Hypoxia hits APOL1 in the kidney

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Individuals of African ancestry carrying two pathogenic variants of apolipoprotein 1 (*APOL1*) have a substantially increased risk for developing chronic kidney disease. The course of APOL1 nephropathy is extremely heterogeneous and shaped by systemic factors such as a response to interferon. However, additional environmental factors operating in this second-hit model have been less well defined. Here, we reveal that stabilization of hypoxia-inducible transcription factors (HIF) by hypoxia or HIF prolyl hydroxylase inhibitors activates transcription of *APOL1* in podocytes and tubular cells. An active regulatory DNA-element upstream of *APOL1* that interacted with HIF was identified. This enhancer was accessible preferentially in kidney cells. Importantly, upregulation of APOL1 by HIF was additive to the effects of interferon. Furthermore, HIF stimulated expression of *APOL1* in tubular cells derived from the urine of an individual carrying a risk variant for kidney disease. Thus, hypoxic insults may serve as important modulators of APOL1 nephropathy.

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Lay Summary

Genetic variants in the gene apolipoprotein 1 (*APOL1*) can lead to chronic kidney disease. Other factors, such as autoimmune diseases or viral infections, influence the course of kidney disease in patients with risk variants. These factors are incompletely understood. Here, we found that low oxygen levels (hypoxia) and drugs that stabilize hypoxia-inducible transcription factors (HIFs) activate transcription of *APOL1* in kidney cells. This regulation was specific for kidney cells and was additive to effects of interferon, another inducer of APOL1. In addition, HIF stimulated the expression of risk variants of *APOL1* in tubular cells that were collected from the urine of a patient. We conclude that hypoxic conditions may serve as important modulators of *APOL1*-associated kidney disease.

The presence of 2 alleles of the coding variants G1 and G2 in the apolipoprotein L1 (*APOL1*) gene increases the risk of developing chronic kidney disease dramatically, with odds ratios ranging from 2.6 to 29, depending on the underlying condition.[1] For example, APOL1 nephropathy manifests as focal segmental glomerular sclerosis or HIV-associated nephrosclerosis in patients with sub-Saharan African ancestry. In these individuals, the frequency of carrying 2 risk alleles is ≈13%.[2] Mechanistically, *APOL1* risk variants are supposed to exert a gain of dysfunction of the APOL1 protein, which affects molecular processes, such as mitochondrial function, inflammasome activation, endoplasmic reticulum stress, autophagy, and ion transport.[2] Thus, any stimulus augmenting the concentration of the APOL1 risk variants will have significant impact on the course of kidney disease. In this respect, high levels of interferons caused by either viral infection or therapeutic application (e.g., in patients with hepatitis C) led to development of collapsing focal segmental glomerular sclerosis in *APOL1* risk carriers.[3] Interestingly, only a minority of risk allele carriers develop APOL1 nephropathy. This supports the hypothesis of a 2-hit model requiring the presence of *APOL1* risk alleles and contribution of environmental or systemic factors, such as viral infection, to initiate and promote APOL1 nephropathy. Apart from the interferon response, there is little evidence so far for the

involvement of other factors influencing *APOL1* expression. The risk of developing APOL1 nephropathy persists in kidney allografts from *APOL1* risk variant–positive donors.[4] This indicates that the deleterious effects of *APOL1* risk variants are generated within the cells of the kidney and, thus, any factors operating on *APOL1* expression in renal cells will modify the risk of developing kidney disease. In the kidney, podocytes, endothelial cells, and proximal tubular cells have been identified to express *APOL1*.[2] In many kidney diseases, including acute kidney injury, inflammation, and fibrosis, these cells are exposed to hypoxia, which leads to stabilization of hypoxia-inducible factors (HIFs).[5] For example, HIF stabilization specifically in podocytes causes nephritis in mice, indicating a prominent role of HIF in this compartment.[6] Recently, HIF stabilizers have been approved for therapy of renal anemia in patients with chronic kidney disease.[7] These compounds were developed to stabilize HIF-2α in interstitial cells of the kidney and to induce the HIF target gene erythropoietin. Here, we explore the effects of hypoxia and HIF stabilization on the regulation of *APOL1* expression in renal cells.

