indirectly with a small-molecule inhibitor, significantly delayed cystogenesis in the mouse. In summary, c-Myc is a central node in the pathogenesis of Pkd1/Pkd2 mouse models and of human ADPKD development and progression.

Key words: Mouse models; Myc; Polycystic kidney disease; Renal development; Signalling

Introduction

Autosomal dominant polycystic kidney disease (ADPKD), a ciliopathy, is characterized by formation of renal tubular cysts that involves increased epithelial cell proliferation and apoptosis, alterations in cell polarity and in tubular basement membrane and abnormalities in trans-epithelial fluid transport. Early on, ADPKD was claimed to be a neoplasia in disguise (1) and has been regarded as a multifocal neoplastic disorder. Molecular genetic analysis of ADPKD implicates one of two loci, PKD1 or PKD2. PKD1 encodes polycystin-1 (PC1), predicted to be a large cell surface receptor, and PKD2 encodes polycystin-2 (PC2), a non-selective calcium channel. Progress in understanding the molecular basis and pathogenesis of ADPKD was greatly enhanced by analyses carried out in murine experimental systems and on the PC1 and PC2 proteins. Studies on the ADPKD proteins have revealed that PC1 and PC2 interact together and with multiple proteins including other cystoproteins. Although the precise functions of the polycystin proteins are an issue not yet resolved, many signalling pathways and transcription factors are activated during ADPKD development and progression of cyst growth. A common key molecular pathogenic effector in the ADPKD network is c-Myc. In fact, there is overwhelming evidence for upregulation of c-Myc in renal cystic diseases. This chapter will address the role of c-Myc and its functional relationships in normal kidneys and ADPKD pathogenesis (2-4).

Insights into Myc biology

Myc is a pleiotropic transcription factor that regulates a multitude of cellular functions. This section gives an overview of its regulation and function at molecular and cellular level.

Regulation of Myc gene expression

c-Myc is among the best-studied proteins in biology. It is a member of a gene family that also includes N- and L-Myc, all of which are basic region/helix-loop-helix/leucine zipper transcription factors. These factors carry out similar functions that are dependent also on their spatial and temporal expression patterns (5). Myc is an immediate early growth response gene that is rapidly induced upon signalling from Wnt, Hedgehog, Notch and many receptor tyrosine kinases like extracellular signal-regulated protein kinase (ERK) (6-9). Expression of c-Myc is under tight regulation not only at the level of transcription and the mRNA itself but also post-translationally with modifications that provide stringent controls. Several transcription factors regulate c-Myc expression including the transcriptional regulator Brd4, which contains a bromodomain and extraterminal domain (BET) that binds to the c-Myc regulatory region (10, 11). Myc transcription is also controlled at both initiation and elongation by RNA polymerase II (12-14). Furthermore, the c-Myc transcripts are modulated by microRNAs (miR): miR-145, miR-34, miR-24 and Let-7 (15-20). The c-Myc protein can be degraded in the nucleus by the ubiquitin-proteasome system via several E3 ligases (21, 22) and it has a very short half-life of approximately 20 minutes (23).

More recently, c- and N-Myc were found to be post-translationally controlled by proteolytic cleavage (24). The Myc protein is cleaved in the cytosol by calcium-activated calpains that produce a protein termed Myc-nick, which lacks the C-terminal region essential for nuclear translocation (NLS) and DNA binding (bHLH, LZ) (24). Myc-nick binds to the microtubular cytoskeleton, and with the histone acetylase (HAT) GCN5 (25) mediates acetylation of α-tubulin involved in trafficking, primary cilia assembly and mitotic spindle formation. Hence, cleavage of Myc reduces levels of transcriptionally-active, nuclear Myc and produces Myc-nick which regulates microtubule dynamics and function to promote cell differentiation. Switching of full-length c-Myc to the cleaved form could explain the paradoxical roles of both inhibition and stimulation of cell differentiation attributed to c-Myc. Therefore, it is presumed that the proportion of full-length c-Myc converted into Myc-nick should be tightly regulated to determine lineage commitment and differentiation.

