Multidrug Therapy for Polycystic Kidney Disease: A Review and Perspective

Gianluca Aguiari[a]   Luigi Catizone[b]   Laura del Senno[a]

[a]Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, and [b]Division of Nephrology, S. Anna Hospital, Ferrara, Italy

Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a renal disorder characterized by the development of cysts in both kidneys leading to end-stage renal disease (ESRD) by the fifth decade of life. Cysts also occur in other organs, and phenotypic alterations also involve the cardiovascular system. Mutations in the PKD1 and PKD2 genes codifying for polycystin-1 (PC1) and polycystin-2 (PC2) are responsible for the 85 and 15% of ADPKD cases, respectively. PC1 and PC2 defects cause similar symptoms; however, lesions of PKD1 gene are associated with earlier disease onset and faster ESRD progression. The development of kidney cysts requires a somatic 'second hit' to promote focal cyst formation, but also acute renal injury may affect cyst expansion, constituting a 'third hit'. PC1 and PC2 interact forming a complex that regulates calcium homeostasis. Mutations of polycystins induce alteration of Ca^{2+} levels likely through the elevation of cAMP. Furthermore, PC1 loss of function also induces activation of mTOR and EGFR signaling. Impaired cAMP, mTOR and EGFR signals lead to activation of a number of processes stimulating both cell proliferation and fluid secretion, contributing to cyst formation and enlargement. Consistently, the inhibition of mTOR, EGFR activity and cAMP accumulation ameliorates renal function in ADPKD animal models, but in ADPKD patients mild results have been shown. Here we briefly review major ADPKD-related pathways, their inhibition and effects on disease progression. Finally, we suggest to reduce abnormal cell proliferation with possible clinical amelioration of ADPKD patients by combined inhibition of cAMP-, EGFR- and mTOR-related pathways.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent hereditary kidney disease accounting for nearly 10% of dialysis patients with high medical care costs [1]. Cysts occur mostly in the kidney, but also in the liver, ovary, pancreas, spleen and central nervous system. Abnormalities of the vascular system are also reported [1]. Cystogenesis, a focal process involving less than 8–5% of the nephrons, leads to hypertension in 60% of ADPKD cases before renal insufficiency and the majority of patients reach ESRD by 55 years of age [1]. Cyst development and enlargement are associated with alterations in epithelial cell growth, fluid secretion and extracellular matrix composition [1]. These processes occur mainly by the impairment of EGFR-, cAMP- and
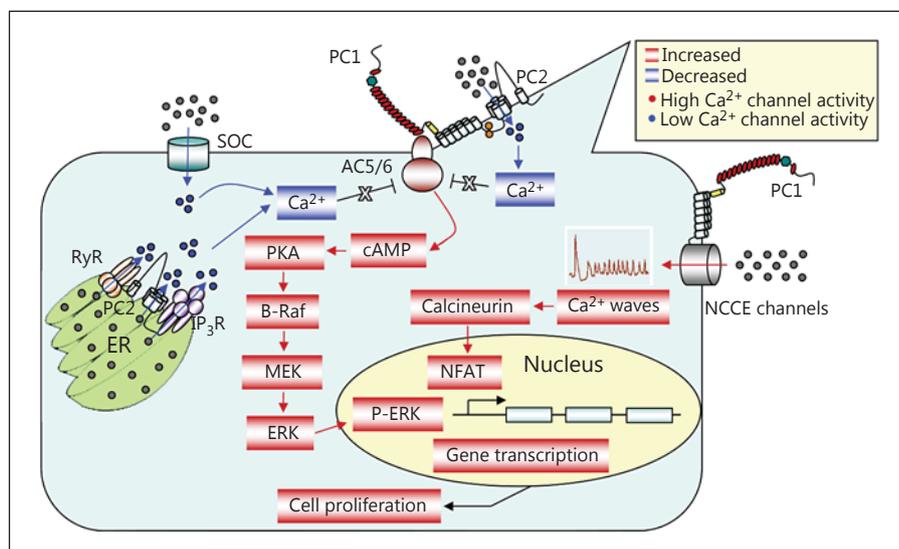
Fig. 1. Abnormal calcium homeostasis and Ca^{2+}-dependent signaling in ADPKD kidney epithelial cystic cell. Polycystin mutations cause abnormal intracellular Ca^{2+} levels by impairing both plasma membrane and endoplasmic reticulum calcium channels. In particular, in primary cilium, polycystin dysfunction reduces intracellular Ca^{2+} influx activated by mechanical bending of cilium which protrudes into the tubular lumen. Thus, reduced flow-sensitive intracellular Ca^{2+} levels and signaling cause a decrease in Ca^{2+} release from both endoplasmic reticulum (ER) and plasma membrane store-operated channels (SOC) in ADPKD cystic cells. Intracellular Ca^{2+} reduction may activate Ca^{2+}-dependent adenylyl cyclases 5 and 6 (AC5/6) resulting in cAMP accumulation that, by stimulation of Ras/Raf/MEK/ERK signaling, causes an increased cell proliferation. In addition, polycystin loss of function causes an increase in intracellular Ca^{2+} oscillations through an abnormal activity of plasma membrane NCCE. These Ca^{2+} oscillations are involved in abnormal cell proliferation of ADPKD cystic cells, by activation of the transcription factor NFAT.