SHORT METHODS

Cell culture

The human podocyte cell line was a gift from M. Saleem and cultured in supplemented Roswell Park Memorial Institute medium. Proliferating podocytes were cultured under 33 °C; for differentiation, subconfluent dishes were transferred to 37 °C and cultured for 10 days. Human urinary primary tubular cells (PTCs) were isolated from a patient with a G0/G1 *APOL1* genotype following a published protocol.[8] HEK293T cells expressing APOL1 G0, G1, and G2 variants lacking the signal peptide under the control of a doxycycline-inducible promoter were cultured in standard Dulbecco's modified Eagle's medium.[9]

Western blotting

Cells were lysed in urea/sodium dodecylsulfate buffer, and proteins were resolved by sodium dodecylsulfate–polyacrylamide gel electrophoresis. Proteins were detected using an HIF-1α antibody (Cay10006421; Cayman Chemicals), an HIF-2α antibody (AF2997; R&D Systems), an APOL1 antibody (Ab108315; Abcam), and a β-actin antibody (A3854; Sigma Aldrich). Horseradish peroxidase–conjugated secondary antibodies were used as applicable (Dako, Agilent Technologies).

Assay for transposase-accessible chromatin

Assay for transposase-accessible chromatin (ATAC) experiments were performed as previously described.[10] A total of 60,000 cells were directly subjected to the Omni-ATAC protocol, as described by Corces *et al.*[11]

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) experiments were performed using the ChIP-IT High Sensitivity kit (Active motif). For immunoprecipitations, 20 μg of chromatin and 3–6 μl of antibodies directed against HIF-1α (Cay10006421; Cayman Chemicals), HIF-1β (NB100-110; Novus Biologicals), HIF-2α (PM9; kind gift from Professor Sir Peter Ratcliffe, Oxford), or H3K27ac (ab4729; Abcam) were used.

RESULTS

To test whether hypoxia controls expression of *APOL1*, we performed transcriptomic analysis of podocytes cultured under hypoxic (16 hours, 1% O_2) or control conditions using RNA sequencing. Strikingly, differential gene expression analysis revealed that according to adjusted *P* values, *APOL1* is the second most significantly induced gene under hypoxic conditions in these cells (Figure 1a). Exposure of podocytes to the HIF-hydroxylase inhibitor dimethyloxalylglycine (DMOG) also increased APOL1 mRNA significantly, indicating involvement of HIF in *APOL1* transcriptional regulation (Supplementary Figure S1). Increased mRNA levels also translated into higher protein levels of APOL1 (Figure 1b). Similar results were obtained in a series of primary tubular cells isolated from non-diseased regions of tumor nephrectomy specimens, indicating a conserved regulation in a broader spectrum of renal cells (Supplementary Figure S2). To confirm the involvement of HIF, we transfected siRNA against HIF subunits (HIF-1α and HIF-2α) into podocytes and measured expression of *APOL1* (Figure 1c; Supplementary Table S1). Induction of APOL1 protein by DMOG was reduced especially in HIF-1α–depleted podocytes (Figure 1c). Similar results were obtained in podocytes targeting HIF using Clustered regularly interspaced palindromic repeats/Cas9 (CRISPR/Cas9) protein together with HIF-subunit specific guide RNAs and in tubular cells using siRNA against HIF (Supplementary Figure S3). To evaluate the overlap of HIF and APOL1 mRNA in different renal cell types, we reanalyzed published single-cell RNA-sequencing (scRNA) data from nondiseased kidneys.[12] This analysis confirmed a high degree of overlap of HIF and APOL1 mRNA, especially in podocytes, vasculature, and, to a lesser extent, tubular cells, which provides a prerequisite for the observed regulation of *APOL1* expression by hypoxia in cells of the human kidney (Figure 1d). Recently, analysis of acute kidney injury in humans by scRNA-seq revealed remarkable transcriptomic changes, especially in tubular cells of the injured kidneys.[13] Hypoxia signaling was among the most significant upregulated pathways in this compartment. We reanalyzed the available data and determined significantly increased levels of APOL1 mRNA in tubular cells from injured kidneys compared with control kidneys, indicating that the aforementioned regulation of *APOL1* by hypoxia occurs *in vivo* (Supplementary Figure S4).

