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Lessons from microRNA biology: Top key cellular drivers of Autosomal Dominant Polycystic Kidney Disease

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ABSTRACT

Background: Numerous microRNAs (miRs), small RNAs targeting several pathways, have been implicated in the development of Autosomal Dominant Polycystic Kidney Disease (ADPKD), which is the most common genetic cause of Chronic Kidney Disease. The hallmark of ADPKD is tissue overgrowth and hyperproliferation, eventually leading to kidney failure.

Scope of the review: Many miRs are dysregulated in disease, yet the intracellular pathways regulated by these are less well described in ADPKD. Here, I summarise all the differentially expressed miRs and highlight the top miR-regulated cellular driver of ADPKD.

Major conclusions: Literature review has identified 35 abnormally expressed miRs in ADPKD. By performing bioinformatics analysis of their target genes I present 10 key intracellular pathways that drive ADPKD progression. The top key drivers are divided into three main areas: (i) hyperproliferation and the role of JAK/STAT and PI3K pathways (ii) DNA damage and (iii) inflammation and NFκB.

General significance: The description of the 10 top cellular drivers of ADPKD, derived by analysis of miR signatures, is of paramount importance in better understanding the key processes resulting in pathophysiological changes that underlie disease.

1. Introduction and scope of review

Is more (data) always better? The answer could be somewhere in the middle. There are times that more data is essential to better understand complex pathogenicity. Examples of this include the 'Cancer Genome Atlas', amongst other important initiatives, which has provided a significantly improved understanding of many cancers. Yet other times having access to a lot of data is not necessarily optimal. According to Professor Sydney Brenner '*We are drowning in a sea of data and thirsting for some theoretical framework with which to understand it*' [1]. Within the field of ADPKD research, which is rapidly expanding, we have generated a vast amount of data. While this is an exciting and essential step towards gaining mechanistic insight into the pathways that lead to disease, it is also clear that we need to put more effort into understanding the data produced with the scope of creating new theory. To begin to understand the extend of miRs dysregulation in ADPKD, I have generated an up-to-date synopsis of all miRs discovered to be differentially regulated in ADPKD by several groups world-wide (Table 1). From this I have extracted key information, in the form of 10 key top intercellular drivers of ADPKD, which can be seen Fig. 1. What is currently known about these 10 top pathways in the context of ADPKD is discussed in subsequent sections.

2. ADPKD

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common genetic disorder affecting the kidneys, often leading to kidney failure by middle age [2]. However, ADPKD is not only a kidney disease, but it can also affect liver and vascular function (extra-renal manifestations) [3]. Patients with ADPKD have no curative medicines and as such they have to rely on either kidney transplantation or lifelong dialysis for survival, both of which have their own limitations and disadvantages. Tolvaptan (a vasopressin V2 receptor antagonist) was recently approved for some patients with ADPKD and is shown to slow down disease progression [4]. As such tolvaptan has provided the first, and much needed, evidence that ADPKD progression can be slowed down with appropriate pathway targeting [5]. However, tolvaptan, at the doses used, can cause lethal side effects such as liver toxicity [6],

Abbreviations: ADPKD, Autosomal Dominant Polycystic Kidney Disease; JAK/STAT, JAnus Kinase / Signal Transducers and Activators of Transcription.

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Table 1
Summary table of all miRs that are reported to be dysregulated in ADPKD. A summary of all the published miRs that are either upregulated or downregulated in ADPKD models of disease.

microRNA name	Up or down	In vitro (1), in vivo (2), both (3)	Species/ model	Methods	Study details
miR-16	Up	2	Human plasma	qPCR	Kulesza A et al, 2021 [13]
miR-192-5p, 194-5p, miR-30a-5p, miR-30d-5p, miR-30e-5p	Down	3	Human urine exosome PKD1nl/nl	RNA-seq	Magayr TA et al, 2020 [14]
miR-214	Up	3	Ksp-Cre, Pkd1fl/ flPkd12fl/fl	Validation of previously identified microRNA	Lakhia R et al, 2020 [15]
miR-17-5p, miR-18a-5p, miR-19a, miR-20a-5p, miR-19b-3p	Up	2	KspCre/ Pkd1F/RC	qPCR	Yheskel M et al, 2019 [16]
miR-192-5p, miR-194-3p or 5p	Down	3	Human kidney, mouse Pkd1f/f; HoxB7-cre	Microarrays (Agilent human microarray - G4471A-021827)	Kim Y et al, 2019 [17]
miR-501-5p	Up	1	Multiple human cell lines	Microarrays (Agilent human microarray - G4470B)	de Stephanis L et al, 2018 [18]
miR-20b-5p, miR-106-5p	Down	2	Mouse - Pkd2F/F	Validation of previously identified microRNAs (study 9)	Shin Y et al, 2018 [19]
up: miR-3907, miR-92a-3p, miR-25-3p miR-21-5p and down: miR-1587 miR-3911	Both	2	Human serum	384 subset qPCR analysis	Kocyigit I et al, 2017 [20]
miR-582-5p, miR-660, miR-193b, miR-182a-2, miR-1228	Down	1	Human ADPKD cell lines	Microarrays (SurePrint G3 Human Gene Expression 8 × 60-K)	Streets A et al, 2017 [21]
miR-17 family	Up	2	Mouse-Ksp/Cre; Pkd1F/F & Pkhd1/Cre; Pkd2F/F	Microarrays validated by in situ hybridization	Hajarnis S et al, 2017 [10]
miR-21	Up	2	Ksp/Cre; Pkd1F/F & Pkhd1/Cre; Pkd2F/F	In situ validation of previous publication	Lakhia R et al, 2016 [22]
miR-199a-5p	Up	3	Human ADPKD tissues and OX161 cells	qPCR of a single miRNA without justification	Sun L et al, 2015 [23]
Up: miR-429, miR-96, miR-182, miR-30a-5p, down: miR-10a, miR-126-5p	Both	2	Mouse Pkd1-null (E 14.5 and E17.5)	Agilent nanochips/ systems biology approach	Pandey P et al, 2011 [24]
miR-17 ~ 92	Up	2	Non ADPKD (Kif-3a-KO)	LC Science microarray	Patel V et al, 2013 [25]

leading to liver failure. As such new treatments are needed, discovery of which depends on continued primary research combined with thorough analysis of published work in a systematic way.

3. microRNAs and their signatures

MiRs are endogenous small (~22 nt) RNAs with ability to target and modulate several genes and as such they have key roles in maintaining health and homeostasis [7]. Thus, it is not surprising that changes in miR expression are associated with several diseases, including ADPKD [8]. Moreover, certain miRs act as biomarkers of disease progression [9]. Finally, miRs have attracted interest because they themselves can be good therapeutic targets [10]. miRs enforce post-transcriptional silencing through the RNA interference pathway [11]. As such miR biology is currently expanding in all fields of research from cancer to chronic kidney disease. Several databases have been generated to dissect the potential roles of miRs. Some of these databases identify miR targets based on computational predictions, which must later be experimentally validated. A good example is MiRTarBase 2020, which is a curated database of miR-mRNA targets with experimental validation. As such mirTarBase is a tool to dissect miR-pathology relationship using pre-existing experimentally validated data. Here, I have performed a literature review of all the published miRs (Table 1) that are either up or down regulated in ADPKD, which resulted in a total of 35 differentially expressed miRs. I then identified the experimentally validated gene targets of these miRs using the miRTarBase platform. This approach generated a list of over 700 potentially altered genes in ADPKD. Using this gene list, I performed hallmark pathway analysis (using the UC San Diego MSigDB collection from the broad institute) [12] to select the most robust targets (Fig. 1). This approach identified three main areas for further analysis (i) inflammation via NFκB (ii) DNA-damage/ apoptosis via p53 and (iii) hyperproliferation via PI3K and STAT5. I then go on to discuss what we know about these top three intracellular drivers in the ever-evolving field of ADPKD.