mTOR-mediated pathways [2–4]. In this regard, several drugs have been developed in order to inhibit the signaling associated with ADPKD, including cAMP and mTOR [5–8]. However, the different molecules, used individually in clinical trials, failed to ameliorate the clinical picture of ADPKD patients.

Here we suggest to treat ADPKD patients with a combination of inhibitors against three major pathways altered in ADPKD such as cAMP, mTOR and EGFR.

Signaling Impaired in ADPKD

PC1 and PC2 interact forming a complex (PC complex) [9] which works in a number of signaling pathways mainly related to calcium, cAMP, mTOR and EGFR which are involved in the control of cell growth, cell adhesion and apoptosis.

Calcium-Related Signaling
In primary cilia of kidney epithelium the PC complex functions as a mechanotransductor which regulates intracellular calcium influx in response to fluid flow variations (fig. 1). Alterations of PC1 or PC2 proteins induce a strong reduction in calcium influx, with aberrant calcium-dependent signals, resulting in altered ciliary function, planar cell polarity and organ morphogenesis [10, 11]. The loss of local calcium affects cAMP homeostasis by de-repressing the Ca^{2+}-sensitive adenylyl cyclases AC5 and AC6, leading to a cAMP increase and the development of renal cysts [12]. Moreover, in PKD1-mutated kidney cystic cells the increase in calcium oscillations, dependent on non-capacitative calcium entry (NCCE) channels (fig. 1), is associated with an increased cell proliferation, via activation of the transcription factor NF-AT [13].