To further explore the role of HIF in regulating *APOL1* expression, we generated HIF ChIP-sequencing data from podocytes exposed to DMOG and observed HIF-DNA interactions of both HIF α-subunits and the β-subunit ≈3 kb upstream of the *APOL1* promoter (Figure 1e). The identified putative regulatory DNA element displayed marked accessibility and activity, as determined by ATAC sequencing and H3K27ac ChIP sequencing, respectively. Interestingly, when comparing ATAC-sequencing data from podocytes with DNAse hypersensitive sites defined in 733 biosamples,[14] we noted open chromatin preferentially in samples from the renal cluster of these samples, suggesting the existence of a kidney-associated enhancer at this position (Figure 1e). Thus,

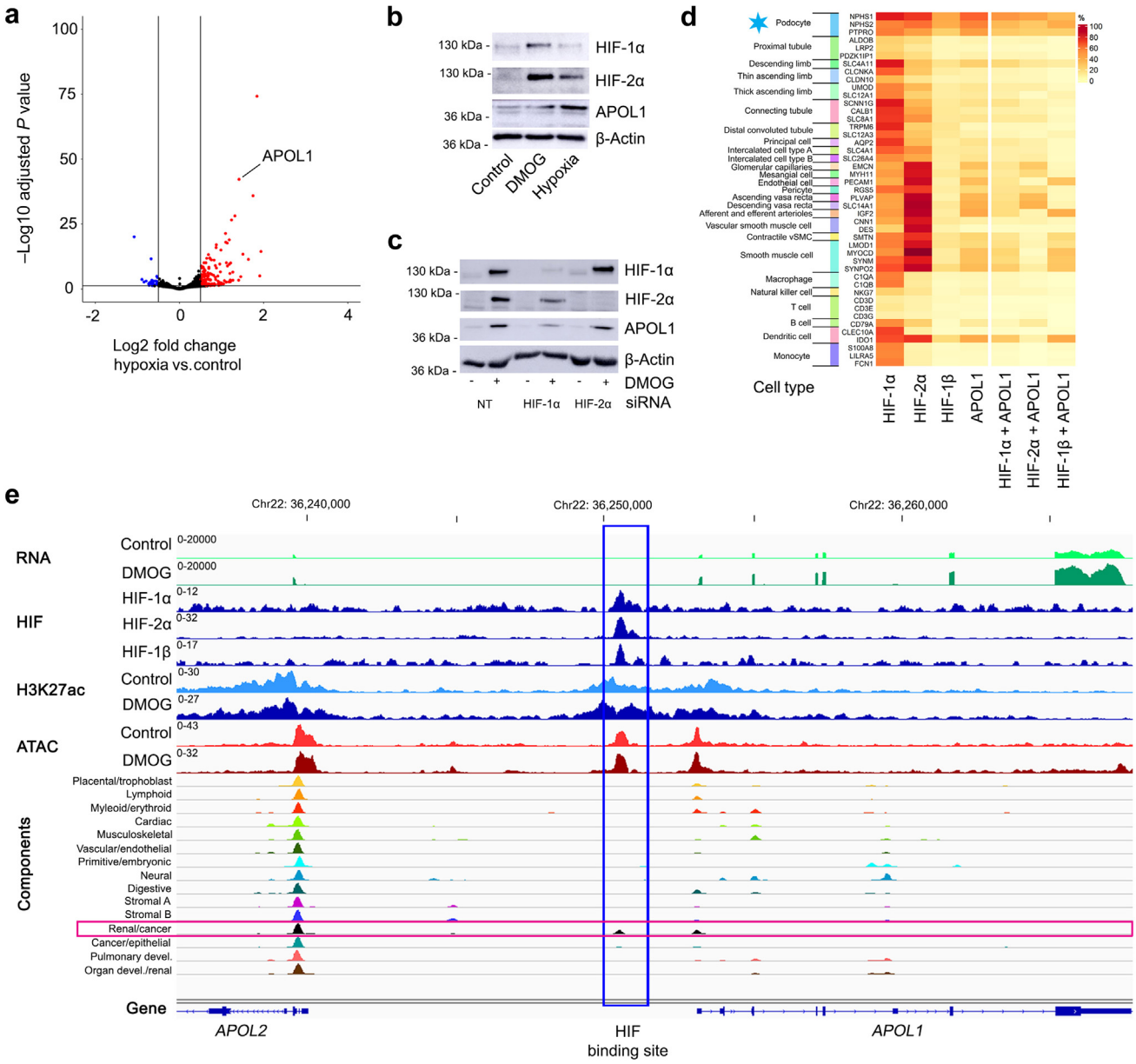

Figure 1 | Hypoxia-inducible factor (HIF) regulates APOL1 expression. (**a**) Volcano blot showing log2 fold change (16 hour of hypoxia at 1% O_2 versus control conditions) of mRNA-sequencing (seq) values in human podocytes on the x axis. The y axis indicates –log10 adjusted *P* value, as calculated by the Benjamini-Hochberg approach in DESeq2. The APOL1 transcript is indicated. (**b**) Immunoblot analysis for HIF-1α, HIF-2α, APOL1, and β-actin in lysates from podocytes exposed to control conditions, 1 mM dimethyloxalylglycine (DMOG), or hypoxia (1% O_2) for 16 hours. (**c**) Immunoblot analysis of lysates from podocytes depleted for the indicated HIF isoform using