Myc molecular functions

c-Myc forms heterodimers with Max and together they bind DNA at one of the most frequent motifs in the genome, the canonical E-boxes. Genome wide mapping of Myc binding sites to chromatin and gene expression profiling identified a considerable number of potential gene targets (26). The Myc-Max heterodimer recruits multiple coactivator complexes and is associated with chromatin modification and gene activation. It is well recognized, however, that Myc gene activation is notoriously modest. In fact, all Myc target genes are not necessarily transcriptionally-responsive. Many studies have shown that Myc cooperates with a diverse set of additional factors to influence expression of target genes. Importantly, Myc promotes transcriptional regulation (27) in a dose-dependent fashion, such that as levels of Myc increase, gene activation becomes more generalized. Myc has been found to operate via a novel transcriptional mechanism, the "amplifier model", that consists of the transcriptional amplification of gene expression already in an activate state to coordinate a growth program (28, 29). As in the case of many transcriptional factors, Myc can also play a role in transcriptional repression when complexed with Miz-1 or through recruitment of histone deacetylases (HDACs). An additional mode of Myc regulation is through a network of microRNAs. c-Myc activates expression of a locus of 6 microRNAs, miR-17~92 via direct binding (30) and represses expression of several microRNAs, miR-23, miR-29, Let-7 (31-33).

Myc cellular functions

The Myc proteins are involved in major biological processes including proliferation, metabolism, cell cycle progression, apoptosis, cell growth and differentiation, fibrosis, and polarity (34-36) (Figure 1). All of these cellular functions are altered and ongoing in ADPKD. Consistent with the cooperative function of c-Myc with other transcription factors in stem cells, c-Myc was shown to promote and maintain pluripotency of stem and progenitor cells (37, 38). While high expression of c-Myc is essential in embryonic development, Myc expression levels normally wane in late developmental and cell differentiation stages.

When upregulated, the c-Myc protein is a potent oncogene involved in malignant transformation and in most types of human tumors (39). The Myc family members, in contrast to several oncogenes, do not need to undergo changes in coding sequence. In fact, Myc overexpression can drive a series of cellular changes, de novo mutations, and genomic instability that promote human malignancies. The c-Myc oncoprotein contributes to the genesis of different forms of human cancer ranging from lymphomas

to solid tumours (40, 41). N-myc is frequently overexpressed in solid cancers like neuroblastoma (42, 43) and L-Myc in small cell lung carcinomas (44). Myc contributes to tumorigenesis through various mechanisms. By compelling transcriptional regulation of many E-box genes and/or non-transcriptionally, Myc can play a role in the stages of initiation, maintenance, and progression of tumorigenesis, which led to the concept of "Myc addiction" (45). This Myc addiction can result, at least in part, from the need for energy metabolism since Myc regulates different genes of the glutaminolytic and glycolytic pathways (32, 46-48).