4. Hyperproliferation and the PI3K and STAT5 pathways

Proliferation, a process whereby a cell grows and divides to produce two daughter cells, is tightly entangled with PKD disease progression. Cell proliferation leads to an increase in cell number and is therefore a mechanism for tissue growth. When cells are proliferating, they control the cellular growth to maintain an approximately constant cell size [26]. Cell cycle (transition from G1 to S to G2 to M) is a cellular process that allows a cell to divide [27]. It is driven by proteins called Cyclin Dependent Kinases (CDKs) that associate with relevant cyclin regulatory proteins at different points of the cell cycle [28]. These specific time points are known as checkpoints, some of which have been reported to be defective in ADPKD [29]. G2-M checkpoint is the specific time during cell division that DNA damage is detected [30]. If DNA damage is detected, then a cascade of events is triggered (some of these events are

discussed in the DNA damage section of this review). In the kidney, hyper-proliferation of tubular cells induces renal cyst formation which can be considered a form of tissue overgrowth syndrome. A reason for the increased proliferation in ADPKD can be: (i) directly related to the loss of the Pkd gene function and the dysregulation in any Pkd-regulated genes and/or (ii) indirectly related to incorrect localisation of Pkd1 also resulting in dysregulation of Pkd1 target genes. Most of the animal and cellular models of ADPKD use the first of these two (i.e. reduced or mutant PKD expression). Yet, it is known that correct localisation of the Pkd1 protein, specifically within the primary cilium, is required to avoid overproliferation [29]. PKD1 is the gene most frequently mutated giving rise to ADPKD. A number of miRs are predicted to regulate PKD1, including Mir-615-3p, miR-484, miR-324-5p and miR-200b-3p (MiRarBase). Patel and colleagues showed that miR-200b/c/429 induce post-transcriptional repression of PKD1 by binding to two conserved 3′ untranslated regions (UTRs) of the Pkd1 gene [31]. Another miR targeting Pkd1 is miR-20, where it was found that the Pkd1-miR-20 interaction may be a basis for cystogenesis [32].

Taken together appropriate cell division is a tightly controlled multi-step process that involves many proteins including PKD1, incorrect control of this process can lead to tissue overgrowth such as that observed in the kidneys of people with ADPKD.

4.1. PI3K in ADPKD

Phosphoinositide 3-kinase (PI3Ks) are a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, and survival. PI3Ks phosphorylate the 3rd position of the hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns) [33]. These enzymes fall under four distinct classes: class I, II, III, and IV and each enzyme belongs to one category based on primary structure, regulation, and lipid substrate specificity [34]. A simplified way of pathway activation involves G-coupled receptor (GPCR) activation followed by PI3K mediated (PIP3) activation of mTORC2 leading to activation and plasma membrane translation of the central AKT protein [35]. AKT is a serine/threonine kinase and a proto-oncogene, while Phosphatase and TENsin homolog (PTEN) is a protein that acts as an inhibitor of AKT and is considered to be a tumour suppressor [36]. Interestingly, PTEN is one of the most frequently mutated tumour suppressor genes [37]. Activated AKT leads to activation of the mammalian target of rapamycin (mTOR) [38], which is overactive in many cancers. Dysregulation of PI3K/AKT pathway is implicated in the pathogenicity of several human over-proliferation related diseases including cystic kidney disease. Because of its central role mTOR and mTOR inhibition (with Sirolimus) have become the focus of a number of studies, some showing promising initial results including: (i) mTOR was inappropriately activated in cyst lining cells and (ii) that its inhibition with Sirolimus resulted in a staggering 50% reduction in cystic index in mice with PKD [39]. Guided by these initial observations, two mTOR inhibitors, namely Sirolimus [40] and Everolimus [41], have been trialled in the clinical setting in patients with ADPKD. Both drugs showed no overall success in preventing kidney function decline or significantly reducing cyst size [40,41] (in clinical trials of 18 months or 2 years duration). Whether combination treatment of mTOR inhibition (at lower dosages) with other drugs may be of benefit is currently debated but essentially unknown. The fact that the PI3K pathway is overactive in cancers has led to huge efforts to generate new PI3K inhibitors. Some new inhibitors have received regulatory approval, including the PI3Kα isoform-selective inhibitor *alpelisib* [42,43], but the effectiveness of these new drugs has not been studied in the setting of ADPKD. An alternative way to target PI3K and mTOR pathways is via using a molecular targeted approach, by for example using mimics and inhibitors of miRs that regulate these pathways delivered by modified mRNA or adenovirus. While it is clear that a number of miRs can act upon and alter the activities of PTEN, PI3K and mTOR, amongst them miR-21 has been shown by many researchers to be a common miR that targets this pathway [44–46]. miR-21 is interesting as it has been found to play a role in ADPKD, specifically it has been shown to aggravate cystic growth [47]. The role of miR-21 in ADPKD has been reviewed previously by Yheskel and Patel [48] and Fragiadaki, Macleod and Ong [5]. Taken together, it clear that the PI3K/AKT is abnormally activated in ADPKD and drives excessive proliferation, whether inhibition along this axis may prove to be beneficial remains to be uncovered in the future.

4.2. JAK2/STAT5 in ADPKD

JAnus Kinase (JAK) and Signal Transducers and Activators of Transcription (STAT) is an evolutionarily conserved pathway mediating key

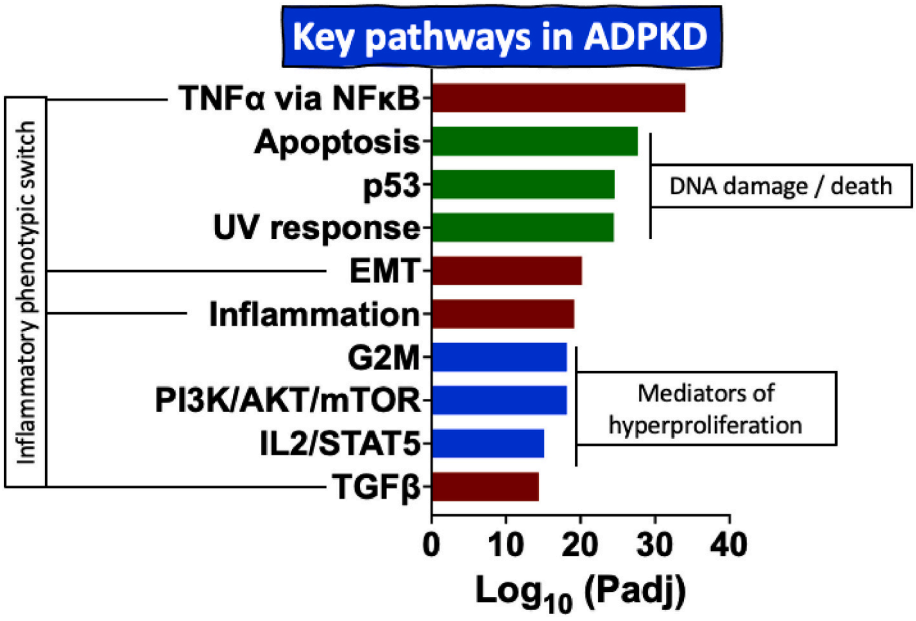

Fig. 1. Key top drivers of ADPKD. GSEA hallmark pathway analysis identified 10 most statistically significantly altered pathways associated with ADPKD. These 10 key drivers of disease were cluster in three groups (1) hyperproliferation and the roles of PI3K and STAT5, (2) DNA damage driving cell death and (3) key mediators of cellular hyperproliferation inflammatory phenotypic switching.