siRNA. Cells were exposed to 1 mM DMOG or control conditions for 16 hours before harvest. (**d**) Cells for each renal compartment were identified using the indicated marker genes from single-cell RNA-seq data of the kidney. Heat map displays percentage of cells expressing mRNA of the different HIF subunits or *APOL1* as well as overlap of expression of HIF subunits and *APOL1*. The blue asterisk indicates markers for podocytes. (**e**) RNA-seq, HIF, and H3K27ac chromatin immunoprecipitation (ChIP)-seq as well as assay for transposase-accessible chromatin (ATAC)–seq tracks at the *APOL1* gene locus generated from podocytes stimulated with 1 mM DMOG or cultured under control conditions. RNA-seq indicates increased expression of APOL1 mRNA under DMOG conditions compared with control. HIF ChIP-seq tracks (only DMOG condition) reveal HIF DNA-interactions with a promoter-distal region of *APOL1*. This site overlaps active (H3K27ac ChIP-seq) and open chromatin (ATAC-seq) signals in podocytes. The enhancer (blue vertical bar) coincides with accessible chromatin defined by DNAse1 hypersensitivity in the compartment "renal/cancer" from the 733 biosamples set (red horizontal bar). Chr, chromosome; NT, nontargeting control; Pulmonary devel., pulmonary development and organ development; vSMC, vascular smooth muscle cell.

our data indicate that HIF regulates APOL1 mRNA expression in podocytes and tubular cells from a kidney-associated enhancer.

