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Polycystic kidney disease: The complexity of planar cell polarity and signaling during tissue regeneration and cyst formation

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is an inherited systemic disease with intrarenal cystogenesis as its primary characteristic. A variety of mouse models provided information on the requirement of loss of balanced polycystin levels for initiation of cyst formation, the role of proliferation in cystogenesis and the signaling pathways involved in cyst growth and expansion.
Here we will review the involvement of different signaling pathways during renal development, renal epithelial regeneration and cyst formation in ADPKD, focusing on planar cell polarity (PCP) and oriented cell division (OCD). This will be discussed in context of the hypothesis that aberrant PCP signaling causes cyst formation.
In addition, the role of the Hippo pathway, which was recently found to be involved in cyst growth and tissue regeneration, and well-known for regulating organ size control, will be reviewed. The fact that Hippo signaling is linked to PCP signaling makes the Hippo pathway a novel cascade in cystogenesis.
The newly gained understanding of the complex signaling network involved in cystogenesis and disease progression, not only necessitates refining of the current hypothesis regarding initiation of cystogenesis, but also has implications for therapeutic intervention strategies. This article is part of a Special Issue entitled: Polycystic Kidney Disease.

General introduction

Polycystic kidney disease presents itself in many different forms. In this review we will focus on Autosomal Dominant Polycystic Kidney Disease (ADPKD). ADPKD is a systemic disease with intrarenal cystogenesis as its primary characteristic. Extra-renal manifestations are hypertension, cardiac valvular abnormalities, cerebral aneurysms, and cysts in liver and pancreas[1]. Usually, in young adults, only a small number of cysts can be detected ultrasonographically, whereas at middle age, thousands of cysts and fibrotic tissue have replaced almost all normal renal parenchyma and renal function declines.

In general, ADPKD patients carry one germ-line mutation and one normal *PKD1,* or *PKD2* allele. Renal cysts develop from somatic inactivation of *PKD1* or *PKD2* by a 'two-hit' mechanism[2]. In addition, haploinsufficiency, stochastic fluctuations in *PKD1*or *PKD2* gene dosage below a tissue-specific threshold, may suffice to cause cyst formation[3]. Importantly, recent studies indicate that the picture can be more complex, since several families have been reported with non- or incompletely penetrant alleles of *PKD1* inherited at a homozygous state, or in *trans* with another mutation[4, 5].

In kidneys the primary defect has been found to occur in the epithelium. A balanced expression level of the *PKD1* and 2 genes, encoding polycystin 1 and 2 (PC1and PC2) is critical to maintain renal epithelial architecture. Both PC1 and PC2 are expressed in several sub-cellular compartments such as the primary cilium, cell-cell contacts and cell-extracellular matrix contacts. They form different multimeric protein complexes in the cell, modulating several signaling pathways, which in concert control essential cellular functions such as proliferation, apoptosis, cell adhesion, and differentiation[6, 7]. Disruption of any of these processes can lead to cyst formation, as demonstrated by a variety of mouse models[8-12]. In this review the role of proliferation, injury and repair in ADPKD, as well as their different signaling pathways will be discussed, with the major focus on planar cell polarity (PCP) and oriented cell division (OCD).

Mechanism of disease

The renal pathogenesis of ADPKD recognizes several phases. In the initiation phase, normal quiescent epithelial cells become hyper-proliferative[13, 14]. Subsequently, a phase characterized by cyst growth and cyst expansion caused by proliferation of cystic epithelial cells and increased fluid secretion, as well as extracellular matrix synthesis, can be recognized. During this phase also additional signaling pathways become either activated, or dysfunctional, resulting in more cyst growth[15]. These cysts detach from the original nephrons and continue to growth as isolated autonomous cysts.

Later on, cysts lining epithelial cells of large cysts stop dividing and apoptotic nuclei can be found in these cysts[16]. In addition, interstitial inflammatory infiltrates become apparent resulting in tubulointerstitial fibrosis and subsequent deterioration of kidney function[17-19].

As the material obtained from kidneys of ADPKD patients mainly allows investigation of the last phase of the disease, mouse models had to be developed in order to study the different stages of the disease.