roles in cell proliferation, differentiation and immunity. The pathway is comprised of 4 tyrosine kinases (JAK1–3 and tyk2) and 7 transcription factors (STAT1-5a, STAT5b and STAT6), which possess a dual role (signal transducers and activators of transcription). Upon cytokine (e.g. oncostatin M) or hormonal activation (e.g. erythropoietin) of a relevant cytokine receptor (e.g. OSMR, EPOR) a conformational change takes place enabling the JAK2 kinases to come into close physical contact and transphosphorylase one another as well as phosphorylate the intracellular portion of the receptor they are associated with (e.g. EPOR). Once the intracellular portion of the receptor becomes phosphorylate it generates a docking site for the recruitment of the latent STAT transcription factors. Upon recruitment STATs become phosphorylated, which allows them to dimerise and move into the nucleus where they act as canonical transcription factors. Once in the nucleus STATs orchestrate the expression of several genes involved in proliferation (e.g. CCDN1, MCL1, MYC, Suvivin, BCL-XL). Of interest, components of the JAK/STAT pathway are abnormally activated in a number of cancers [49]. Notably, a gain-of-function mutation in the JAK2 kinase (JAK2 V617F) is known to cause polycythaemia vera, a type of blood cancer [50,51]. Moreover, STAT3 is found elevated in several solid tumours and as such STAT3 targeting has been proposed as a cancer immunotherapy [52]. Amongst the JAK/STAT family members perhaps the ones with most relevance to tissue growth are two transcription factors STAT5a/b. Indeed, STAT5b knockout animals exhibit impaired growth due to loss of growth hormone responsiveness [53], while mutations within STAT5b also leads to growth hormone insensitivity [54] and result in short stature. Epigenetic silencing of the *STAT5A* gene, and STAT5A protein, is essential for oncogenesis as it permits uninterrupted transcription of MPM1-ALK [55], identifying STAT5A as a tumour suppressor gene. Moreover, it was shown that loss of STAT5A leads to tumour growth through miR-23a which activates the AKT pathway, suggesting a cross-talk between JAK/STAT and ATK in controlling cancer tissue growth mediated by miRs [56]. A number of miRs have been found to directly target STAT5 including miR-221 [57] and miR-1469 [58].

Several groups have reported that the expression and/or nuclear translocation of other members of the STAT family is elevated in ADPKD mouse and cellular models of disease [59–61]. Specifically, STAT6 was shown to be activated in murine ADPKD cystic epithelium and its genetic deletion leads to improved kidney function and reduced cystic growth [62]. Interestingly, it was shown that STAT3 and STAT6 co-localise with PKD1/2 at primary cilia and co-migrate to the nucleus in the absence of urinary flow [63]. Importantly, using genetic deletion of STAT3 in PKD1 deficient cells, it was shown that STAT3 is not a critical mediator of cyst growth, but rather STAT3 limits renal inflammation in ADPKD [64]. It follows that inhibition of STAT3 is predicted to have a long-term negative effect in ADPKD as prolonged inflammation (e.g. triggered by STAT3 blockade) may exacerbate kidney damage over time. Moreover, it has been shown that JAK2 physically interacts with PKD1 to promote cell cycle inhibition by activating STAT1 which in turn lead to activation of the cell cycle inhibitor p21 [65], suggesting a potentially protective role for STAT1 and its target genes in ADPKD.

Given the fact that many groups have reported altered JAK/STAT activity in ADPKD, our group took an unbiased approach. By performing siRNA screening we deciphered which of the core JAK/STAT components control cell proliferation in ADPKD. We identified that a number of JAK/STAT family members participate in proliferation in ADPKD-derived cells. Amongst them, JAK2/STAT5 was identified as strong positive regulators of proliferation in murine and human cellular models of ADPKD [66]. This is consistent with previous reports identifying STAT5 as a pro-proliferative gene promoting cell growth and an anti-apoptotic gene signature [67]. This was also consistent with previous findings showing that growth hormone activates STAT5 to coordinate body growth [68]. We showed that in addition to STAT5, JAK2 - the tyrosine kinase upstream of STAT5 - is highly elevated in cellular models of ADPKD and its pharmacological inhibition (using curcumin or tofacitinib) led to decreased cystogenesis [69]. A number of miRs have been reported to control STAT5, including miR-204 which was shown to inhibit proliferation of cells and to target the 3′ UTR of STAT5 [70]. Likewise, STAT5 can transcriptionally control the expression of a number of miRs. Interestingly, genome wide analysis revealed the miR-17/92 cluster as a STAT5 target [71]. Moreover, STAT5 was found to positively control miR-21 in vitro and in vivo [72]. It is interesting that both miR-17 and miR-21 are under the control of STAT5, as both of these miRs are elevated in ADPKD and participate in cyst growth [10,22,25]. Taken together, the cell cycle control pathway JAK/STAT is overactive in ADPKD and may explain some of the tissue overgrowth phenotypes seen in ADPKD.

5. DNA damage response (DDR)

Each one of the cells of the human body is subjected to thousands of DNA lesions per day. These alterations can result in stalled genome replication and/or transcription, which could predispose cells to genomic instability with detrimental consequences. Genome instability is the tendency of the genome to undergo permanent and transmittable mutations of the DNA [73]. Given that our nuclear DNA has only two copies for each of our genes, its integrity can only be maintained via constant DNA repair, since it cannot be re-made from a previous copy. To overcome this inherent problem, we have evolved an elaborate network of highly coordinated proteins that work together to ensure genome stability and are known as the DNA repair and Damage Response (DDR) [74]. On a daily basis DNA lesions arise due to several challenges including genetic and environmental stimuli as well as due the natural process of aging. Aging is a complex and multifaceted process leading to functional tissue decline [75] and is therefore the cause of most chronic diseases resulting a major burden worldwide. Ultraviolet light (UV) for example is a ubiquitous and pervasive environmental DNA-damaging agent, which results in transcriptional changes that activate DDR. The DDR then in turn triggers the activation of a combination of mechanisms that are designed to (i) recognise the DNA damage, (ii) recruit mediators and effectors and (iii) execute a cellular response (which may include apoptosis, repair, senescence, or cell transdifferentiation). There are several initial steps in the DDR, including the phosphorylation of H2AX (γH2AX) which is involved in the early steps leading to chromatin decondensation after DNA double-strand breaks have been detected [76–78]. Following rapid chromatin remodeling two kinases, ATM and ATR, are activated within minutes after DNA is damaged [79]. ATM responds to DNA double-strand breaks and/or disruptions in chromatin structure, while ATR primarily responds to accumulation of single-stranded DNA (ssDNA), which can be generated due to prolonged stalling of replication forks [80,81]. The overall outcome is that the combined effect of ATM and ATR is the activation of downstream signaling leading to cell cycle arrest [82]. P53 is an important downstream target of ATM and ATR, and its activation is required for triggering apoptosis following detection of DNA damage [30]. P53 is a tumour suppressor, sometimes referred to as ‘the guardian of the genome’ that is at the interconnection of several cellular pathways that sense DNA damage, senescence, and cellular stress [83,84]. The role of this important gene is to integrate such signals and by doing so to promote either growth arrest, apoptosis or DNA repair in a context-dependent manner [83,85]. Given its central role in genome stability the regulators of p53 have been studied extensively. Amongst these miRs