cAMP-Related Signaling
In normal kidney epithelial cells cAMP elevation has an inhibitory effect on cell growth, while in ADPKD cysts it stimulates cell proliferation. This occurs by activating ERK signaling through the sequential phosphorylation of PKA, B-Raf and MAPK kinases [14] (fig. 1, 2). Moreover, cAMP may also stimulate fluid secretion and cyst en-
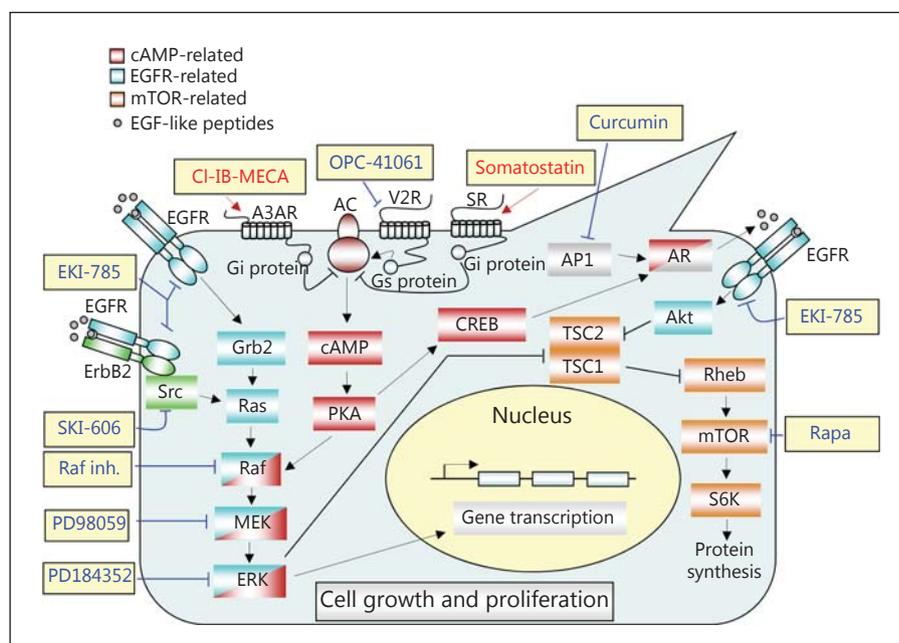
Fig. 2. Altered cAMP-, EGFR- and mTOR-dependent signaling pathways in ADPKD cystic cell and related inhibitors. Polycystin loss of function causes an increase in cell proliferation by alteration of different pathways mostly including cAMP, EGFR and mTOR signaling. The activation of EGFR, which was found to be translocated to the apical membrane in cystic cells, by interaction with its ligands, increases the activity of the Ras/Raf/ERK signaling pathway which stimulates cell growth. EGFR activation is also enhanced by AR which is overexpressed in cystic cells through a CREB- and AP1- dependent signaling. Abnormal cAMP accumulation also contributes to the activation of the Ras/Raf/ERK signal. Furthermore, this pathway may be stimulated also by activation of Src, which is able to interact with EGFR in its EGFR/ErbB2 heterodimer form [53]. In addition, altered EGFR and cAMP signaling stimulate mTOR activity by activation of Akt and ERK kinases that inhibit the TSC1/TSC2 complex. Inhibitors (blue) and agonists (red) are indicated. Colors refer to the online version only.

largement, activating the apical CFTR channel and specific basolateral transporters [14, 15]. Consistently, adenylyl cyclase agonists, beside promoting cell proliferation, are known to be able to stimulate electrolyte secretion in human ADPKD cells in vitro [3].

mTOR-Related Signaling

ADPKD patients carrying deletions of PKD1 and TSC2 adjacent genes show an earliest cystogenesis, therefore we can assume that both PC1 and tuberin (the TSC2 gene product) function in a common cystogenic pathway. TSC2 is known to inactivate the signaling of the Ser/Thr kinase mTOR (fig. 2) which, in fact, is found abnormally activated in ADPKD cyst-lining cells [4, 16]. mTOR activation results in increased protein translation via phosphorylation of S6K and 4EBP1 leading to cell growth stimulation and proliferation [4]. PC1 inhibits mTOR activity by interacting with tuberin, thus blocking its inactivation which usually occurs through phosphorylation by ERK and Akt kinases [17–19].

EGFR-Related Signaling

ADPKD cell proliferation may be stimulated also by EGFR through a mechanism involving the sequential activation of Ras, Raf-1, MEK and ERK signaling [14], where the EGFR signal may converge on the same pathway activated by cAMP, which leads to activation of ERK kinases (fig. 2). Consistently, cyst-derived epithelial cells are susceptible to proliferative stimuli of EGF-related peptides which are secreted into the apical medium of cultured ADPKD epithelia as well as in cyst fluids of ADPKD patients [20].