Genetic mechanism in mice

Different *Pkd1*- or *Pkd2*-mutant mouse models have been created, which are supporting the genetic mechanisms mentioned above. The first support for the second hit model was provided by the *Pkd2*$^{ws25,-}$ model. In this model, with one null allele and one instable *Pkd2* allele, somatic rearrangements are accelerating renal cystic disease[20], while mice that are heterozygous for an inactivating *Pkd1* or *Pkd2* mutation, show only a very mild phenotype. Homozygous *Pkd1*- and *Pkd2*-deficient mice, however, die at about embryonic stage 15.5 or directly after birth because of severe cystic disease, vascular defects, and/or abnormalities of the placental labyrinth[20-29]. On the other hand, mice carrying one *Pkd1* as well as one *Pkd2* mutant allele are viable and show accelerated renal cystic disease compared to the single gene phenotypes[29].

Also hypomorphic mouse models for polycystic kidney disease that express *Pkd1* or *Pkd2* at a reduced level are viable, although they develop severe PKD[30-32]. Extra-renal manifestations such as cysts in liver and pancreas[21-23, 28, 33-35], and aortic dissecting aneurysms have been reported as well[30, 36]. However, the severity of the phenotype and lifespan is dependent on the genetic background of the mouse strain (unpublished results). The models with expanded life span are especially useful for studying the later phases in PKD.

For proper renal development the levels of *Pkd1* and *Pkd2* gene expression are critical, since over-expression leads to a renal cystic phenotype[35, 37-39], although in one model *Pkd2*-overexpression did not cause cystic disease but renal tubulopathy[40]. In addition, extra-renal manifestations similar to those observed in patients have been described in a *Pkd1* over-expression model[38], and over-expression of a truncated *Pkd2* gene caused polycystic kidney disease and retinal degeneration in transgenic rats[41].

Inducible conditional deletion models

To overcome embryonic lethality and to study tissue specific effects of *Pkd1*-inactivation, inducible and conditional *Pkd1* and *Pkd2* deletion mice have been created, using the Cre-*loxP* system [42-48]. These models have provided valuable information on the mechanisms involved in the initiation phase of cyst formation. Inducible *Pkd1*-deletion mice were used to analyze the kinetics of cyst formation upon *Pkd1*-gene disruption at different time-points after birth. In mice the progressiveness of cystogenesis depends on the age of *Pkd1*-gene disruption, e.g. in young mice the highest progression can be observed, while adult mice show a very slow phenotype[43, 45]. The difference in progression of cystic renal disease correlates with the developmental status of the renal tissue at the moment of gene disruption as well as with its proliferative status[43, 45].

Around neonatal day 13, renal development enters its completion in mice and a critical switch in gene expression was observed[45]. Also renal epithelial cell proliferation is dramatically higher in neonatal compared to adult mice[43]. Knocking-out *Pkd1* at postnatal day 4 results in progressive cyst formation with the major involvement of the distal nephron. At the moment of gene-inactivation, the distal nephron is still elongating at that time, and shows many proliferating cells[43]. Interestingly, *Pkd1*-gene disruption in young adult mice, well beyond the above-mentioned developmental switch in gene transcription, leads to a disease progression rate intermediate to that of neonatal and adult mice, while young adults also show levels of renal epithelial cell proliferation between neonatal and adult mice[45, 49]. Therefore, proliferation in the absence of functional polycystins seems to be a trigger for cyst formation [49].

As proliferation seems to be an accelerating factor in cystogenesis in young *Pkd1*-deletion mice, proliferation should also provide an accelerating trigger for cyst formation in the kidneys from adult *Pkd1*-deletion mice with low renal epithelial proliferation rates at the moment of gene disruption. Indeed renal epithelial injury accelerates the progression of cystogenesis in adult *Pkd1*-deletion mice[49, 50]. However, it cannot be excluded that proliferation is not the only accelerating factor, as epithelial regeneration probably shares (parts of) the cellular programs involved in renal development.

Also in non-orthologous inducible, conditional knockout models for renal cystic disease, in which *kif3* or *hnf1β* is inactivated, injury accelerates the cystic phenotype[51, 52]. Although the mechanism underlying cyst formation in the *hnf1β* conditional knock-outs probably differs from the model in which *kif3* was deleted. In all of these models the nephron segment that is targeted by the injury, and in which regeneration occurs, determines the origin of the majority of the cysts.

