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Can we cure HIV-1 associated nephropathy in transgenic mice?

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Abstract

HIV-1 associated nephropathy is a rapidly progressive form of focal segmental glomerulosclerosis typically seen in patients of African ancestry. HIV-transgenic mice can develop an HIVAN-like renal disease. Zhong *et al.* show that the oral administration of a cyclic nucleotide phosphodiesterase 4 inhibitor and a retinoic acid receptor-alpha agonist can prevent the development of HIVAN in transgenic mice, acting through a cAMP dependent mechanism that is independent of HIV-1 genes. These findings suggest that endogenous host factors play a critical role in HIVAN.

The late physician-scientist Dr. Judah Folkman, considered by many to be the father of anti-angiogenic cancer therapies, showed that certain types of experimental cancers in mice could be cured by disrupting their blood supply without directly attacking the tumor cells. His revolutionary treatment is currently being tested in patients with angiogenic tumors, including AIDS-Kaposi's Sarcoma (KS). Although the pathogenesis of HIV-associated nephropathy (HIVAN) is clearly different from AIDS-KS, HIVAN is characterized by the excessive proliferation of renal glomerular and tubular epithelial cells, leading to the microcystic transformation of renal tubules and rapid development of focal segmental glomerulosclerosis (FSGS). Therefore, the new study by Zhong *et al.*¹ (this issue), challenges us to ask a similar type of question: Can we cure HIVAN in transgenic (Tg) mice without attacking the HIV-1 genes?

Briefly, the study by Zhong *et al.* show that the oral administration of two drugs that increase the intracellular levels of cAMP, Roflumilast and Am580, are capable of curing HIVAN in Tg mice without affecting the expression of HIV-1 genes¹. Roflumilast is a cyclic nucleotide phosphodiesterase 4 (PDE4) inhibitor that increases the intracellular concentration of cAMP by blocking its degradation. Am580 is a specific retinoic acid receptor-alpha (RAR α) agonist that has several systemic and renal effects, including increasing the intracellular levels of cAMP. Both drugs, acting in a synergistic manner, prevented the progression of the renal disease in HIV-Tg mice by blocking the de-differentiation and proliferation of podocytes through a cAMP/PKA/CREB dependent

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None

pathway¹ (Fig. 1, red color). This pathway requires the participation of the RAR- α in the nucleus of podocytes, in addition to the direct activation of cAMP and PKA in the cytoplasm of these cells. The mechanism by which retinoic acid receptor agonists induce a direct activation of cAMP in the cytoplasm is not well understood, but similar findings have been documented in acute myeloblastic leukemia cells¹. However, Am580 did not prevent the progression of HIVAN in dual HIV-Tg / RAR α knock-out mice², suggesting that the nuclear RAR α plays a key role in this process. Thus, more studies are needed to determine how the RAR α may induce the rapid activation of cAMP in the cytoplasm, and to validate this pathway in normal human podocytes. Finally, Zhong *et al.* concluded that the beneficial effects of Roflumilast and Am580 were independent of HIV-viral genes. Overall, these provocative findings raise several interesting issues that need to be discussed.

First, we should mention that Zhong *et al.* used a new colony of HIV-Tg mice that was re-derived from the parent HIV-Tg₂₆ mouse line. Therefore, to understand how HIVAN can be cured in these mice, it is necessary to review the natural history of the renal disease in Tg₂₆ mice. Briefly, these mice carry a replication-deficient gag/pol PNL4-3 HIV proviral genomic DNA driven by the endogenous HIV-1 promoter³⁻⁴. The mRNA expression of HIV-1 is detected in a wide range of cells, including glomerular and tubular epithelial cells³⁻⁵. Viral proteins cannot however, be detected in the circulation or peripheral blood mononuclear cells. Heterozygous mice, are born with normal kidneys, but develop progressive renal disease during their first months of life³⁻⁴. Usually, male mice develop a more rapid and progressive renal disease when compared to female mice³⁻⁵. Severe edema and nephrotic syndrome occur approximately between 60 and 250 days of age³⁻⁵. At this time point, Tg₂₆ mice usually showed BUN levels exceeding 200 mg/dl and elevated serum creatinine (> 1 mg/dl) and die of uremia and ascitis³⁻⁴. However, not all mice develop renal disease, and others do it in an asynchronous and unpredictable manner³⁻⁵. Thus, a remarkable finding of the study of Zhong *et al.* is that all untreated control HIV-Tg mice, both males and females, developed chronic renal failure in a synchronized manner during the first nine weeks of life. Moreover, the prevalence of the renal disease in the control group was increased relative to the parent Tg₂₆ mouse line³⁻⁴, and to the expected prevalence (~ 80%) in the re-derived mouse colony. We should considered that Zhong *et al.* pre-selected mice at four weeks of age based on urine dipsticks protein values of 1+ / 2+ (30 –100 mg/dl). However, similar levels of proteinuria can be detected in Tg₂₆ mice without renal disease³, due to the normal excretion of low molecular weight tubular proteins and pheromones. In summary, one limitation of the study by Zhong *et al.* is that we do not know how many mice in each group had pre-existing renal disease at the beginning of the study.