In this regard, the increased expression of TGF-α is also observed in ADPKD cells, and transgenic mice overexpressing TGF-α develop cystic kidneys [21]. Moreover, EGF-related peptides amphiregulin (AR) and heparin-binding EGF are also increased in cystic cells of PKD patients [2]. In particular, AR expression is associated with activation of transcription factors CREB, a cAMP downstream effector, and AP1 (fig. 2) [22]. Furthermore, in ADPKD cystic cells, the apical plasma

Table 1. Molecular inhibitors with the relative targets against cAMP-, mTOR- and EGFR-dependent signaling pathways

Compound	Target	In vitro	In vivo	Clinical trials	Outcome	References
Sirolimus	mTOR	yes	yes	yes	failed clinical trials	7, 35, 37, 48
Everolimus	mTOR	yes	yes	yes	failed clinical trials	8, 36, 38
Tolvaptan	V2R	yes	yes	yes	in progress clinical trials	5, 26
Octreotide	SR	yes	yes	yes	in progress clinical trials	6, 27–29
Bosutinib	c-Src	yes	yes	no	good in PKD cell and animal models	42
Sorafenib	Raf-1	yes	yes	no	good in PKD cell and animal models	31
MEK inhibitors	MEK	yes	yes	no	good in PKD cell models, but failed in PKD animals	32, 33
PD184352	ERK	yes	yes	no	good in PKD cell and animal models	34
Cl-IB-MECA	A3AR	yes	no	no	good in PKD cell models	30
Curcumin	AP1	yes	yes	no	good in PKD cell and animal models	22, 40, 41
EGFR inhibitors	EGFR	yes	yes	no	good in PKD cell and animal models	24, 39

The therapeutic agents currently used in clinical trials for the treatment of ADPKD patients are also indicated.

membrane mislocalization of EGFR, instead of its normal basal localization, as well as the heterodimerization of EGFR with ErbB2 (fig. 2), play a further role in cyst enlargement [23, 24]. Therefore, the EGFR activation may generate an autocrine loop resulting in increased cell proliferation and cyst expansion in ADPKD cystic cells [23].

ADPKD Treatment

Currently, different molecules able to reduce cell proliferation and renal cystic volume in ADPKD animal models are being used for clinical trials in ADPKD patients. For instance, inhibitors of mTOR, such as rapamycin (sirolimus) or its derivate everolimus, are already used in clinical trials, while other compounds like cAMP and EGFR inhibitors are still in study on animal models or have to complete clinical trial phases. Here, we describe the effects of some drugs developed for ADPKD treatment (see also fig. 2 and table 1).

cAMP Signal Inhibitors

In preclinical studies the V2R antagonist OPC-31260 reduces cyclic AMP levels, renal cystogenesis, kidney enlargement and renal dysfunction in the *Pkd*$^{-/\text{tm1Som}}$ Pkd2 mouse model [25]. Consistently, another V2R antagonist, OPC-41061 (tolvaptan), with a high affinity and strength for the receptor, inhibits ERK pathway, cAMP production, Cl$^-$ secretion and the in vitro cyst growth of three-dimensionally cultured AVP-induced ADPKD cells [26].

These findings encouraged the setting of clinical trials in ADPKD patients using V2R inhibitors. In fact, 3 years of treatment with tolvaptan slows the increase in total kidney volume and kidney decline function in ADPKD patients compared with placebo. However, different side effects as thirst, polyuria, and related adverse events in some patients were observed [5].

Also the octreotide, an analogue of somatostatin that inhibits cAMP accumulation (see fig. 2), has been evaluated for its potential inhibition of cyst growth in ADPKD animal models. Octreotide treatment results to reduce cAMP accumulation and the progression of liver and kidney cysts in a PKD rat model [27]. In a randomized, double-blind, placebo-controlled clinical trial with 42 ADPKD patients, octreotide treatment after the first year maintains TKV almost unchanged, while it increases in the placebo group. Similar benefits are also observed in the second year of treatment, suggesting that octreotide administration may reduce kidney cyst growth [6, 28, 29].

Furthermore, cAMP levels may be also reduced by the stimulation of adenosine type 3 receptors (A3AR) which inhibits adenylyl cyclase and are overexpressed in ADPKD cystic cells and tissues [30]. The specific A3AR agonist Cl-IB-MECA is able to reduce cell proliferation by reduction of cAMP levels and ERK activity in ADPKD cystic cells [30]. Future studies on ADPKD animal models are necessary to establish if Cl-IB-MECA is effective also in vivo.