There are several possible answers to the question why proliferation accelerates cyst formation. One explanation may reside in the fact that in quiescent cells epithelial junction complexes, where also the polycystins can be found, are relatively stable but undergo extensive remodeling, when renal epithelial cells are forced to change their quiescent state into either proliferation, or migration[53]. Another possibility is that the polycystins themselves are not stable, and disappear from the junction complexes, but are crucial for proper (re-)establishment of adhesion complexes[54, 55].

Altered ciliary signaling and planar cell polarity signaling

The primary cilium is a common denominator in renal cystic diseases. This organelle harbors many of the proteins encoded by the genes mutated in a variety of renal cystic diseases[56] i.e. genes mutated in Bardet-Biedl syndrome[57], Meckel-Gruber syndrome[58-63], nephronophthisis[64, 65] or oral-facial-digital-syndrome[66].

During development the primary cilia on renal epithelial cells sense flow upon the start of urine production. This is accompanied by up-regulation and altered localization of the protein inversin. These changes result in a switch from canonical to non-canonical Wnt signaling, also known as the planar cell polarity (PCP) pathway[67].

PCP is polarity within the plane of an epithelial monolayer, parallel to the basement membrane and perpendicular to apical-basal polarity, which represents as the (identical) spatial arrangement of cells or sub cellular structures of cells within an epithelial plane. The PCP pathway is the signaling pathway that affects planar polarization of cells.

In PKD, however, defects in PCP signaling are found, as well as increased canonical Wnt signaling[49, 68, 69]. A proper balance of these pathways may be crucial for normal renal development and maintenance of tissue homeostasis since persistent activation of the β-catenin-mediated Wnt signaling itself also causes renal cyst formation in transgenic mouse models [10, 70].

Ciliary signaling and PCP signaling are closely linked; these two pathways act bidirectional in regulating PCP and cilial/basal body orientation. At least for motile cilia, PCP roughly provides cues for the cilium since PCP dictates the orientation of the basal body, resulting in synchronization of the beating direction of motile cilia in specific tissues. On the other hand, orientation of cilia is optimized by hydrodynamic forces, i.e. flow[71-73], and PCP is required for positioning/proper localization of cilia[74]. From these studies Marshall concluded that the cilia have a role in sensing the direction of fluid flow and by that can provide cues for planar orientation of the cilium/basal body[75]. Correspondingly, proper orientation and positioning of the basal body of non-motile primary cilia may also be regulated by PCP-signaling and hydrodynamic forces, although this remains to be confirmed.

PCP and oriented cell division in development

It is proposed that PCP signaling is needed during renal development in order to ensure the correct morphology and diameter of the nephrons. Defects in PCP signaling may therefore cause an increase in tubular diameter[76, 77]. Fischer *et al.* concluded that renal epithelial cells were planary polarized as cell division was oriented during renal development. Orientation of cell division was indicated by the orientation of the nuclear spindle along the longitudinal tubular axis, depicted as the Y-direction in the tubule shown in Figure 1A[77]. Therefore orientation of the nuclear spindle, measured by determining mitotic angle of the nuclear spindle relative to the tubular longitudinal axis, is now commonly used as a read out for PCP signaling.

Using this method, misoriented cell division was observed in developing kidneys and dilated tubules and in cysts in two murine models for renal cystic disease[51, 77]. These findings are consistent with the concept that aberrant PCP signaling causes cyst formation. This hypothesis was further supported by the development of renal cysts in mice in which Fat4, a PCP component, was knocked-out[11]. In addition, in many mouse models presenting renal cysts, tubular dilation and cyst formation are accompanied by aberrant oriented cell division (OCD). However, these models are non-orthologous models for PKD in which the targeted epithelial cells lack cilia[51, 78], non-canonical Wnt-signaling is influenced by Wnt9b deficiency[79] or in which cysts are formed upon unilateral ureteral obstruction[80].

Also for a *Pkd1* conditional knockout model, misorientation of cell division has been reported in pre-cystic epithelia[81]. This contrasts a parallel publication, in which orientation of cell division was studied in a *Pkd1*, *Pkd2* and *Pkdhd1* model. Aberrant OCD could not be observed in pre-cystic tubules of *Pkd1* and *Pkd2*-deletion mice. It was only observed upon dilation of the tubules[82]. These different findings between two independent studies on *Pkd1* deletion mice may be explained by the differences in analysis methods.