have been found to both regulate p53 [86,87] as well as p53 is found to regulate cellular processes by altering certain miR clusters [88]. For example, p53 has been shown to induce the expression of miR-192 resulting in the cell cycle inhibitor p21 accumulation and cell cycle arrest [89]. Interestingly miR192 is one of the miRs that is differentially expressed in ADPKD (Table 1), yet its role in p21 mediated blockage of cell cycle has not yet been studied in ADPKD. Taken together, while DNA damage happens all the time, we have evolved elaborate mechanisms to correct these mistakes, some of the mechanisms of DDR involve key transcription factors such as p53 and miRs including miR-192.

A growing body of evidence suggests that DNA damage and activation of the DDR pathway is altered in ADPKD and other ciliopathies. Original evidence comes from analysing lymphocytes from patients with ADPKD in which the authors examined whether there is increased molecular or cytogenic damaged associated with this disease. Chromosome analysis identified that some ADPKD patients exhibited increased DNA damage after 0.5 Gy dose of gamma radiation [90], which is an ionizing radiation capable of breaking molecular bonds (unlike UV radiation). Intriguingly some ciliopathies including ADPKD have been associated with defects in DDR. Zhang and colleagues showed that in human kidneys with end-stage ADPKD there is activation of three markers of DNA damage, namely phosphorylated γ-H2AX, ATR and ATM [91]. A few years later, the same team also reported that two pharmacological inhibitors of ATM/ATR, namely AZD0156 and VE-821, reduced the cystic growth in cellular three-dimensional assays (using MDCKII cells and human cells). These drugs also led to a decrease in proliferation *in vivo* (measured by ki67) and an increase in the protective tumour suppressor p53 gene expression in the $Pkd1^{RC/RC}$ model after dosing with either 5 mg/kg or 20 mg/kg of AZD0156 for two weeks. Yet this treatment was not sufficient to reduce cystic growth or control kidney enlargement, partly owning to the short duration of the treatment. The authors did go on to genetically delete ATM, one of the DDR members, yet neither ATM heterozygosity nor ATM homozygosity were able to reduce the progression of kidney disease or suppress renal enlargement at three months after deletion, suggesting that ATM is not a suitable drug target at least in the $Pkd1^{RC/RC}$ model [92]. It is interesting to note that deletion of ATM did not worsen the kidney phenotype, as may have been expected given the cytoprotective role of the DDR pathway. Evidence of a more protective role for the DDR pathway in ciliopathies comes from Choi et al. who showed that NPHK9 (NEK8), a ciliary kinase associated with nephronophthisis (NPHP) and PKD, is a key effector of ATR-mediated replication stress response, while mutations in NPHP9 lead to accumulation of DNA damage and replication stress [93]. Taken together, Zhang J and colleagues have made the first interesting observation that parts of the DDR system are increased in ADPKD opening the field of DNA damage and repair in ADPKD. The DDR pathway is primarily considered a cytoprotective pathway, however given the literature from Zhang et al. it is clear that we do not fully understand the relative contributions of evading DNA damage repair especially in the context of a genetic disease.

A number of studies have investigated the potential role of p53 in ADPKD. Nishio and colleagues showed that cystic disease progression is associated with decreased levels of p53 activity, on the contrary PKD1 wild type cells show strong induction of p53. The authors found that p53 activity prevented wild type cells from becoming hyperproliferative [94]. More recently it was shown that in PKD1 mutant mice there was increased proliferation and cystic expansion partly via the actions of a deacetylase sirtuin 1 (sirt1) resulting in altered levels of cell death via a mechanism involving deacetylation of p53 [95]. The direct effect of loss of the disease-causing gene, Pkd1, on p53 levels was examined by Kim H and colleagues by using antisense oligonucleotides (ASO) to inhibit Pkd1 which led to a reduction of p53 in cells irradiated with UV light. The authors concluded that Pkd1 is involved in the regulation of cell cycle progression checkpoints [96]. Interestingly, miR-501-5p was found to be upregulated in ADPKD, this upregulation was linked with p53 ubiquitination and degradation via the proteosome [18]. Taken together several groups have suggested a strong link between Pkd1 inactivation and/or Pkd1 mutations and altered DDR activity with a focus on inactivation/reduction of p53-mediated signaling, this in turn suggests a strong link between cell cycle progression the Pkds and essential checkpoints.

6. NFκB as a driver of renal inflammation

Nuclear factor kappa B (NFκB) is a family of transcription factors that together regulate multiple aspects of immunity and as such are major mediators of inflammation [97]. The NFκB family is composed of five structurally related members, including NFκB1 (p50), NFκB2 (p52), RelA (p65), RelB and c-Rel. NFκB proteins are normally sequestered to the cytoplasm by a family of proteins known as IκB which are characterised by the presence of ankyrin repeats. The best-known member of the IκB family is IκBa, which contains 6 ankyrin repeats [98]. Upon activation, such via the TNF receptor, IκBa becomes ubiquitinated and degraded allowing rapid and transient nuclear translocation of NFκB subunits leading to fast activation of inflammatory genes (such as IL1, Il6 and IL12, MCP1, RANTES and CXCL1). Menon V and colleagues have shown that in 50 patients with ADPKD there is a significant increase in the inflammatory cytokine IL6 amongst ADPKD patients, potentially suggesting abnormal NFκB activity in this population of patients [99]. Indeed, several NFκB subunits were found to be highly expressed in human and rodent models of PKD. Ta M and colleagues found that p105, p65, p50, cRel and RelB were all present in cystic kidneys and were phosphorylated, thus activated [100]. They also found elevated MCP1 levels consistent with transcriptionally active NFκB. Given that NFκB is activated in ADPKD the question becomes what triggers aberrant NFκB activity? Since TNFα is a major activator of NFκB pathway, the level of TNFα has been studied in murine and human models of ADPKD. Elevated TNFα was first demonstrated to play a role in patients with ADPKD by Gardner and colleagues. They performed ELISA and found elevated TNFα (as well as IL1β, IL2 and PGE_2) [101]. More recently, TNFα was shown to trigger increased id2 protein expression resulting in a decrease in the cell cycle inhibitor p21, further implicating the TNF pathway in the control of cell cycle in the polycystic kidney [102]. Given the pathogenic activation of NFκB subunits in ADPKD, it follows that NFκB inhibition may offer a therapeutic benefit. While specific NFκB knockout animals have not yet been studied in ADPKD, a dietary approach has been reported. Specifically, treatment with resveratrol, a natural phenol found in the skin of grapes and blueberries, was able to reduce TNFα levels in turn resulting in decreased p50/p65 activity in male Han:SPRD (cy/+) rats with PKD [103] leading to reduced cyst growth in cellular models of ADPKDs and in a Zebrafish model. As such a pathogenic role of abnormal activation of NFκB subunits in ADPKD has been established. Given the important functions of the NFκB subunits in the control of inflammation and immunity over 750 inhibitors of the NFκB pathway have been identified [104], some of which are tested in animal models [105]. While several of these inhibitors generally block NFκB activity, some inhibit specific pathways of induction, and it is these inhibitors that may be important to study in the context of ADPKD. Taken together, inflammation especially via abnormally increased activity of the NFκB has been demonstrated in ADPKD and it remains to be established whether selective subunit inhibition may be of benefit in this disease setting.