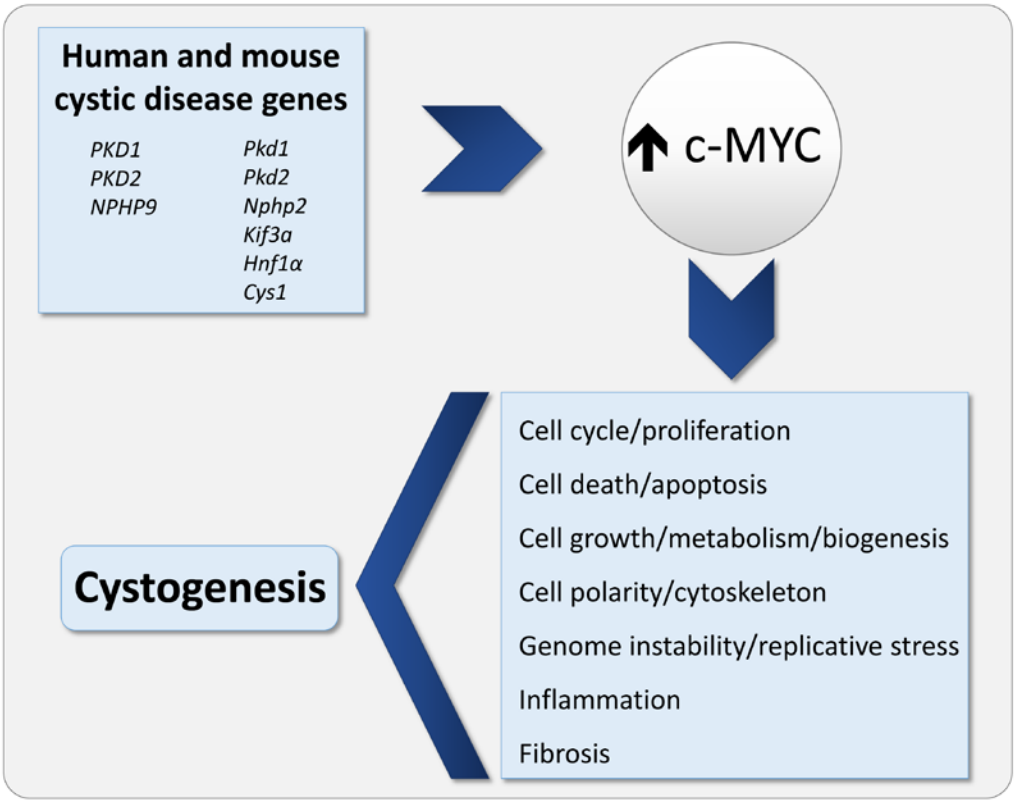

Figure 1. c-Myc at the node of PKD signalling. This schematic representation shows a cystogenic mechanism that could be operative in PKD. The c-Myc protein is depicted at the centre of the PKD pathogenic mechanism. Human ADPKD and nephronophthisis as well as cystic mouse models, orthologous and non-orthologous, induce upregulation of c-Myc expression. The well-studied c-Myc protein functions to elicit key cystogenesis features.

Role of c-myc in kidney development and homeostasis

Myc is central to the formation of the metanephros, the definitive kidney that initiates from embryonic day 10.5 (e10.5) in mice. Renal organogenesis involves mutual cellular inductive interactions between the metanephric mesenchyme and the ureteric bud that incite a subset of metanephric mesenchyme cells to condense and form the cap mesenchyme where stem cells are localized (49). The Myc family members are expressed during nephrogenesis but have distinct cellular patterns of expression in renal development. Normally, endogenous c-Myc is expressed early in uninduced renal mesenchyme, but also in the comma-shaped bodies, S-shaped bodies, and elongating tubules from e11.5-16.5. Upon renal maturation, c-Myc expression decreases to undetectable levels postnatally in differentiated renal tubular epithelium (50, 51). In comparison to c-Myc, N-Myc expression is even more transient with high levels upon renal mesenchyme induction, and is undetectable once the transition to epithelium is completed, whereas L-myc expression is confined to epithelium of the ureteric bud derivatives, in particular the collecting ducts (50).

Insights on the role of the Myc genes in the kidneys were provided from genetic studies using gene targeting. Mice with targeted disruption of the L-Myc gene were viable and had no renal histological anomalies (52), suggesting that L-Myc plays no essential role in the kidneys. Inactivation of N-Myc is embryonic lethal between e10.5-e12.5 with underdeveloped heart and neuronal defects (53). Embryos with a hypomorphic N-Myc allele resulted in hypoplastic kidneys at e13.5 with normal developing structures, due to reduced cell proliferation in mesenchymal cells and ureteric bud (54). Germline c-Myc inactivation leads to small and developmentally-retarded embryos that die at e9.5-e10.5 (55), and similarly epiblast-restricted c-Myc conditional inactivation resulted in fetal demise at e11.5 (56, 57) which precluded further analysis of c-Myc function in renal development as well as in adult renal homeostasis.