Another limitation of this study is the definition of chronic renal failure (BUN levels > 30 mg/dl). Previous publications in Tg₂₆ mice reported similar BUN levels in mice without renal disease, and used cut-off BUN values above 45 mg/dl³⁻⁴. These BUN values, revealed a much better correlation with the renal histological findings associated with chronic renal failure, showing all glomeruli with segmental or global glomerulosclerosis in combination with microcystic tubular dilatation³⁻⁴. In contrast, 30% to 40 % of the mice treated with Roflumilast or Am580 alone, were reported as having chronic renal failure, but showed only “mild” glomerular sclerotic changes, very few collapsed glomeruli, and mild tubulo-

interstitial injury scores. The data presented by Zhong *et al.* also appear to exclude the possibility that these mice suffered weight loss, or pre-renal failure secondary to drug-induced diarrhea, dehydration, severe edema, nephrotic syndrome, or ascitis. Thus, it is unclear why these mice developed chronic renal failure with such renal histological and clinical findings. Moreover, a previous study done by the same group, showed that a more prolonged treatment with Am580 alone was capable of preventing the progression of HIVAN in Tg₂₆ mice². Finally, for the purpose of comparing the outcome of the renal disease in mice belonging to the original Tg₂₆ line³⁻⁴ vs. those used in this study, both the renal histological and clinical findings at nine weeks of age appear to be more severe in Tg₂₆ mice³⁻⁵.

Nevertheless, when the findings of Zhong *et al.* are considered in the context of previous studies⁶⁻⁸, it is clear that Roflumilast and Am580 have the potential to improve the outcome of HIVAN acting through different mechanisms and renal cell types (Fig.1). For example, Zhong *et al.* began their treatment when mice were four weeks of age. Renal electron microscopy studies done in Tg₂₆ mice with renal disease at this early time point, show effacement of foot processes, expansion of the mesangial matrix narrowing the lumen of glomerular capillaries, simplification and atrophy of tubular epithelial cells, and thickening of glomerular and tubular basement membranes^{3,5}. No renal epithelial proliferative changes however, are detected at this early stage^{3,5}. Thus, in addition to the pro-differentiation changes in podocytes described by Zhong *et al.*, these drugs have the potential to improve the outcome of HIVAN by blocking the synthesis and accumulation of renal extracellular matrix proteins. Previous studies suggest that these changes can be mediated by cAMP and/or TGF- β dependent mechanisms⁸⁻⁹. In addition, blocking the synthesis of heparan sulfate proteoglycans (Fig. 1), may prevent the renal accumulation of heparin binding growth factors, including VEGF-A and FGF-2, which can induce renal proliferative changes during the late stages of HIVAN in Tg₂₆ mice⁵. Moreover, previous studies showed that drugs that increase the intracellular levels of cAMP, can also decrease the mitogenic activity of FGF-2 in cultured human primary renal epithelial cells by inhibiting the MAPK signaling pathway¹⁰ (Fig 1). In summary, it is tempting to speculate that retinoids, in combination with PDE4 inhibitors, may ameliorate the progression of HIVAN independently of HIV-genes, by blocking the de-differentiation of podocytes and the synthesis of extracellular matrix proteins during the early stages of the renal disease, and by preventing the mitogenic activity of heparin binding growth factors during the late stages of HIVAN.

Finally, Roflumilast and Am580 have powerful systemic effects, which could also modulate the outcome of HIVAN in humans and transgenic mice. For example, retinoids can decrease the blood pressure of mice with renal disease⁸, and may improve their renal outcome by modulating the activity of NO synthase, endothelin-1, and/or angiotensin II type 1 receptors⁶. Most notably, these drugs have powerful anti-inflammatory activity, and can affect the recruitment of inflammatory cells, as well as the transcription of inflammatory genes acting through AP-1 or NF κ B related mechanisms⁶. (Fig.1). Roflumilast also inhibits the expression of the cytokine monocyte chemoattractant protein 1 (MCP-1), and the generation of reactive oxygen species⁷. Based on its anti-inflammatory activity, Roflumilast was approved by the FDA to treat flares of chronic obstructive pulmonary disease.

Unfortunately, this drug cannot be used in patients with liver disease, and has known adverse interactions with several HIV-medications. Alternatively, retinoids are being used in clinical trials to treat skin lesions and tumors, including AIDS-KS, and in patients with minimal change disease or FSGS. Overall, we hope that these studies will provide valuable information to assess the clinical value of Roflumilast and Am580 in patients with HIVAN.