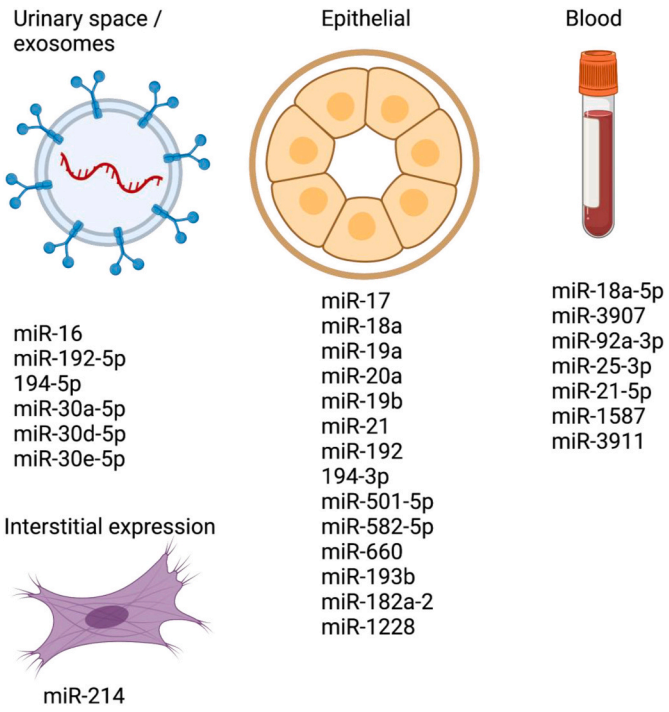

Fig. 2. Expression of key microRNAs in ADPKD. The majority of the microRNAs were discovered either by sequencing (or microarray analysis) using (a) urine or exosomes, (b) kidney lysates/epithelial lysates or (c) blood, with serum being the commonest form used from the studies. The exception is miR-214 whose expression is found in the interstitium, where it is thought to suppress inflammation via inhibition of the toll like receptor 4 (tlr4) [15].

7. Concluding comments

Taken together, analysis of the 35 differentially altered miRs resulting in over 700 altered genes in ADPKD has identified three key pathways that are critical drivers of cystogenesis: (i) proliferation, (ii) DNA damage and repair and (iii) inflammation. The analysis was done on miRs that are differentially regulated in disease, the majority of which are expressed in renal epithelial cells (Fig. 2). The enormous variety of genes regulated by miRs (e.g. proliferation, inflammation and DDR related genes) in all animals reflects the widespread importance of the RNA interference pathway utilised by miRs. Indeed, increasing our knowledge of how miRs interact with their target genes to promote or inhibit disease via altering cellular processes is important in our attempt to better understand critical points in the disease process(es) where intervention may be of benefit. The fast pace at which dysregulated miRs and the pathways they affect have been discovered in all fields of research, including ADPKD, gives hope that one day we will accurately integrated miR function into models of disease, an initiative that may also lead to therapies. A good example of this is the anti-miR-17 oligonucleotide RGLS4326, which shows very promising initial results [106]. It is not surprising that proliferation, inflammation and DNA damage are key drivers of ADPKD, as they also have very well-established roles in a variety of other pathologies. Now that these pathways have been identified as key drivers the challenge will become to find the best ways to selectively suppress pathogenic arms of these processes with the hope to extend renal function without causing detrimental systemic side effects.

Declaration of competing interest

The author declares the following financial interests/personal relationships which may be considered as potential competing interests:

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References

[1] S. Brenner, Turing centenary: life's code script, Nature 482 (2012) 461, https://doi.org/10.1038/482461a.
[2] C.L. Chow, A.C. Ong, Autosomal dominant polycystic kidney disease, Clin. Med. (Lond.) 9 (2009) 278–283, https://doi.org/10.7861/clinmedicine.9-3-278.
[3] R.L. Luciano, N.K. Dahl, Extra-renal manifestations of autosomal dominant polycystic kidney disease (ADPKD): considerations for routine screening and management, Nephrol. Dial. Transplant. 29 (2014) 247–254, https://doi.org/10.1093/ndt/gft437.
[4] V.E. Torres, et al., Tolvaptan in patients with autosomal dominant polycystic kidney disease, N. Engl. J. Med. 367 (2012) 2407–2418, https://doi.org/10.1056/NEJMoa1205511.
[5] M. Fragiadaki, F.M. Macleod, A.C.M. Ong, The controversial role of fibrosis in autosomal dominant polycystic kidney disease, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21238936.
[6] J.L. Woodhead, et al., Application of a mechanistic model to evaluate putative mechanisms of tolvaptan drug-induced liver injury and identify patient susceptibility factors, Toxicol. Sci. 155 (2017) 61–74, https://doi.org/10.1093/toxsci/kfw193.
[7] N.C. Lau, L.P. Lim, E.G. Weinstein, D.P. Bartel, An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans, Science 294 (2001) 858–862, https://doi.org/10.1126/science.1065062.
[8] A. Abak, S. Amini, E. Sakhinia, A. Abhari, MicroRNA-221: biogenesis, function and signatures in human cancers, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 3094–3117, https://doi.org/10.26355/eurrev_201805_15069.
[9] P.S. Mitchell, et al., Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 10513–10518, https://doi.org/10.1073/pnas.0804549105.
[10] S. Hajarnis, et al., microRNA-17 family promotes polycystic kidney disease progression through modulation of mitochondrial metabolism, Nat. Commun. 8 (2017) 14395, https://doi.org/10.1038/ncomms14395.
[11] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297, https://doi.org/10.1016/s0092-8674(04)00045-5.
[12] A. Liberzon, et al., The Molecular Signatures Database (MSigDB) hallmark gene set collection, Cell Syst. 1 (2015) 417–425, https://doi.org/10.1016/j.cels.2015.12.004.
[13] A. Kulesza, et al., miRNA-16 as a predictive factor for intracranial aneurysms in autosomal dominant polycystic kidney disease, Neurol. Neurochir. Pol. 55 (2021) 306–309, https://doi.org/10.5603/PJNNS.a2021.0026.