APOL1 expression in the kidney is triggered by inflammatory stimuli, such as interferon gamma. To test whether HIF signaling interferes with the interferon pathway on

APOL1 expression, we exposed podocytes and tubular cells to DMOG, interferon gamma, or a combination of both (Figure 2a and b; Supplementary Figure S5). Strikingly, a combination of both stimuli induced APOL1 mRNA and protein expression to higher levels than each stimulus alone. This indicates that HIF and interferon gamma regulate expression of *APOL1* independent from each other in renal cells. To further corroborate this finding, we introduced the sequence containing the hypoxia-responsive element of the HIF-binding enhancer into a reporter plasmid. On exposure of the transfected HEK293T cells to DMOG, we measured increased activity of the reporter. This effect was unchanged on interferon gamma treatment, suggesting that interferon gamma does not operate from the HIF-binding kidney-associated enhancer (Figure 2c).

Harmful effects of APOL1 on kidney function are mediated by the 2 *APOL1* risk alleles. We identified one individual heterozygous for the variant G1 and isolated tubular cells from the urine (human urinary PTCs). Again, these cells demonstrated increased APOL1 mRNA expression on HIF stabilization by DMOG (Figure 2d). The allele ratio for *rs60910145* did not change in the cDNA on HIF stabilization, indicating that HIF induces expression from both alleles (Figure 2e).

HIF stabilizers have been licensed for clinical use to treat patients with renal anemia. To evaluate an effect of these compounds on *APOL1* regulation, we exposed human urinary PTCs from the G0/G1 individual to a broad selection of these substances and detected increased levels of APOL1 mRNA and protein in HIF-stabilized cells (Figure 2f and g).

In these experiments, we noticed differences in the potency of the inhibitors in stabilizing HIF-1α and in increasing APOL1 mRNA. For example, 100 μM daprodustat and 100 μM vadadustat led to comparable induction of APOL1 mRNA also in time course experiments in these cells, but daprodustat stabilized HIF-1α protein and increased APOL1 protein more potently, which agrees with an earlier study on the cellular effects of HIF stabilizers (Supplementary Figure S6).[15] When adjusting the amount of daprodustat to lower concentrations (1 and 10 μM) in dose-response experiments in human urinary PTCs and podocytes, effects on HIF stabilization and APOL1 protein induction were comparable to the effects of higher concentrations of vadadustat (Supplementary Figures S7 and S8). The reason why differences in HIF-1α protein levels do not entirely reflect the increases in APOL1 mRNA remain unclear but may point to additional transcriptional or post-transcriptional mechanisms of *APOL1* regulation caused by HIF-stabilizing substances. In this respect, we generally observed higher levels of APOL1 mRNA when treating cells with the pan-hydroxylase inhibitor DMOG, which inhibits prolyl hydroxylase domain (PHD) proteins and factor-inhibiting HIF-1 (FIH-1), an HIF hydroxylase that limits HIF transcriptional activity by catalyzing asparagine hydroxylation at ASN803 of HIF-1α, thereby reducing p300 coactivator recruitment. In line with a role of FIH-1 in regulating *APOL1* expression, a combination of the PHD-specific inhibitor 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetate (ICA) and the FIH-1–specific inhibitor dimethyl N-oxalyl-D-phenylalanine (DM-NOFD) led to increased APOL1 mRNA in PTCs when compared with PHD inhibition alone (Supplementary Figure S9). Thus, *APOL1* appears to be an HIF-1α target gene that is sensitive to FIH-1 regulation.

To gain insights on whether increased levels of APOL1 risk variants and concomitant stabilization of HIF have an impact on cell survival, we resorted to an available HEK293T cell model in which APOL1 variants lacking the signal peptide can be overexpressed by a doxycycline-inducible vector.[9] In these cells, endogenous *APOL1* expression is not regulated by HIF, which allows for an unbiased assessment of effects of simultaneous HIF stabilization and intracellular *APOL1* risk variant overexpression (Supplementary Figure S10). Doxycycline-induced overexpression led to comparable protein levels of G0, G1, and G2 variants in the cells (Supplementary Figure S10). As shown in a previous report, expression of G1 and G2 variants reduced cell viability over time (Figure 3a).[9] Strikingly, exposure of the APOL1-overexpressing cells to the HIF stabilizers roxadustat and vadadustat further decreased cell viability (Figure 3a). The effect of the HIF stabilizers was most prominent in G2-overexpressing cells and not observed in non–doxycycline-induced control cells (Figure 3b). In addition to the regulatory role of HIF in *APOL1* expression, this suggests that coincidence of both stimuli (e.g., in acute kidney injury of patients with a high inflammatory status) can have detrimental effects on renal cell survival.

