JAK-STAT 1:3, 216–218; July/August/September 2012; © 2012 Landes Bioscience

Is suppression of cyst growth in PKD enough to preserve renal function?

STAT6 inhibition is a novel promising target

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Keywords: ADPKD, signal transduction, cyst formation, STAT6 inhibition, cell proliferation

Submitted: 06/08/12

Revised: 07/26/12

Accepted: 07/26/12

http://dx.doi.org/10.4161/jkst.21634

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Commentary to: Olsan EE, Mukherjee S, Wulkersdorfer B, Shillingford JM, Giovannone AJ, Todorov G, 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 2011; 108:18067–72; PMID:22025716; http://dx.doi.org/10.1073/pnas.1111966108

The autosomal dominant form of polycystic kidney disease (ADPKD) is one of the most frequent monogenic disorders and the most frequent among inherited kidney disorders. In fact it has a prevalence in the population of about 1/1,000 individuals, therefore it does not even satisfy the definition for rare diseases. It is mainly characterized by the formation of multiple cysts filled with fluid that over time develop in number and size leading to the distraction of the structure and function of the kidneys and eventually leading to chronic kidney disease/end stage kidney disease (CKD/ESKD), usually between the 4th and 7th decade of life. There are two known forms of the autosomal dominant type of polycystic kidney disease, type 1 and type 2, caused by mutations in the *PKD1* and *PKD2* genes, located on chromosomes 16 and 4 respectively. The polycystin 1 protein, encoded by *PKD1* and mutated in ~85% of patients, is a huge protein of 4,302 amino acids with multiple transmembrane domains, 200 residues intracytoplasmic part and a huge extracellular part with multiple Ig-like PKD repeats, which probably acts as a receptor to an unknown ligand. Polycystin 1 has been shown to interact with and participate in multiple signal transduction pathways, including the G-protein coupled receptor, cAMP pathway, Wnt, mTOR, MAPK/ERK, AP1 and JAK-STAT pathway, while its intracytoplasmic C-terminal domain has been shown to be cleaved and translocated to the nucleus where it plays a role in gene transcription, in concert with P100 and STAT6.[1]

The polycystin 2 protein is a much smaller protein of 960 amino acids and mutations in this protein are responsible for about 15% of cases of ADPKD. It causes a milder form of the disease as regards later age of onset of ESKD and fewer complications. It has been shown to act as a calcium channel protein, but also it has been shown to interact with polycystin 1 through the intra-cytoplasmic C-terminal coiled-coil domains of both proteins.

We and others have shown that at least in some cases, the inherited germinal mutation in either the *PKD1* or the *PKD2* gene may be a necessary but not sufficient event for cystogenesis. Specifically, it has been shown that second hits as acquired somatic mutations in the form of loss of heterozygosity (LOH) or classical mutations in the allele inherited from the healthy parent, or even mutations in the other implicated gene (trans-heterozygous second hits), are found in the DNA of tubular epithelial cystic cells.[2] Recent studies highlight an important distinction in the mechanism of ADPKD pathogenesis. The process of cyst formation and cyst growth or expansion is regulated by different cellular pathways. Interestingly, in a rat model of PKD we showed that cyst formation precedes hyper-proliferation of cells, an event that has been closely associated with cyst formation per se.[3] Therefore, the finding of treatments that can effectively cease cyst formation and/or uncontrolled growth, has been the target of many works in the recent years.

Teriflunomide, the active metabolite of leflunomide, is a known tyrosine kinase inhibitor and has been shown to be an

effective inhibitor of transcription factor STAT6. It is an approved drug for the treatment of arthritis. Olsan et al., in a recent manuscript in the *Proceedings of the National Academy of Sciences*, use cell culture systems and murine models of autosomal dominant polycystic kidney disease and show that knockout of STAT6 expression or inhibition of STAT6 activity is able to suppress renal cyst growth and improve overall kidney function.[4] STAT6 is a signal molecule which is activated through phosphorylation, upon activation of IL4R and IL13R receptors which act in concert with JAK tyrosine kinases (**Fig. 1**). Phosphorylated STAT6 is translocated to the nucleus where it acts as a transcription factor in various downstream effects including the T-helper Type II (Th2) cell differentiation and in airway inflammation and airway hyper-responsiveness and mucus production. The same inhibitor of STAT6 phosphorylation had been used as a drug for asthma.[5]