Since cAMP elevation activates B-Raf/MEK/ERK pathway in ADPKD cells (fig. 1, 2), its inhibition should reduce renal cyst expansion. Accordingly, the treatment

with sorafenib, a Raf inhibitor, reduces proliferation of cystic cells from human ADPKD kidneys by reducing ERK, B-Raf and MEK/ERK activation. Sorafenib may thus be used as therapeutic molecule to reduce cyst expansion [31].

Although the MEK pathway appears to be crucial in cAMP- and EGFR-dependent cyst formation, the efficacy of MEK inhibitors in PKD cell and animal models results controversial. In fact, the treatment with the MEK inhibitor PD98059 completely inhibits ADPKD cell proliferation in response to cAMP agonists [32]; however, the MEK inhibitor U0126 does not retard the cyst development in a PKD mouse model [33]. Although U0126 does not reduce cyst progression, the treatment with the ERK inhibitor PD184352 slows cyst growth in polycystic kidney disease PKD mice [34]. These contradictory findings indicate that further studies in other PKD animal models are needed to evaluate the efficacy of these molecules.

mTOR Inhibitors

Since in ADPKD kidneys mTOR kinase activity is increased [4], mTOR inhibition should delay cystic growth and expansion in ADPKD kidneys. Actually, treatments with mTOR inhibitors rapamycin/sirolimus or everolimus decrease renal cyst size and improve kidney function in ADPKD animal models [35, 36]. Results of a randomized clinical trial suggest that rapamycin administration in ADPKD patients does not modify the GFR compared with standard care-treated group [7]. Moreover, the TKV does not change in sirolimus-treated patients and controls, while urinary albumin excretion rate is higher in the sirolimus-treated group. Thus, 18 months of sirolimus treatment does not improve renal function in ADPKD patients with early chronic kidney disease [7]. Other studies show that after 6 months of treatment with higher doses of sirolimus cyst volume does not change, while it increases in conventional therapy-treated subjects [37]. On the contrary, parenchymal volume increases by sirolimus administration while it is stable with standard therapy. Thus, high doses of sirolimus inhibit cyst growth and increases parenchymal volume in patients with ADPKD after a short period of monitoring. Further studies are needed to understand whether these effects may ameliorate renal function and the quality of life in ADPKD patients [37].

Clinical trials have also been assessed by administration of everolimus which are used in a 2-year study that includes placebo controls and ADPKD patients at a late stage [8]. In everolimus-treated patients, both TKV cyst volume and renal parenchymal volume increase more slowly than in those treated with placebo. However, after 24 months the estimated GFR does not change in the everolimus group. Findings of a 2-year study suggest that everolimus slows the increase in TKV, but does not improve renal function of ADPKD patients [8]. This discouraging result may be due to late stage of disease, therefore clinical trials should be conducted on patients with early stage of chronic kidney disease, so that everolimus therapy may actually improve the renal function [38].

EGFR Inhibitors

Administration of EGFR inhibitors EKI-785 and EKB-569 in a rat model for ADPKD lowers kidney weights and cyst volumes, suggesting a therapeutic potential of EGFR inhibition for ADPKD treatment [39]. Also the inhibition of EGF-like growth factors such as TGF-α, AR, and heparin-binding EGF, found abnormally expressed in human ADPKD epithelial cells [2, 22], may be taken in account for ADPKD treatment. In fact, the inhibition of AR by specific antibodies restores normal cell growth in human and mouse ADPKD cystic cells [22]. In addition, the inhibition of both CREB and AP1 activity, which stimulates AR expression in these cells, also reduces cystic cell growth [22]. Consistently, curcumin, a potential AP1 inhibitor that is known to reduce activities of several signaling proteins including ERK and fos, significantly reduces cyst development in an in vitro ADPKD model [40]. Moreover, in Pkd1 gene conditionally inactivated mice, curcumin induces a slower progression of proliferation index, cystic index and kidney growth [41]. The inhibition of Src, a kinase linked to EGFR signaling, by the SKI-606 molecule (bosutinib) also reduces cell proliferation in human ADPKD cystic cells and delays renal cystic growth and progression in a Pkd1 heterozygous mouse model, suggesting that the inhibition of this way may also contribute to amelioration of renal function [42].