However, it is also possible that the accepted hypothesis, that misoriented cell division is the cause of cyst formation, is incorrect or incomplete, since a quite contrasting observation to the accepted hypothesis was made in the latter study. In *Pkhd1*$^{del4/del4}$ mice, defective OCD, including out of the epithelial plane cell division, into the lumen was found. However, these mice failed to develop dilated tubules or cystic kidneys.

Mitotic angles may deviate from the normal orientation in two directions, described by two angles: the first angle (α1) describes the deviation from the longitudinal tubular axis which is represented by Y in Figure 1A, but parallel to the epithelial plane (XY-plane) as shown in Figure 1B. The second angle (α2) describes the angle between the mitotic spindle axis and the basal membrane, a cell division in the YZ-plane as shown in Figure 1C. Thus α2 defines how much the mitotic spindle axis comes out of the epithelial plane. The paper of Nishio *et al.*, studying OCD in *Pkhd1* mutant and *Pkd1* and *Pkd2*-deletion mice, as discussed above, is the first that (clearly) discriminated between these two angles. They showed that misorientation of cell division occurs in both directions, at least clearly shown for mildly dilated tubules in *Pkd1* and *Pkd2*-deletion mice[82]. In addition, they suggested that cell division out of the plane of the epithelium in *Pkhd1*^{del4,del4}, was probably corrected by a convergent extension-like movement into the epithelial plane of cells, resulting in an increased number of tubular circumferential cells but not in cystic kidneys[82]. It cannot be excluded however, that other factors may explain the lack of cyst formation in *Pkhd1*$^{del4/del4}$, e.g. the complex processing of the *Pkhd1* transcript.

Thus, misorientation of cell division includes cell divisions out of the plane of the epithelium. Not in every model misoriented cell division can be designated as the cause of cyst formation, since cytogenesis can develop without loss of OCD. In addition, aberrant orientation of cell division alone seems not to be sufficient for cyst formation and rather seems to be a permissive condition or an accelerating factor for tubular dilatation.

It cannot be excluded, however, that cyst formation and renal cystic disease in the commonest and more adult-onset form of polycystic disease, ADPKD, are mechanistically different from the processes occurring in other types of (congenital) cystic kidney diseases, particularly the ciliopathies.

PCP and oriented cell division in renal epithelial regeneration

It is likely that the complex interplay of processes involved during renal development; e.g. PCP signaling, OCD, convergent extension and ciliary signaling are also involved during regeneration of tubular epithelia. PCP signaling has already proven to be important for proper tubular epithelial repair[80]. To ensure correct tubular morphology during repair, the majority of the mitotic angles have to be parallel to the tubular longitudinal axis.

Reports in which OCD was examined after injury in two different models for PKD, a *Pkd1* and an *Hnf1β* deletion model, showed that the orientation of mitotic spindles during repair in controls is preferentially directed along the tubular (longitudinal) axis[52, 81]. This suggests that, like in developing nephrons, there is planar polarization of the epithelial cells.

As in renal development, the *Pkd1* and an *Hnf1β* deletion model show misoriented cell division during regeneration[52, 81]. However, measurement of the mitotic angle can only be done in dividing cells. As the centrosomes form the spindle poles during cell division, OCD and centrosome position are linked[78], the position of centrosomes may be used as a read-out for PCP in non-dividing cells. This is supported by the observation that disrupting PCP components, lead to aberrant positioning of the basal body/centrosome and cilium[73, 74, 83, 84]. Also in adult *Pkd1*-deletion mice mispositioning of the centrosome was observed during the pre-cystic phase after completion of repair[49]. These data indicate that aberrant PCP precedes cyst formation, although mispositioning of the centrosome was also found in tubules of adult *Pkd1*-deletion mice that were not subjected to epithelial injury, and develop cysts rather late[49].