[14] T.A. Magayr, et al., Global microRNA profiling in human urinary exosomes reveals novel disease biomarkers and cellular pathways for autosomal dominant polycystic kidney disease, Kidney Int. 98 (2020) 420–435, https://doi.org/10.1016/j.kint.2020.02.008.
[15] R. Lakhia, et al., Interstitial microRNA miR-214 attenuates inflammation and polycystic kidney disease progression, JCI Insight 5 (2020), https://doi.org/10.1172/jci.insight.133785.
[16] M. Yheskel, R. Lakhia, P. Cobo-Stark, A. Flaten, V. Patel, Anti-microRNA screen uncovers miR-17 family within miR-17~92 cluster as the primary driver of kidney cyst growth, Sci. Rep. 9 (2019) 1920, https://doi.org/10.1038/s41598-019-38566-y.
[17] D.Y. Kim, et al., Impact of miR-192 and miR-194 on cyst enlargement through EMT in autosomal dominant polycystic kidney disease, FASEB J. 33 (2019) 2870–2884, https://doi.org/10.1096/fj.201800563RR.
[18] L. de Stephanis, et al., MicroRNA501-5p induces p53 proteasome degradation through the activation of the mTOR/MDM2 pathway in ADPKD cells, J. Cell. Physiol. 233 (2018) 6911–6924, https://doi.org/10.1002/jcp.26473.
[19] Y. Shin, D.Y. Kim, J.Y. Ko, Y.M. Woo, J.H. Park, Regulation of KLF12 by microRNA-20b and microRNA-106a in cystogenesis, FASEB J. 32 (2018) 3574–3582, https://doi.org/10.1096/fj.201700923R.
[20] I. Kocyigit, et al., Serum micro-rna profiles in patients with autosomal dominant polycystic kidney disease according to hypertension and renal function, BMC Nephrol. 18 (2017) 179, https://doi.org/10.1186/s12882-017-0600-z.
[21] A.J. Streets, et al., Parallel microarray profiling identifies ErbB4 as a determinant of cyst growth in ADPKD and a prognostic biomarker for disease progression, Am. J. Physiol. Renal Physiol. 312 (2017) F577–F588, https://doi.org/10.1152/ajprenal.00607.2016.
[22] R. Lakhia, et al., MicroRNA-21 aggravates cyst growth in a model of polycystic kidney disease, J. Am. Soc. Nephrol. 27 (2016) 2319–2330, https://doi.org/10.1681/ASN.2015060634.
[23] L. Sun, et al., Inhibition of MiR-199a-5p reduced cell proliferation in autosomal dominant polycystic kidney disease through targeting CDKN1C, Med. Sci. Monit. 21 (2015) 195–200, https://doi.org/10.12659/MSM.892141.
[24] P. Pandey, S. Qin, J. Ho, J. Zhou, J.A. Kreidberg, Systems biology approach to identify transcriptome reprogramming and candidate microRNA targets during the progression of polycystic kidney disease, BMC Syst. Biol. 5 (2011) 56, https://doi.org/10.1186/1752-0509-5-56.
[25] V. Patel, et al., miR-17~92 miRNA cluster promotes kidney cyst growth in polycystic kidney disease, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 10765–10770, https://doi.org/10.1073/pnas.1301693110.
[26] R.J. A, et al., Cell-size dependent progression of the cell cycle creates homeostasis and flexibility of plant cell size, Nat. Commun. 8 (2017) 15060, https://doi.org/10.1038/ncomms15060.
[27] C.J. Sherr, J.M. Roberts, CDK inhibitors: positive and negative regulators of G1-phase progression, Genes Dev. 13 (1999) 1501–1512, https://doi.org/10.1101/gad.13.12.1501.
[28] C.J. Sherr, G1 phase progression: cycling on cue, Cell 79 (1994) 551–555, https://doi.org/10.1016/0092-8674(94)90540-1.
[29] F. Hildebrandt, T. Benzing, N. Katsanis, Ciliopathies, N. Engl. J. Med. 364 (2011) 1533–1543, https://doi.org/10.1056/NEJMra1010172.
[30] W.R. Taylor, G.R. Stark, Regulation of the G2/M transition by p53, Oncogene 20 (2001) 1803–1815, https://doi.org/10.1038/sj.onc.1204252.
[31] V. Patel, et al., MicroRNAs regulate renal tubule maturation through modulation of Pkd1, J. Am. Soc. Nephrol. 23 (2012) 1941–1948, https://doi.org/10.1681/ASN.2012030321.
[32] M.P. Bartram, et al., Conditional loss of kidney microRNAs results in congenital anomalies of the kidney and urinary tract (CAKUT), J. Mol. Med. (Berl.) 91 (2013) 739–748, https://doi.org/10.1007/s00109-013-1000-x.
[33] M.P. Wymann, L. Pirola, Structure and function of phosphoinositide 3-kinases, Biochim. Biophys. Acta 1436 (1998) 127–150, https://doi.org/10.1016/s0005-2760(98)00139-8.
[34] D. Liu, P. Hou, Z. Liu, G. Wu, M. Xing, Genetic alterations in the phosphoinositide 3-kinase/Akt signaling pathway confer sensitivity of thyroid cancer cells to therapeutic targeting of Akt and mammalian target of rapamycin, Cancer Res. 69 (2009) 7311–7319, https://doi.org/10.1158/0008-5472.CAN-09-1077.
[35] M.E. Feldman, et al., Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2, PLoS Biol. 7 (2009), e38, https://doi.org/10.1371/journal.pbio.1000038.
[36] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501, https://doi.org/10.1038/nrc839.
[37] J.R. Molina, et al., PTEN, NHERF1 and PHLPP form a tumor suppressor network that is disabled in glioblastoma, Oncogene 31 (2012) 1264–1274, https://doi.org/10.1038/onc.2011.324.
[38] A. Hahn-Windgassen, et al., Akt activates the mammalian target of rapamycin by regulating cellular ATP level and AMPK activity, J. Biol. Chem. 280 (2005) 32081–32089, https://doi.org/10.1074/jbc.M502876200.
[39] J.M. Shillingford, et al., The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 5466–5471, https://doi.org/10.1073/pnas.0509694103.
[40] A.L. Serra, et al., Sirolimus and kidney growth in autosomal dominant polycystic kidney disease, New Engl. J. Med. 363 (2010) 820–829, https://doi.org/10.1056/NEJMoa0907419.