The role of c-Myc during renal development and homeostasis was determined by using a conditional c-Myc allele (58) at two distinct stages of development (2). Inactivation of c-Myc in early metanephric mesenchyme (e11.5) (59) showed that c-Myc plays an early and crucial role in the renal cap mesenchymal cells. Ablation of c-Myc resulted in depletion of the renal stem/progenitor cell population caused by a major decrease in proliferation that likely impaired their self-renewal potential (2). Interestingly, loss of c-Myc leads to a significant reduction of kidney size but with a normal branching pattern, cellular commitment, and architecture, revealing the preservation of normal renal developmental cues or developmental program. In fact, this renal c-Myc inactivation reproduced the typical pathologic condition of renal hypoplasia. In contrast, ablation of c-Myc at a later stage of renal epithelialisation (e17.5) (60) determined that c-Myc deficiency from e17.5

onward had no detectable impact on renal differentiation, maturation or homeostasis (2). This finding indicated that c-Myc is not essential from the late phase of renal development to adulthood, consistent with barely detectable c-Myc expression in mature kidneys. Hence, c-Myc is only essential in the cap mesenchymal cells within a critical developmental window. Therefore, the presence of c-Myc expression in mature kidneys likely results from stress signals (e.g. metabolic, DNA damage), environment stimuli (ischemia) or genetic conditions and could play a critical role in renal pathologies like PKD. Given that renal homeostasis can occur in a c-Myc independent manner, this implies that abrogation of renal c-Myc expression specifically can be a therapeutic strategy to prevent or stop the development of human adult pathologies such as PKD or renal cell carcinoma.

Upregulation of c-myc in human ADPKD

ADPKD pathogenesis in humans is presently thought to be a gene dosage dependent mechanism based on characterization of mouse models (61). In ADPKD patients, expression levels of the PKD1/PKD2 mutated allele can be strongly or mildly reduced. Paradoxically, PKD1/PKD2 and PC1/PC2 are found overexpressed in human ADPKD kidneys (3, 62-67). The severity of the ADPKD cystogenic mechanism(s) is likely to depend on modulation of the remaining non-mutant copy of PKD1/PKD2 potentially through de novo mutations, a stochastic event, altered functional role of polycystins and/or epigenetic inheritance that are actively under study.

Nonetheless, signalling pathways, networks and downstream effectors have been investigated in human ADPKD. Quantification of c-Myc and N-Myc expression in renal tissues from ADPKD patients revealed that all human ADPKD kidney biopsies showed highly elevated c-Myc levels (up to 15-fold) whereas N-Myc expression is unaffected (68). Analysis from ADPKD kidney biopsies using microarrays confirmed increased c-Myc expression (69, 70). Significantly, expression levels of c-Myc in fetal ADPKD kidneys were increased by approximately 40-fold, indicating that c-Myc is induced in both early and advanced stages of ADPKD. In addition, expression of c-Myc was detected by a strong signal intensity over renal ADPKD cystic epithelial cells by in situ hybridization (3). High expression of c-Myc indicated an active state of c-Myc during cystogenesis and correlated with a role in disease development and/or progression (Figure 1). Consistent with c-Myc function, ADPKD kidneys undergo increased epithelial proliferation (10 to 100-fold) and show an elevated apoptotic index (up to 100-fold) mainly localized in the cystic epithelium (3, 71). The apoptotic pathway involved is most likely independent of p53 and Bcl-2 since the expression levels of the pro-apoptotic p53 were low to undetectable whereas those of the anti-apoptotic Bcl-2 increased ~10 to 20-fold (3). Both proliferation and apoptosis in

epithelial cysts demonstrated a focal distribution with frequent cell clusters, implicating paracrine regulation via cell-cell interaction or disruption of basement membrane via cell-matrix interaction.