Taken together, our data suggest that hypoxia and HIF stabilization drive expression of *APOL1* and its pathogenic variants in renal cells and that the presence of HIF together with intracellular APOL1 risk variants has harmful effects on cell survival (Figure 3c).

DISCUSSION

Together with the involvement of HIF in many kidney diseases, our findings are compatible with a model in which hypoxic insults (e.g., observed in acute kidney injuries) drive progression of kidney disease by augmenting expression of *APOL1* risk variants. In this respect, a recent retrospective cohort study among patients with African ancestry in the Million Veteran Program revealed that development and severity of coronavirus disease 2019 (COVID-19)–related acute kidney injury correlated with the presence of 2 APOL1 risk variants.[16] Although the primary insults in this disease would have been viral infection and inflammation, it is tempting to speculate that a hypoxic environment in the acutely injured kidneys promoted expression of the pathogenic variants and progression of kidney disease. Similarly, chronic hypoxia may influence expression of *APOL1* variants. In this respect, it is noteworthy that in patients with sickle cell disease, a condition of chronic hypoxia, APOL1-associated nephropathy, is frequently observed.[17] Our data indicating

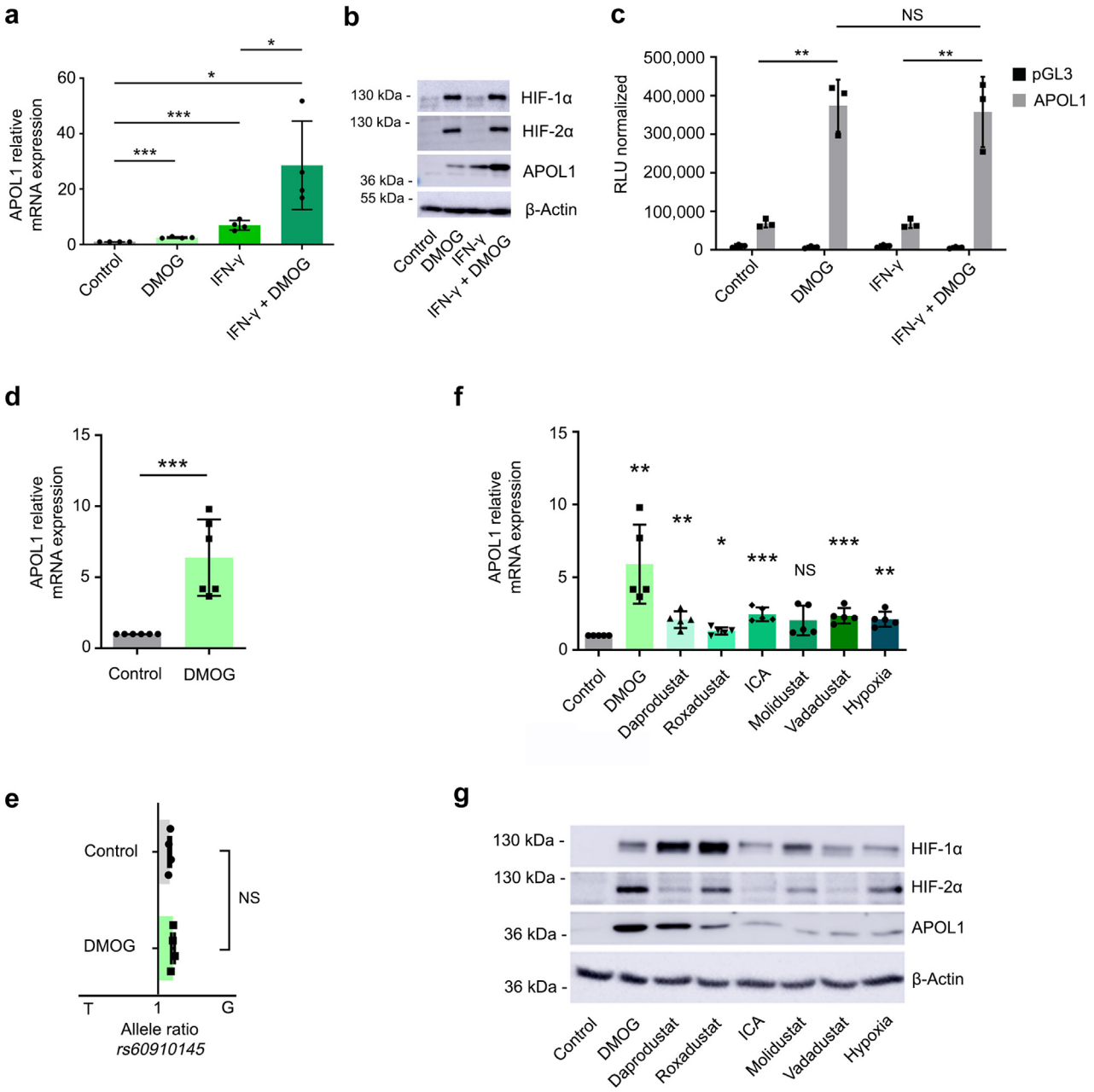

Figure 2 | Hypoxia-inducible factor (HIF) stabilization increases effects of interferon and induces an *APOL1* pathogenic variant. (**a**) Expression quantitative polymerase chain reaction (qPCR) analysis for APOL1 mRNA in lysates from podocytes exposed to dimethyloxalylglycine (DMOG), interferon gamma (IFN-γ; final concentration, 50 ng/ml), or a combination of both stimuli for 16 hours. Data are from n = 4 independent experiments. Mean values ± SD. One-sample *t* test was performed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (**b**) Immunoblot