[41] G. Walz, et al., Everolimus in patients with autosomal dominant polycystic kidney disease, N. Engl. J. Med. 363 (2010) 830–840, https://doi.org/10.1056/NEJMoa1003491.
[42] F. Andre, et al., Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer, N. Engl. J. Med. 380 (2019) 1929–1940, https://doi.org/10.1056/NEJMoa1813904.
[43] I.A. Mayer, et al., A phase ib study of alpelisib (BYL719), a PI3Kalpha-specific inhibitor, with letrozole in ER+/HER2- metastatic breast cancer, Clin. Cancer Res. 23 (2017) 26–34, https://doi.org/10.1158/1078-0432.CCR-16-0134.
[44] L.J. Bai, et al., MicroRNA-21 regulates PI3K/Akt/mTOR signaling by targeting TGF beta I during muscle development in pigs, Plos One 10 (2015), e0119396, https://doi.org/10.1371/journal.pone.0119396.
[45] L. Fang, M. Tamm, D. Stolz, M. Roth, Supportive interaction of PI3K-akt-mTOR and STAT3-miR21-PTEN controls IgE induced airway remodeling, Eur. Respir. J. 52 (2018), https://doi.org/10.1183/13993003.congress-2018.PA2179.
[46] B.H. Xiong, Y. Cheng, L. Ma, C.Q. Zhang, MiR-21 regulates biological behavior through the PTEN/PI-3 K/Akt signaling pathway in human colorectal cancer cells, Int. J. Oncol. 42 (2013) 219–228, https://doi.org/10.3892/ijo.2012.1707.
[47] R. Lakhia, et al., MicroRNA-21 aggravates cyst growth in a model of polycystic kidney disease, J. Am. Soc. Nephrol. 27 (2016) 2319–2330, https://doi.org/10.1681/Asn.2015060634.
[48] M. Yheskel, V. Patel, Therapeutic microRNAs in polycystic kidney disease, Curr. Opin. Nephrol. Hypertens. 26 (2017) 282–289, https://doi.org/10.1097/Mnh.0000000000000333.
[49] H. Yu, H. Lee, A. Herrmann, R. Buettner, R. Jove, Revisiting STAT3 signalling in cancer: new and unexpected biological functions, Nat. Rev. Cancer 14 (2014) 736–746, https://doi.org/10.1038/nrc3818.
[50] R. Kralovics, et al., A gain-of-function mutation of JAK2 in myeloproliferative disorders, N. Engl. J. Med. 352 (2005) 1779–1790, https://doi.org/10.1056/NEJMoa051113.
[51] C. James, et al., A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera, Nature 434 (2005) 1144–1148, https://doi.org/10.1038/nature03546.
[52] S. Zou, et al., Targeting STAT3 in cancer immunotherapy, Mol. Cancer 19 (2020) 145, https://doi.org/10.1186/s12943-020-01258-7.
[53] G.B. Udy, et al., Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 7239–7244, https://doi.org/10.1073/pnas.94.14.7239.
[54] E.M. Kofoed, et al., Growth hormone insensitivity associated with a STAT5b mutation, N. Engl. J. Med. 349 (2003) 1139–1147, https://doi.org/10.1056/NEJMoa022926.
[55] Q. Zhang, H.Y. Wang, X.B. Liu, M.A. Wasik, STAT5A is epigenetically silenced by the tyrosine kinase NPM1-ALK and acts as a tumor suppressor by reciprocally inhibiting NPM1-ALK expression, Nat. Med. 13 (2007) 1341–1348, https://doi.org/10.1038/nm1659.
[56] Y.B. Jiang, et al., Loss of STAT5A promotes glucose metabolism and tumor growth through miRNA-23a-AKT signaling in hepatocellular carcinoma, Mol. Oncol. 15 (2021) 710–724, https://doi.org/10.1002/1878-0261.12846.
[57] X. Jiang, et al., MicroRNA-221 sensitizes chronic myeloid leukemia cells to imatinib by targeting STAT5, Leuk. Lymphoma 60 (2019) 1709–1720, https://doi.org/10.1080/10428194.2018.1543875.
[58] C. Xu, et al., MiRNA-1469 promotes lung cancer cells apoptosis through targeting STAT5a, Am. J. Cancer Res. 5 (2015) 1180–1189.
[59] A. Takakura, et al., Pyrimethamine inhibits adult polycystic kidney disease by modulating STAT signaling pathways, Hum. Mol. Genet. 20 (2011) 4143–4154, https://doi.org/10.1093/hmg/ddr338.
[60] W.N. Leonhard, et al., Curcumin inhibits cystogenesis by simultaneous interference of multiple signaling pathways: in vivo evidence from a Pkd1-deletion model, Am. J. Physiol. Renal Physiol. 300 (2011) F1193–F1202, https://doi.org/10.1152/ajprenal.00419.2010.
[61] C. Formica, et al., Characterisation of transcription factor profiles in polycystic kidney disease (PKD): identification and validation of STAT3 and RUNX1 in the injury/repair response and PKD progression, J. Mol. Med. (Berl.) 97 (2019) 1643–1656, https://doi.org/10.1007/s00109-019-01852-3.
[62] E.E. Olsan, et al., Signal transducer and activator of transcription-6 (STAT6) inhibition suppresses renal cyst growth in polycystic kidney disease, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 18067–18072, https://doi.org/10.1073/pnas.1111966108.
[63] J.J. Talbot, et al., Polycystin-1 regulates STAT activity by a dual mechanism, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7985–7990, https://doi.org/10.1073/pnas.1103816108.
[64] A. Viau, et al., Tubular STAT3 limits renal inflammation in autosomal dominant polycystic kidney disease, J. Am. Soc. Nephrol. 31 (2020) 1035–1049, https://doi.org/10.1681/ASN.2019090959.
[65] A.K. Bhunia, et al., PKD1 induces p21(waf1) and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2, Cell 109 (2002) 157–168, https://doi.org/10.1016/s0092-8674(02)00716-x.
[66] M. Fragiadaki, et al., STAT5 drives abnormal proliferation in autosomal dominant polycystic kidney disease, Kidney Int. 91 (2017) 575–586, https://doi.org/10.1016/j.kint.2016.10.039.
[67] J.C. Morcinek, C. Weisser, E. Geissinger, M. Schartl, C. Wellbrock, Activation of STAT5 triggers proliferation and contributes to anti-apoptotic signalling mediated by the oncogenic Xmrk kinase, Oncogene 21 (2002) 1668–1678, https://doi.org/10.1038/sj.onc.1205148.