Several genetic diseases cause renal cystic disease in human. Among them nephronophthisis 9 or NPHP9/NEK8 was found to downregulate expression of the orthologous PKD1 and PKD2 genes and to increase c-Myc expression levels (72) (Figure 1). Other ciliopathies including other nephronophthises, Bardet-Biedl syndrome, Meckel-Gruber syndrome and oro-facial-digital type1 syndrome develop renal cysts but the molecular role and intracellular interactions remain to be investigated for a potential association with Myc dysregulation in these conditions.

Upregulation of c-Myc in renal cysts of human ADPKD results most likely from dysregulation of key developmental signalling pathways. Insights into possible human polycystin networks and downstream effector pathways were acquired from global and candidate approaches on human ADPKD kidneys and cell lines. These studies reported activation of developmental pathways including Wnt, Sonic Hedgehog, Notch, Hippo, bone morphogenic protein/transforming growth factor-β (BMP/TGFβ), ERK and transforming growth factor-α (TGFα) signaling (69, 70, 73-75) that are known to regulate the c-Myc early growth response gene (6-9). Studies from human ADPKD tissues pointed to one or several signaling pathways that converge on c-Myc as a key "cystogenic" factor. Consistent with c-Myc being a central node in ADPKD, renal cellular energetics in ADPKD and even in the autosomal recessive form of PKD, ARPKD rely on activation of the glutaminolytic and glycolytic pathways, known to be c-Myc molecular metabolic targets in cancer (70, 76).

c-Myc, a key cystogenic factor in murine PKD

Presently, it is thought that c-Myc upregulation is a hallmark of PKD and cystogenesis in general. c-Myc is almost universally upregulated in cystic kidney diseases and virtually, independently of the underlying mutated genes.

c-Myc as an inducer of PKD in the SBM mouse model

While several mouse models with c-Myc overexpression promote carcinogenesis in collaboration with other factors as p53 and the Bcl2 gene family, c-Myc upregulation in the kidneys is cystogenic (4, 77) and reproduces human ADPKD as evidenced by the SBM mice (4). The unique SBM transgenic mouse model was generated by expression of the murine

c-Myc gene driven with the "SB" regulatory elements that target specifically renal epithelial cells. Mice derived from the 18 independent SBM transgenic lines consistently developed a PKD phenotype with 100% penetrance (4, 68, 78-81). The SBM mice exhibit all of the typical PKD renal morphologic and physiologic features. SBM kidneys are enlarged with numerous tubular and glomerular cysts that initiate in utero from e16.5 and progress with age. All nephron segments are affected in SBM kidneys as observed in human ADPKD (79). Developing cysts displayed extensive glomerular and tubular epithelial hyperplasia, in particular in fetal and young kidneys (postnatal day 0 to 20). Consistently, SBM kidneys have shown a markedly increased renal proliferation index of ~5 to 20-fold (78) and increased apoptosis of ~3 to 10-fold. The proliferation and apoptosis frequently occurred in cell clusters, suggesting cell-cell and/or cell-matrix interactions. SBM renal epithelia exhibit abnormal cell polarity by mislocalization of Na/K-ATPase, fodrin, ankyrin, and E-cadherin, and even loss of marker identity for a minority cystic tubules (79, 80) (Figure 1). These cellular characteristics could be compatible with a conflict in signals elicited from elevated full-length c-Myc and Myc-nick levels, resulting in microtubule hyperacetylation and altered cell differentiation (24). The SBM cystic kidneys showed persistence of immature renal epithelium either undifferentiated or dedifferentiated (80). As also found in ADPKD patients, SBM mice develop high blood pressure (~200mmHg) associated with vascular abnormalities (81). Interestingly, SBM renal parenchyma displays regions of fibrosis and focal infiltrates. SBM kidneys show evidence of renal epithelial chromosomal abnormalities, multipolar spindles, adenomas at young age, but no adenomas or carcinomas in adulthood (78). Consequent to the pathologic features, SBM died of renal insufficiency at ~3 to 5-months with severe renal damage and proteinuria.