Olsan et al. showed that in total kidney lysates and cyst lining epithelial cells from two murine pkd models, the STAT6 is significantly activated by phosphorylation and this activation is mediated by interleukins IL4 and IL13. Importantly the bpk/bpk model for pkd, when crossed with the STAT6$^{-/-}$ model, showed less severe kidney disease and had preservation of kidney function, alluding to the negative role of STAT6. Importantly, when the mice were administered with a STAT6 inhibitor, leflunomide, it ameliorated renal cystic disease in bpk mice, as evidenced by strong suppression of renal cystic growth, lower kidney weight and cystic index, while renal function was largely preserved as assessed by BUN. Finally, upon treatment with this drug the strong phosphorylated STAT6 signal in untreated cyst lining epithelial cells is nearly ablated in the treated animals, suggesting that this chemical indeed acts through inhibition of STAT6 in the kidney. The authors observed also significant degree of toxicity in young mice which they attributed to the inhibition of pyrimidine synthesis which is the known mechanism of action of this drug. This activity of the drug is reasonably expected to affect more severely young growing animals compared with adult animals, as the authors point out. Another issue that needs to be addressed is the timing of treatment. The authors treat the animals with 1.4 mg/kg teriflunomide every 2 d from postnatal day 7 to day 21. This entails that if such a treatment is translated to humans, the drug needs to be administered at a very young age in order to be effective. This in conjunction with the vast molecular targets of the drug and especially its effect on T and B cell differentiation may weaken leflunomide potential as therapy for PKD.

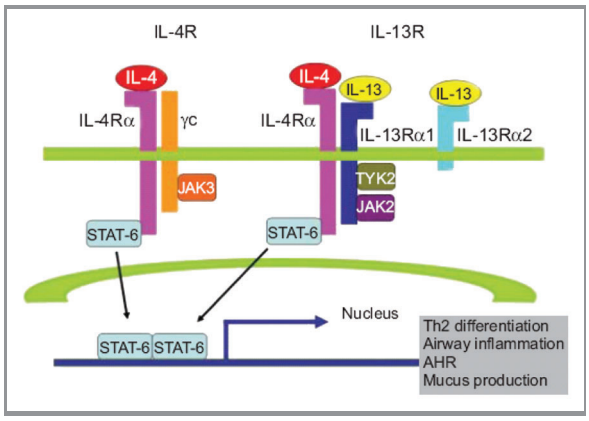

Figure 1. Schematic diagram of the interleukin (IL)-4/IL-13/signal transducer and activator of transcription factor (STAT)-6 signaling pathways. Both IL-4 and IL-13 signal via the IL-4Ra, a component of the type I (IL-4Ra and γc) and type II receptors (IL-4Ra and IL-13Ra1). IL-4 signals via both type I and II receptor pathways, whereas IL-13 signals only via the type II IL-4R. IL-13 also binds to the IL-13Ra2 chain, which does not contain a transmembrane-signaling domain and is thought to act as a decoy receptor. γc activates Janus kinase (JAK)3, whereas IL-13Ra1 activates tyrosine kinase 2 (TYK2) and JAK2. Activated JAKs then phosphorylate STAT-6. Phosphorylated STAT-6 dimerizes, migrates to the nucleus, and binds to the promoters of the IL-4 and IL-13 responsive genes, such as those associated with T-helper type 2 (Th2) cell differentiation, airway inflammation, airway hyperresponsiveness (AHR) and mucus production. Reproduced with permission of the European Respiratory Society (Eur Respir Rev March 2010 19:46–54; doi:10.1183/09059180.00007609)[5]

Researchers attempted repeatedly to treat polycystic kidney disease using substances with relevant pharmacological activity. Previous attempts to use rapamycin, an inhibitor of mTOR (everolimus, sirolimus) provided mixed results on clinical trials.[6] In fact it was quite disappointing that treatment with everolimus had a very positive effect on kidney size but not on kidney function. In other words, everolimus was able to retard the growth of cystic kidneys in patients with ADPKD but was not significantly able to slow the decline in renal function, while it was also associated with a high rate of side effects, similar to those observed in patients who were administered everolimus after undergoing kidney transplantation. It is interesting that the growing of renal cysts per se is not necessarily inherently associated or absolutely associated with a loss of kidney function. In a previous work we showed that cyst formation in a pkd2 transgenic rat model, precedes deregulation of proliferation related pathways, thereby indicating that cell proliferation and perhaps other pathways related to loss of kidney function, start later than the noticeable phenomenon of cysts formation.[3]

It is very comforting that basic research of this kind identifies potential targets for treatment aimed at preventing renal cyst formation and decline of renal function. It is even more encouraging that the availability of several murine and rat models allows investigators to perform a significant amount of work before moving to clinical trials with human subjects. Not withstanding this availability, however, it turns out that the positive results seen in animals do not automatically reflect expected promising results in humans.

Will it be possible to modify this STAT6 inhibitor molecule and remove its toxic activity while maintaining its cyst

growth suppression activity? Will leflunomide display the same effectiveness in older animals? Is cyst suppression activity an adequate read-out point to go after for PKD treatment in humans? As mentioned before, previous similar experience was unfortunately gravely disappointing. One hopes that an effective future treatment would be admittedly safe and easy to administer fairly early and able to guarantee the absolute prevention of onset of renal failure.

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