[68] J. Herrington, L.S. Smit, J. Schwartz, C. Carter-Su, The role of STAT proteins in growth hormone signaling, Oncogene 19 (2000) 2585–2597, https://doi.org/10.1038/sj.onc.1203526.
[69] F. Patera, A. Cudzich-Madry, Z. Huang, M. Fragiadaki, Renal expression of JAK2 is high in polycystic kidney disease and its inhibition reduces cystogenesis, Sci. Rep. 9 (2019) 4491, https://doi.org/10.1038/s41598-019-41106-3.
[70] L. Wu, et al., MicroRNA-204 targets signal transducer and activator of transcription 5 expression and inhibits proliferation of B-cell lymphoma cells, Mol. Med. Rep. 11 (2015) 4567–4572, https://doi.org/10.3892/mmr.2015.3298.
[71] Y. Feuermann, et al., The miR-17/92 cluster is targeted by STAT5 but dispensable for mammary development, Genesis 50 (2012) 665–671, https://doi.org/10.1002/dvg.22023.
[72] Y. Feuermann, K. Kang, A. Shamay, G.W. Robinson, L. Hennighausen, MiR-21 is under control of STAT5 but is dispensable for mammary development and lactation, PLoS One 9 (2014), e85123, https://doi.org/10.1371/journal.pone.0085123.
[73] A. Tubbs, A. Nussenzweig, Endogenous DNA damage as a source of genomic instability in cancer, Cell 168 (2017) 644–656, https://doi.org/10.1016/j.cell.2017.01.002.
[74] J.E. Haber, Deciphering the DNA damage response, Cell 162 (2015) 1183–1185, https://doi.org/10.1016/j.cell.2015.08.034.
[75] M. Yousefzadeh, et al., DNA damage-how and why we age? elife 10 (2021) https://doi.org/10.7554/eLife.62852.
[76] I. Hickson, et al., Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM, Cancer Res. 64 (2004) 9152–9159, https://doi.org/10.1158/0008-5472.CAN-04-2727.
[77] M. Stucki, et al., MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks, Cell 123 (2005) 1213–1226, https://doi.org/10.1016/j.cell.2005.09.038.
[78] L.J. Mah, A. El-Osta, T.C. Karagiannis, gammaH2AX: a sensitive molecular marker of DNA damage and repair, Leukemia 24 (2010) 679–686, https://doi.org/10.1038/leu.2010.6.
[79] S. Matsuoka, et al., ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage, Science 316 (2007) 1160–1166, https://doi.org/10.1126/science.1140321.
[80] C. Bruhn, M. Foiani, A model of DNA damage response activation at stalled replication forks by SPRTN, Nat. Commun. 10 (2019) 5671, https://doi.org/10.1038/s41467-019-13610-7.
[81] L. Zou, DNA replication checkpoint: new ATR activator identified, Curr. Biol. 27 (2017) R33–R35, https://doi.org/10.1016/j.cub.2016.11.025.
[82] J. Bartkova, et al., DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis, Nature 434 (2005) 864–870, https://doi.org/10.1038/nature03482.
[83] D.P. Lane, Cancer. p53, guardian of the genome, Nature 358 (1992) 15–16, https://doi.org/10.1038/358015a0.
[84] K.M. Ryan, p53 and autophagy in cancer: guardian of the genome meets guardian of the proteome, Eur. J. Cancer 47 (2011) 44–50, https://doi.org/10.1016/j.ejca.2010.10.020.
[85] C.J. Brown, S. Lain, C.S. Verma, A.R. Fersht, D.P. Lane, Awakening guardian angels: drugging the p53 pathway, Nat. Rev. Cancer 9 (2009) 862–873, https://doi.org/10.1038/nrc2763.
[86] H. Bouamar, et al., MicroRNA 155 control of p53 activity is context dependent and mediated by Aicda and Socs1, Mol. Cell. Biol. 35 (2015) 1329–1340, https://doi.org/10.1128/MCB.01446-14.
[87] J. Liu, C. Zhang, Y. Zhao, Z. Feng, MicroRNA control of p53, J. Cell. Biochem. 118 (2017) 7–14, https://doi.org/10.1002/jcb.25609.
[88] Z. Feng, C. Zhang, R. Wu, W. Hu, Tumor suppressor p53 meets microRNAs, J. Mol. Cell Biol. 3 (2011) 44–50, https://doi.org/10.1093/jmcb/mjq040.
[89] C.J. Braun, et al., p53-responsive micrornas 192 and 215 are capable of inducing cell cycle arrest, Cancer Res. 68 (2008) 10094–10104, https://doi.org/10.1158/0008-5472.CAN-08-1569.
[90] A. Cebulska-Wasilewska, et al., Monitoring of molecular and cytogenetic damage in lymphocytes from three persons with polycystic kidney disease, Arch. Med. Res. 30 (1999) 23–28, https://doi.org/10.1016/s0188-0128(98)00011-6.
[91] J.Q.J. Zhang, et al., Up-regulation of DNA damage response signaling in autosomal dominant polycystic kidney disease, Am. J. Pathol. 191 (2021) 902–920, https://doi.org/10.1016/j.ajpath.2021.01.011.
[92] J.Q.J. Zhang, S. Saravanabavan, G.K. Rangan, Effect of reducing ataxia-telangiectasia mutated (ATM) in experimental autosomal dominant polycystic kidney disease, Cells 10 (2021), https://doi.org/10.3390/cells10030532.
[93] H.J. Choi, et al., NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies, Mol. Cell 51 (2013) 423–439, https://doi.org/10.1016/j.molcel.2013.08.006.
[94] S. Nishio, et al., Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and JNK activation, J. Clin. Invest. 115 (2005) 910–918, https://doi.org/10.1172/JCI22850.
[95] X. Zhou, et al., Sirtuin 1 inhibition delays cyst formation in autosomal-dominant polycystic kidney disease, J. Clin. Invest. 123 (2013) 3084–3098, https://doi.org/10.1172/JCI64401.
[96] H. Kim, Y. Bae, W. Jeong, C. Ahn, S. Kang, Depletion of PKD1 by an antisense oligodeoxynucleotide induces premature G1/S-phase transition, Eur. J. Hum. Genet. 12 (2004) 433–440, https://doi.org/10.1038/sj.ejhg.5201136.
[97] K. Van der Heiden, S. Cuhlmann, A. Luong le, M. Zakkar, P.C. Evans, Role of nuclear factor kappaB in cardiovascular health and disease, Clin. Sci. (Lond.) 118 (2010) 593–605, https://doi.org/10.1042/CS20090557.
[98] S.C. Sue, C. Cervantes, E.A. Komives, H.J. Dyson, Transfer of flexibility between ankyrin repeats in I kappa B alpha upon formation of the NF-kappa B complex, J. Mol. Biol. 380 (2008) 917–931, https://doi.org/10.1016/j.jmb.2008.05.048.
[99] V. Menon, et al., Inflammation, oxidative stress, and insulin resistance in polycystic kidney disease, Clin. J. Am. Soc. Nephrol. 6 (2011) 7–13, https://doi.org/10.2215/CJN.04140510.
[100] M.H. Ta, et al., Constitutive renal Rel/nuclear factor-kappaB expression in Lewis polycystic kidney disease rats, World J. Nephrol. 5 (2016) 339–357, https://doi.org/10.5527/wjn.v5.i4.339.
[101] K.D. Gardner Jr., J.S. Burnside, L.W. Elzinga, R.M. Locksley, Cytokines in fluids from polycystic kidneys, Kidney Int. 39 (1991) 718–724, https://doi.org/10.1038/ki.1991.87.
[102] J.X. Zhou, L.X. Fan, X. Li, J.P. Calvet, X. Li, TNFalpha signaling regulates cystic epithelial cell proliferation through Akt/mTOR and ERK/MAPK/Cdk2 mediated Id2 signaling, PLoS One 10 (2015), e0131043, https://doi.org/10.1371/journal.pone.0131043.
[103] M. Wu, et al., Resveratrol delays polycystic kidney disease progression through attenuation of nuclear factor kappaB-induced inflammation, Nephrol. Dial. Transplant. 31 (2016) 1826–1834, https://doi.org/10.1093/ndt/gfw058.
[104] T.D. Gilmore, M. Herscovitch, Inhibitors of NF-kappaB signaling: 785 and counting, Oncogene 25 (2006) 6887–6899, https://doi.org/10.1038/sj.onc.1209982.
[105] M. El-Salhy, et al., Amelioration of severe TNBS induced colitis by novel AP-1 and NF- kappa B inhibitors in rats, ScientificWorldJournal 2014 (2014), 813804, https://doi.org/10.1155/2014/813804.
[106] E.C. Lee, et al., Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease, Nat. Commun. 10 (2019) 4148, https://doi.org/10.1038/s41467-019-11918-y.