The importance and specificity of c-Myc in PKD development and renal proliferation was demonstrated by several studies. First, spontaneous revertants in several SBM transgenic lines with mutations or partial deletions of the transgene in germinal cells were generated that did not develop a PKD phenotype (82). The absence of PKD demonstrated that the intact c-Myc transgene is necessary and sufficient to produce the SBM phenotype. In addition, the specificity of c-Myc in PKD was shown by substitution of c-Myc in the SBM transgene by the c-Fos early response gene and well-characterized proto-oncogene, linked to the "SB" regulatory elements producing the SBF transgene. None of the mice from the eight SBF transgenic lines developed renal abnormalities despite high levels of transgene renal expression (68). Furthermore, the c-Myc gene in the SBM transgene was also substituted by the well-studied growth factor TGFα coding sequence (68). The five SBT transgenic lines generated had no gross or microscopic renal abnormalities. These findings showed that the upregulation of c-Fos and TGFα in cells of human ADPKD cysts (69, 70, 83, 84) are not necessarily causative. Moreover these studies indicated that the PKD

phenotype in SBM mice depends on the specific functions inherent to c-Myc itself and not simply on a general mitogenic deregulation of the renal epithelial cells (4, 82).

In addition to proliferation, c-Myc was shown to promote cellular apoptosis, a prevalent process in PKD (3) (for review (85)) (Figure 1). c-Myc can function by altering a number of pro- and anti-apoptotic molecular mechanisms, in particular p53, Fas, and members of the Bcl2 gene family (86-88). Consistently, SBM kidneys showed increased programmed cell death affecting most severely the tubular cystic epithelium (68). The apoptotic mechanism induced by c-Myc in SBM mice appears independent of the well-known pathways. Successive matings of SBM mice with p53-null mice generated several $p53^{-/-}$ SBM mice. All of these adult mice developed renal tubular cysts similar to SBM, died at the same age, had similar renal apoptotic index, and had no evidence of carcinomas. Transgenic mice carrying the major suppressor of apoptosis Bcl-2 linked to the "SB" regulatory elements expressing high Bcl-2 transgene levels mated to the SBM lines revealed that Bcl-2 expression did not modify the apoptotic rate, slow down PKD or even develop carcinomas (68, 89). Moreover, the SBM mice with c-Myc induced apoptosis were not rescued even partially with a deficient FasL pathway (89). Collectively, the SBM apoptotic mechanism is independent of p53, Bcl-2 and FasL/Fas, consistent with the human ADPKD studies. This novel and atypical *in vivo* c-Myc apoptotic pathway may play a critical role in PKD and potentially in the various human cystic diseases. As such, the SBM transgenic mice demonstrated a definitive causal connection between c-Myc and cyst formation in PKD.

c-Myc, a critical regulator in non-orthologous cystic mouse models

One of the most extensively studied spontaneous murine model of PKD is the autosomal recessive congenital polycystic kidney (cpk) mutant that closely resembles ARPKD. The cpk gene encodes Cystin-1 that is expressed in the cilia of collecting duct epithelia (90). Significantly, the cpk mice were shown to have elevated levels of c-Myc expression (91) and of the c-Fos and Ki-ras proto-oncogenes in kidneys (92). Interestingly, metabolomic analyses on cpk mice revealed renal hyperactivation of the glutaminolytic pathway, consistent with a c-Myc role in cellular metabolism (76). The cpk mouse model is also associated with overexpression of Pkd1 in both the cystic kidneys and pancreas (93). More recently, Cystin-1 was shown to interact with Necdin, a DNA binding factor that can activate c-Myc promoter (94). Cystin-1 in a complex with Necdin antagonizes stimulation of c-Myc expression. Importantly, a study on treatment of the cpk mice using c-Myc antisense oligonucleotides led to marked decrease and delay of cyst severity, attenuating PKD significantly (95).