Other Therapeutic Attempts

Significant findings have been obtained in preclinical trials by using triptolide, an active diterpene used in Chinese medicine. Triptolide induces intracellular calcium release by a PC2-dependent mechanism and inhibit cell proliferation by restoring calcium signaling in mouse ADPKD cells [43]. Furthermore, triptolide improves renal function inhibiting early phases of cyst development in a mouse model of ADPKD [44].

Retrospective studies to evaluate the renoprotective effect of calcium channel blockers (CCB) and/or renin-angiotensin-aldosterone system inhibitor (RAAS-I) by monitoring glomerular filtration rate (GFR) in ADPKD

patients were also performed. No changes in GFR rate after treatment of ADPKD patients with RAAS-I compared with untreated were observed, but the administration of CCB causes an unfavorable effect on renal function with respect to ADPKD patients not receiving CCB treatment [45].

Recently, it was also reported that serum HDL-cholesterol may affect ADPKD progression. Thus, the management of cholesterol should be carefully considered in ADPKD patients [46].

In ADPKD patients the antidiuretic hormone, arginine vasopressin (AVP) stimulates cAMP accumulation contributing to cyst formation. Therefore, water intake should decrease cAMP levels by reduction of AVP activity. Consistently, acute water loading in ADPKD patients decreased urine cAMP concentration, but no significant decreasing in urine cAMP excretion after chronic water loading was observed [47]. Further studies will be needed to test the efficacy of water therapy.

Future Potential Therapies

Could a Multitarget Therapy Become More Successful in the Treatment of ADPKD?

Despite the positive results in preclinical models, findings obtained at the end of clinical trials by using sirolimus, everolimus, and related molecules currently in progress are not completely satisfactory. The mild results of sirolimus in human ADPKD patients can also be due to the administration of this compound at doses that are much too low. In fact, long-term treatment with sirolimus at low doses in ADPKD mouse models is not sufficient to inhibit mTOR activity in renal cystic tissue [48]. However, in Pkd1^{nl,nl} ADPKD mice which express reduced levels of Pkd1 gene, the treatment with high- and low-dose sirolimus in the early disease stage significantly accelerated cyst regression [48]. A high dose of everolimus in clinical trials significantly reduces TKV, but it also induces side effects and does not improve renal function [8]. Therefore, further experiments researching new roads for the cure of ADPKD are required. In this regard, a combined treatment with different compounds blocking simultaneously more signaling pathways may be necessary, which should be more effective in restoring normal kidney parameters and minimizing side effects [48, 49]. This new approach should be addressed to the inhibition of cAMP, mTOR and EGFR pathways strongly involved in ADPKD development. Multitarget therapeutic approaches for the treatment of different pathologies especially for cancer are emerging. In fact, combined inhibition of mTOR and ERK or mTOR and EGFR pathways shows synergistic effects and better efficacy than single-target therapy in preclinical models for cancer and other diseases [50–52]. This goal could be, for instance, obtained by the combined use of sirolimus/everolimus, tolvaptan and EKI-785 that individually showed good results in ADPKD preclinical models. To evaluate possible synergistic effects, the procedure for multidrug treatment in preclinical models of ADPKD should provide for simultaneous or sequential administration of compounds, taking into account that the combined administration may have antagonizing or toxic effects [Aguiari, unpubl. data]. Compounds could be administrated daily or by pulse modality, and administration timing should be carefully evaluated in order to minimize side effects. It is also possibly necessary to try different combinations of targeted inhibitors for each selected pathway to test the best targeted drugs in terms of higher efficacy and lower toxicity. The treatment of ADPKD animals should be programmed in the early stage of disease, when the renal function is not yet compromised.

Conclusions

In this review, we propose alternative roads to treat ADPKD mainly addressed to the inhibition of cAMP, mTOR and EGFR pathways that are strongly involved in increased cell proliferation, fluid secretion and renal cyst development. Recently, multitargeted therapies for the treatment of different pathologies are emerging, especially when conventional single-target therapy failed. Therefore, for the future, multidrug approaches may provide new opportunities for the treatment of ADPKD.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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