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Are renal ciliopathies (replication) stressed out?

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Juvenile renal failure is commonly caused by the ciliopathy nephronophthisis (NPHP). Since all NPHP genes regulate cilia function, it has been assumed that NPHP onset is due to cilia loss. However, recent data suggest that DNA damage caused by replication stress, possibly concomitant with or upstream of cilia dysfunction, causes NPHP.

Renal ciliopathies: not just loss of cilia

The leading genetic causes of pediatric as well as adult kidney failure can be traced back to an organelle called the cilium. Cilia loss of function is thought to be the cellular defect responsible for two classes of renal ciliopathies: the common autosomal dominant polycystic kidney disease (ADPKD), which affects adults; and the rare, recessive pediatric/juvenile ciliopathies collectively referred to as NPHP-related ciliopathies (NPHP-RCs) (Box 1). For example, all 19 NPHP-associated genes reported to date encode gene products known to localize to primary cilia and regulate ciliary structure or function. In addition, many NPHP-associated proteins possess extraciliary functions that potentially contribute to the development of disease, and these functions have only recently been explored.

Recent molecular evidence argues that the nuclear/DNA damage response (DDR) functions of some NPHP proteins may be critical in disease onset or progression. DDR signaling includes mechanisms to detect DNA damage (lesions), signal the presence of damage, arrest the cell cycle, and promote repair. The question is raised of whether nuclear effects of NPHP gene mutations are upstream, downstream, or independent of cilia dysfunction [1–3]. Initial studies of the NPHP genes *ZNF423* (*NPHP14*), and *CEP164* (*NPHP15*) revealed that they colocalize with the DDR proteins SC-35 (a splicing factor), checkpoint kinase 1 (CHK1), and Tat-interactive protein 60 (TIP60) in the nucleus [2]. Additionally, morpholino injection of zebrafish embryos targeting *znf423* or *cep164* induced sensitivity to DNA damage-causing reagents [2], suggesting that a role for NPHP proteins in DDR signaling may contribute to the pathophysiology of NPHP. Moreover, CEP164 interacts with the cell cycle checkpoint proteins ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) during activated DDR signaling [4] and depletion of *Cep164* increases phosphorylation of H2AX in healthy, undamaged cells *in vitro* and *in vivo* [5]. Supporting this hypothesis, a *Sdccag8* (*Nphp10*) mouse model showed increased DNA damage signaling, as evidenced by increased phosphorylation of ATM and H2AX *in vivo* and *in vitro* and disturbed cell cycle progression [3]. These findings strengthen the link between DNA damage signaling and NPHP but fail to address the molecular mechanism of how enhanced DDR signaling is initiated in NPHP. Is DDR signaling activated by phosphorylation of proteins followed by repair and cell cycle arrest or are DNA breaks actually accumulating?

Replication stress at the root of the stalk

Recent studies with cells depleted for CEP164, SDCCAG8, and NEK8 are beginning to address the relation between the DDR and NPHP onset [1–3,5]. Mutation in one of the less-frequently mutated NPHP proteins, the ciliary kinase NEK8 (NPHP9), which associates with NPHP and polycystic kidney disease, results in replication stress as evidenced by accumulating DNA damage in S-phase cells and increased replication fork defects in mouse embryonic fibroblasts derived from *Nek8* mutant *jck* mice [1]. Replication stress is the slowing or stalling of the replication fork progression and/or DNA synthesis, which can lead to decreased cell survival and genome stability [1]. Stalled replication forks are unstable and can collapse, leading to double-strand break formation and chromosomal rearrangements. Moreover, immunohistochemistry of kidneys from 3-week-old *jck* mice shows higher basal levels of phosphorylated H2AX compared with wild type siblings [1], suggesting that these effects are early.

What are the molecular mechanisms linking NPHP proteins to DNA damage? Depletion of NEK8 results in DDR signaling through enhanced cyclin A-associated cyclin-dependent kinase (CDK) activity, leading to replication stress, and activated ATR–CHK1 signaling upon replication stress induction in S phase. Similarly, live-cell imaging of *CEP164*-depleted cells showed delayed S phase progression that could be rescued with wild type but not *CEP164* patient alleles [5], suggesting that these variants are causative for cell cycle impairment in patients. Since cells in S phase do not have cilia, an increase of unciliated cells in S phase would mimic a loss-of-cilia condition (Figure 1). Ciliation was rescued in Nek8-depleted cells treated with CDK inhibitors, although architectural changes of 3D renal spheroids were not rescued, possibly due to cell migration defects [1]. It is important to note that, while NEK8 depletion results in enhanced CDK activity, the role of other NPHP proteins in CDK activity remains to be tested [1]. Furthermore, pharmaceutical reduction of CDK activity by inhibitors of CDK requires further investigation to determine its role in ameliorating cell cycle defects associated with depletion of other NPHP-proteins. Cell cycle progression can also be indirectly