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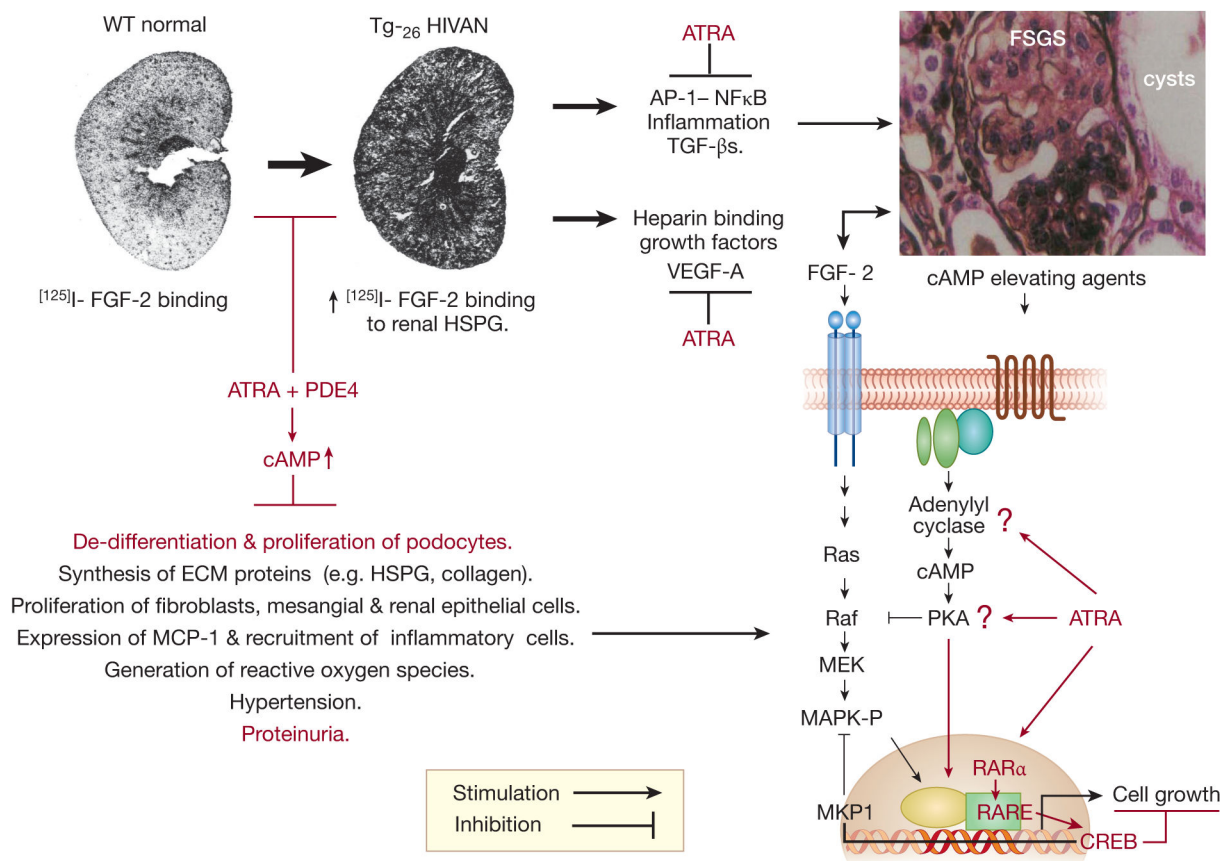


Figure 1. A summary of the possible roles that all-trans retinoic acid (ATRA) and Roflumilast (PDE4) may have to prevent the progression of HIVAN in HIV-Tg₂₆ mice

Zhong *et al.*¹ showed that ATRA + PDE4 prevented the progression of HIVAN in Tg mice by blocking the de-differentiation and proliferation of podocytes through a cAMP/PKA/CREB dependent mechanisms that is independent of HIV-1 genes (highlighted in red color). Based on the pathogenesis of the renal disease in Tg₂₆ mice³⁻⁵, and the known beneficial effects of these drugs in other experimental models of renal diseases⁶⁻⁸, we have highlight additional pathological pathways (black color) that might be affected as well. Abbreviations: ATRA = all-*trans* retinoic acid analogs; PDE4 = cyclic nucleotide phosphodiesterase-4 inhibitor; cAMP = cyclic adenosine monophosphate; FGF-2 = Fibroblast Growth Factor-2; HSPG = heparan sulfate proteoglycans; ECM = extracellular matrix proteins; MCP-1 = Monocyte Chemoattractant Protein 1; AP-1= Activator Protein-1; NFκB = Nuclear Factor Kappa B; TGF-β = Transforming Growth Factor Beta; VEGF-A = Vascular Endothelial Cell Growth Factor-A; FSGS= Focal Segmental Glomerulosclerosis; Ras = rat sarcoma G protein; Raf = rapidly accelerated fibrosarcoma protein kinase; MEK = mitogen activated protein kinase / Erk kinase; MAPK-P = mitogen-activated protein kinase - phosphorylated / ERK-P; MKP-1 = mitogen activated protein kinase phosphatase-1; PKA = protein kinase A; RAR α = retinoic acid receptor alpha; RARE = retinoid acid responsive element; CREB = cAMP responsive element binding protein.