Planar cell polarity and apical-basal polarity

OCD is accepted as a read-out for PCP as PCP-signaling regulates OCD. However, OCD is not regulated by the PCP pathway alone. PCP-signaling provides spatial information within the epithelial sheet. Other signaling pathways provide information concerning apical-basal polarity and regulate the direction of cell division perpendicular to the epithelial sheet, depicted as the Z-direction shown in Figure 1A and D (resembling asymmetric cell division known from stem-cell biology and neuronal development[85, 86]). Figure 1D shows a cell division in Y-direction, parallel with the plane of the epithelium, and one in the Z-direction, parallel to apical-basal polarity.

The Par3/Par6 complex, including Cdc42 and aPKC, is required for establishing and maintaining polarized cortical domains in a wide variety of epithelia[87-90]. Thus this complex is implicated in apical-basal polarity and also regulates OCD[91]. Additionally, signaling complexes connected to adherens junctions, and cell-extracellular matrix contacts also provide input for the orientation of cell division[92-95]. At these locations, polycystin-1 and 2 modulate signaling of multi-protein complexes[6].

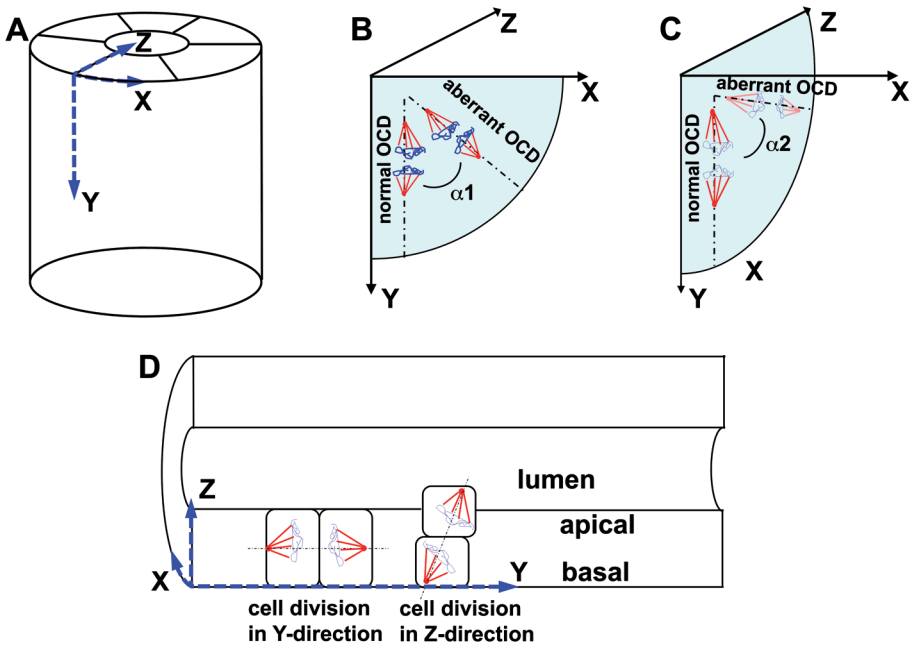

Figure 1. Oriented and misoriented cell division in renal tubules. (A) Cell division within renal tubular epithelium is possible in three directions: in the direction of the Y-axis; parallel to the longitudinal axis of the tubule; in direction of the X-axis parallel to basal membrane and perpendicular to the tubular longitudinal axis or in the direction of the Z-axis; perpendicular to the basal membrane and the tubular longitudinal axis (B) Aberrant OCD with α1 describing the deviation from normal OCD in X-direction within the XY-plane (in the plane of the epithelium). (C) Aberrant OCD with α2 describing the deviation from normal OCD in Z-direction within the YZ-plane (out of the plane of the epithelium). B and C both depict axis as represented in the vertical tubule shown in A. (D) Correct OCD in Y-direction (left) and aberrant OCD with a deviation into Z-direction (right) i.e. cell division out of the epithelial plane. OCD: oriented cell division.

Signaling pathways involved in cyst expansion

A large variety of cellular changes in cyst-lining cells has been observed, and in the last decade it became clear that the polycystins transmit extracellular signals to the nucleus via multiple signaling pathways. A prominent role in cyst growth has been proposed for reduced Ca^{2+} influx, cAMP accumulation and aberrant Ras/Raf/ERK activation[96, 97]. Other proposed mechanisms involve activation of G-proteins, mTOR, PI3-kinase, Jak2-STAT1/3, NFAT (nuclear factor of activated T-cells), and NF-kB (nuclear factor kappa B) signaling[98-100]. It cannot be excluded that some of these pathways or second messengers, e.g. Ca^{2+}, are involved in the early initiation steps of cyst formation. However, these pathways are to a large extend involved in proliferation, and thereby in cyst expansion.