Evidence that c-Myc regulates downstream targets implicated in a cystogenic network was provided by both upregulation and inactivation of miR-17~92. Transgenic mice overexpressing miR-17~92 developed renal tubular and glomerular cysts. Conversely, inactivation of miR-17~92 caused delay of cystic progression in non-orthologous mouse models (96).

Few non-orthologous cystic mouse models known to target Pkd2/PC2 or cilia structure also implicate upregulation of c-Myc. The autosomal dominant mutation in the rat model, Han:SPRD-cy, which is considered a model for human ADPKD, displayed highly elevated levels of renal c-Myc in cystic epithelia from a young age (97). The hepatocyte nuclear factor-1β (Hnf-1β), which directly modulates expression of the orthologous Pkd2 gene and the Pkhd1 gene responsible for autosomal recessive PKD, produce upon inactivation a mouse model with diabetes and renal cysts. Notably, kidneys in this mouse model displayed substantial increase in c-Myc expression (96). The motor protein kinesin family 3A, Kif3a, mediates intraflagellar transport in the primary cilium and interacts with PC2 (98). Renal inactivation of Kif3a causes loss of primary cilia and formation of renal tubular cysts (60). This phenotype was associated with elevated c-Myc and β-catenin expression and suggests the activation of the Wnt canonical pathway as detected in human ADPKD (96, 99). The truncated Inversin mouse mutant lacking the C-terminus (InvΔC), a mouse model of nephronophthisis type 2, NPHP2, has high levels of c-Myc expression that plays a key role in renal cyst formation (100). Treatment of the InvΔC mice with an ERK inhibitor reduced not only the level of phosphorylated ERK but also markedly reduced c-Myc expression associated with decreased proliferation and slower cyst enlargement.

Orthologous PKD mouse models co-associate with c-Myc overexpression

At present, animal models of the two known human genes responsible for ADPKD point to c-Myc as major mediator of cytogenesis *in vivo*. Orthologous dosage-reduced Pkd2 mouse models caused by conditional ablation exhibit increased renal expression of c-Myc (96). Similar to the Pkd2 mouse models, two Pkd1 orthologous dosage-reduced mouse models by conditional Pkd1 ablation, and by Pkd1 hypomorphic mutation, develop renal cysts and also induce elevated c-Myc mRNA and protein (101). The transcriptional upregulation of c-Myc resulted from increased expression of the epigenetic regulator Brd4 that binds to the c-Myc promoter. It was discovered that Brd4 upregulation is caused by the heat shock protein-90 (Hsp90) chaperone activity that protects Brd4 from proteasomal degradation (101), consistent with increased Hsp90 expression in Pkd1 mouse models (102). Most importantly, the use of the BRD inhibitor JQ1 in two Pkd1 mouse models suppressed c-Myc expression and p21 signaling that reduced proliferation and delayed renal cyst growth in both renal deficient and hypomorphic Pkd1 mouse models (101).

Two orthologous dosage-increased Pkd1 mouse models, including one with extrarenal manifestations, reproduce all of the typical PKD characteristics including fibrosis and inflammation (103, 104) (Figure 1). Strikingly, renal analysis in both the $Pkd1_{TAG}$ and $SBPkd1_{TAG}$ mouse models revealed upregulation of c-Myc mRNA expression and protein that is markedly enhanced in the cystic epithelia (104). Pkd1 dysregulation in these mice promotes cellular responses typical of full-length c-Myc. Moreover, the enhanced cilia length quantified in renal epithelial cells of both these Pkd1 dosage-increased models is also consistent with Myc-nick function on α-tubulin acetylation (24, 105). Interestingly, a mouse model that reproduces a naturally-occurring human Pkd1 truncating mutation, $Pkd1_{extra}$, develops slowly progressive renal cysts with elevated c-Myc expression (106). Notably, overexpression of Pkd2 in transgenic mice leads to increased c-Myc levels in kidneys with cystic anomalies (107, 108).