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Box 1. Disease etiology of renal ciliopathies, ADPKD, and NPHP-RC

There are two classes of renal ciliopathies: the common ADPKD (MIM 173900) affecting adults and the rare, recessive pediatric/juvenile ciliopathies including NPHP (MIM 256100), Joubert syndrome (MIM 213300), Meckel–Grüber syndrome (MIM 249000), Senior–Løken syndrome (MIM 266900), and Bardet Biedel syndrome (MIM 615993), collectively referred to as NPHP-RCs. In ADPKD, kidney tissue is damaged by hyperproliferative cystic tissue with some fibrotic tissue, which continues to grow over the life of the patient. In NPHP-RC, cyst development does not always occur but when it does it is limited to the corticomedullary junction. Furthermore, NPHP-RC kidneys remain small throughout life and are primarily characterized by excessive interstitial fibrosis. To date, it is unknown how loss of NPHP genes leads to rapid development of fibrosis [5,8,9]. While genes affecting both diseases have been shown to localize to ciliary components, presumably exercising some local function to regulate cilia and/or their signaling, the distinct phenotypes of ADPKD and NPHP suggest different mechanisms. Extraciliary functions of gene products mutated in ciliopathies remain to be investigated for a role in disease pathophysiology; four NPHP genes – NEK8, CEP164, SDCCAG8, and ZNF423 – have been implicated in DDR signaling to date [1–3].

regulated through ciliary signaling mediated by growth factor receptors and mechanosensation [6]. Therefore, NPHP-associated DDR signaling could interfere with normal cell cycle progression via both nuclear DNA damage-associated cell cycle control and cilia-associated cell cycle control. While the details are far from complete, the present data support the notion that cell cycle disturbances consistent with replication stress are present in many ciliopathies [7].

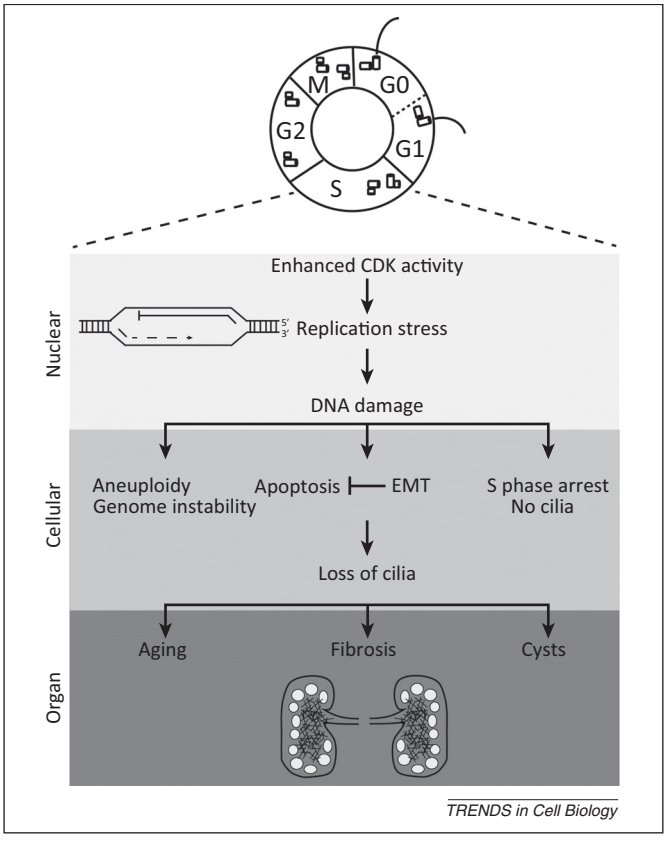

Figure 1. Overview of molecular signaling, cellular responses, and organ pathology in nephronophthisis-related ciliopathies (NPHP-RCs). The organ-, cellular-, and molecular/nuclear-level understanding of the pathophysiology leading to NPHP is illustrated. Besides the cilium, expressed in G_0 and G_1, replication stress in S phase is central in NPHP-RC development. Abbreviations: DDR, DNA damage response; CDK, cyclin-dependent kinase; EMT, epithelial-to-mesenchymal transition.