Tubulointerstitial fibrosis is a major process contributing to the pathology towards end-stage renal disease and is a feature of later stages in ADPKD disease progression. A key regulator of fibrosis is the TGFβ-signaling cascade. Indeed, in human tissues and in mouse models phosphorylation and

nuclear translocation of the TGFβ cascade actor, SMAD2 has been detected in more progressive but not in early stages of the disease[101].

Recent data also indicated the involvement of the Hippo pathway in renal cystic disease. This pathway was first known to be important in organ and tissue size control in *Drosophila*. The core components of the *Drosophila* Hippo pathway are well conserved and also function as tumor suppressors in mammals in addition to regulating growth in terms of organ size control[102]. The Hippo signaling pathway exerts its actions in mammals by controlling the transcription of genes involved in proliferation and apoptosis through the transcriptional co-activator and final effector molecule, YAP and its ortholog TAZ.

In PKD, Hippo signaling showed altered activity[103]. YAP, a transcriptional co-activator and final effector molecule of the Hippo pathway, was observed in nuclei of dilated tubules and cyst in injury-induced *Pkd1*-deletion mice. Even more, disturbed Hippo signaling caused by, e.g. deregulation of YAP[104, 105] or TAZ (Wwtr1) deficiency[105-107], resulted in cyst formation. The observed altered activity of the Hippo pathway can provide a proliferative driving force for cyst expansion. Fascinatingly, YAP/TAZ are transcriptional co-activators for a variety of transcription factors, e.g. TEAD, Glis3, SMAD, ErbB4-CTF, p73, Runx2, many of them implicated in polycystic kidney disease[69, 102, 108-110]. Also it has been reported that the canonical Wnt signaling, one of the signaling cascades altered in cystic epithelia, is modulated by Hippo–signaling[49, 68, 69, 111].

As PCP signaling provides directional information, Hippo signaling is known to control organ size, and possibly provides information about the number of tubular circumferential cells. In other words, PCP signaling tells which direction, Hippo signaling dictates how many cells in each direction. In order to coordinate these processes there must be crosstalk between the Hippo-pathway and the PCP pathway. In fact, crosstalk between these pathways is possible since the upstream regulators of the Hippo pathway are shared with the PCP pathway; i.e. Fat, Four-jointed and Dachsous[112].

Although, nuclear YAP was not observed in tubular epithelial cells prior to tubular dilation and cyst formation[103], it cannot be excluded that reduced activity of the Hippo pathway is already involved in the initiation of cyst formation. As mentioned above, Fat4 is in addition to a PCP component, also a regulator of the Hippo pathway. Therefore, it is possible that cyst formation in *fat4*$^{-/-}$ mice may in fact be attributed to a disbalance of Hippo and PCP signaling[11].

Also in tissue regeneration the Hippo pathway is involved. Nuclear and strong cytoplasmic accumulation of YAP is observed during epithelial regeneration in the gut[113] and a similar phenomenon was observed for the kidneys[103]. Overall these data imply that the delicate interplay between PCP and Hippo signaling may also be important for tissue regeneration after injury.

Future prospects in ADPKD research

The animal models showed us the vastness and complexity of signaling pathways involved in cyst formation and disease progression. These pathways act in a concerted fashion as components of an interconnected signaling network. Experimental data also provided us, sometimes, with apparently conflicting results, which can mostly be explained by differences in experimental conditions and data interpretation. The conflicting results concerning PCP-signaling demand us to systematically (re-)analyze our data concerning the orientation of mitotic spindle axis. This will enable us to refine our hypothesis further regarding the role of PCP-signaling in the initiation of cyst formation, and help us dissect the role of the pathways implicated.

The complexity of the involved signaling networks suggest that it may be too ambitious and probably naive to think that prevention of cyst formation and delay of renal failure can be accomplished by targeting a single pathway. In patients cyst formation is not synchronized and areas with different phases of the disease can be found in the kidneys of an individual patient. Probably, therapy with drugs that target more than one signaling pathway will be necessary and probably more successful than treatment with single-target medication.

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