Molecular PC1 dysregulation identified stimulation of effectors from the Wnt, Hippo, Sonic Hedgehog, Notch, ERK and BMP/TGFβ cascades as uncovered by global profiling and candidate approach analyses (74, 75, 96, 109). Remarkably, the signalling pathways activated in the orthologous mouse models virtually mimic those identified in human ADPKD studies, some of which are also deregulated in non-orthologous mouse models and result in c-Myc upregulation. Recently, accumulating evidence has shown that effectors of Wnt and Hippo pathways for example, are often shared and crosstalk through multiple mechanisms (for review (110)). Significantly, targeting several effectors of these pathways led to renal cyst formation in the mouse (111-117). Similarities between dysregulation of polycystin(s) and c-Myc suggest that these proteins are involved in a network or in common signalling pathways essential in renal development and in the ADPKD adult pathologic condition.

In the mouse, c-Myc is not only a target of polycystin(s) but also a critical mediator of cystogenesis. Definitive causal connection between c-Myc and cyst formation was established from the SBM mouse model and from the therapeutic benefit of c-Myc downregulation in the treatment of Pkd1 dosage-dependent mouse models.

Conclusion

Upregulation of c-Myc is considered a typical characteristic hallmark of PKD in the mouse. In fact, one cannot seem to dissociate c-Myc upregulation from renal cystic diseases (Figure 1). The molecular basis for c-Myc activation in PKD1/Pkd1 and PKD2/Pkd2 dysregulation remains incompletely understood. It is most likely that the polycystins PC1 and PC2 influence the activity of c-Myc through interaction within one pathway, or a

network of pathways. Perhaps most significantly, studies abrogating c-Myc expression in particular in orthologous mouse models was therapeutically beneficial with no side effects detected.

On the principle that mouse signalling studies are often validated in humans, c-Myc ought to be a major player in ADPKD signalling from the substantial accumulation of data that has repeatedly placed c-Myc at the centre of the ADPKD scene. The parallel between the characteristic pathologic features of human ADPKD and murine PKD to the typical functions of the c-Myc oncogene is impressive. ADPKD displays numerous cancer-like characteristics, but probably with less severe changes to genome integrity and fewer multistep lesions. The notion that ADPKD appears dependent on the many functions of c-Myc activation at different steps of cyst initiation, progression and expansion suggests the concept of "c-Myc addiction" for cyst development.

Future directions

Although our knowledge of the mechanisms and genes that govern ADPKD has substantially increased over the last years, several outstanding questions remain to be resolved. From a fundamental perspective, it is unclear what molecular mechanisms underlie the fetal-like phenotype in cystic epithelial cells. The immediate downstream effector(s) of polycystin have not yet been determined. One key question is the exact mechanism associated with the integration of the polycystin signalling interactions and networks leading to c-Myc upregulation in PKD. Etiology of the polycystin pathways will provide powerful support to better preventive and therapeutic measures and to the development of treatments. Molecular understanding of the genetic basis and progression mechanisms of PKD will have crucial translational applications for discovery of novel targets to design drug screening. These studies will provide a rational basis for tailored therapy.

From a translational perspective, ADPKD appears to be characterized by cumulative c-Myc-distinct functions that would warrant development of combinatorial therapies. Clearly, multi-targeting various complementary mechanistic functions in ADPKD for clinical therapeutic interventions would counteract a number of external (environmental, epigenetics) and internal (genetic) variations. An integrated ADPKD therapy may not require complete abrogation but moderate inhibition of most c-Myc-distinct functions that could be as efficient and have tremendous impact in a clinical setting with minimized adverse effect.

Conflict of Interest

The author declares that she has no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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