Why excessive fibrosis?

Cyst formation is attributed to loss of primary cilia, planar cell polarity defects, and hyperproliferation [8]; however, molecular evidence linking cilia loss to the hallmark feature of NPHP, renal fibrosis, has been largely lacking. In addition to cell cycle progression defects, mutations in the NPHP gene *CEP164* appear to increase apoptosis and epithelial-to-mesenchymal transition (EMT) of renal inner medullary collecting duct cells (typically found at the corticomedullary border of the kidney), coupled with a profibrotic response in fibroblasts [5]. Similar regulatory functions are described for *GLIS2* (*NPHP7*) regarding apoptosis and fibrosis [9] (Figure 1). One recent study in myofibroblasts suggests that while the cilium is required for the initiation of EMT, cilia are lost thereafter, suggesting that the profibrotic tissue environment characterized by EMT and cilium loss requires two triggers: disassembly of cellular contacts and transforming growth factor beta (TGF-β) exposure [10].

The question remains: what causes NPHP-associated fibrosis? One explanation could be sought in the association of NPHP proteins with an enhanced DDR [1–3] coupled with apoptosis and fibrosis-associated EMT [5]. Linking ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies supports the suggestion that replication stress is complicit in the pathophysiology of NPHP-RCs. From the clinical perspective, this new hypothesis is interesting since, in contrast to cysts, replication stress and ciliation are reversible to a certain extent. Children diagnosed with NPHP-RC often have a 5–10-year window of therapeutic opportunity before requiring renal replacement therapy. The effect of CDK inhibition on renal fibrosis will still need to be thoroughly addressed, because fibrosis is the major damage inflicted on the kidney and is not typically reversible. The CDK inhibitor roscovitine has been shown to rescue renal cystic phenotypes in jck (*Nek8*/*Nphp9*), cpk (*Cys1*) [11], and *Pkd1* mouse models [12]. The mechanism of action investigated in these murine models suggests that roscovitine induces cell cycle arrest and transcriptional inhibition. Regulation of apoptosis by roscovitine remains disputed; nevertheless, apoptosis is decreased in jck and *Pkd1* mice [11,12]. However, the effect of CDK inhibition on primary cilia and fibrosis remains to be investigated in these murine models.

Concluding remarks

We contend that replication stress is an essential element of the origin of NPHP pathophysiology, which consequently results in decreased cell survival, EMT, and genome instability, leading to the fibrosis observed in NPHP (Figure 1 and Box 1). Loss of cilia in these 'ciliopathies' may therefore be partly or entirely a secondary effect of nuclear events leading to cell cycle retardation initiated by replication stress as a result of CDK activation. Accordingly, effectors of replication stress and the DDR in the nucleus partly overlap or interact with centrosomal

components. Many proteins have multiple localizations, and nuclei and cilia share molecular, structural, and mechanistic components that regulate import [13]; it is conceivable that NPHP proteins utilize the same import mechanism for both the nucleus and the cilium.

The roles of the replication stress response and DNA damage signaling in NPHP-RCs link this disease to inappropriate fibrosis and age-related diseases associated with DDR genes. We argue that DNA damage syndromes such as Seckel (MIM 210600) may be closer in etiology to NPHP-RCs than ADPKD. Some phenotypic overlap between Seckel syndrome and Joubert syndrome (JBTS) (MIM 213300), such as the characteristic cerebellar vermis hypoplasia ('molar tooth sign'), suggests a common etiology. A new avenue for NPHP therapeutic strategies focusing on the upstream molecular signaling defects, such as CDK1/2 inhibitors, could therefore be justified [11,12,14]. Rescue of the ATR–CHK1 DDR may also be a viable option when determining targets for fibrosis and age-related characteristics. One extra allele of *Chk1* prolonged the survival of ATR–Seckel mice [15]. Differences in extraciliary protein functions among the NPHP proteins might be at least part of the explanation of why the different ciliopathies have different clinical manifestations. In addition, the extent of cilia and nuclear dysfunction could be related to the variability between ciliopathies. Looking beyond the cilium and focusing on alternative mechanisms of disease etiology is opening an exciting new chapter for 'ciliopathy' research.

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