analysis for HIF and APOL1 protein in lysates from podocytes exposed to 1 mM DMOG, IFN-γ, or a combination of both for 48 hours. (**c**) Reporter assay using a sequence covering the HIF-binding site at the *APOL1* gene locus. HEK293T cells were transfected with the pGL3 promoter vector or the pGL3 promoter vector containing the *APOL1* enhancer. Cells were exposed to the indicated conditions, and reporter activity was measured after 16 hours of stimulation. Values of luciferase activity were from 3 independent transfections and normalized to activity of cotransfected β-galactosidase. Mean values ± SD. Student *t* test was performed. **$P < 0.01$. (**d**) Expression qPCR analysis for APOL1 mRNA in lysates from human urinary primary tubular cells (huPTCs) isolated from an individual carrying one allele of G1 variant. Values are mean ± SD from 6 independent experiments. One-sample *t* test was performed. ***$P < 0.001$. (**e**) Allelic ratios of *rs60910145* in cDNA from huPTCs exposed to control conditions or 1 mM DMOG for 16 hours. Values indicate mean from one experiment performed in quadruplicates. Results were normalized to the allele ratio measured in the genomic DNA of the patient. Student *t* test was performed. (**f**) Expression qPCR analysis for APOL1 mRNA in lysates from huPTCs isolated from an individual carrying one allele of G1 variant exposed to 1 mM DMOG, hypoxia (1% O_2), or HIF stabilizers (each 100 μM) for 16 hours. Values are mean ± SD from 5 independent experiments. One-sample *t* test was performed. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (**g**) Immunoblot for HIF, APOL1, and β-actin using lysates from huPTCs exposed to the different HIF stabilizers, as described in (**f**). Chr, chromosome; ICA, 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetate; NS, not significant; RLU, relative light unit.

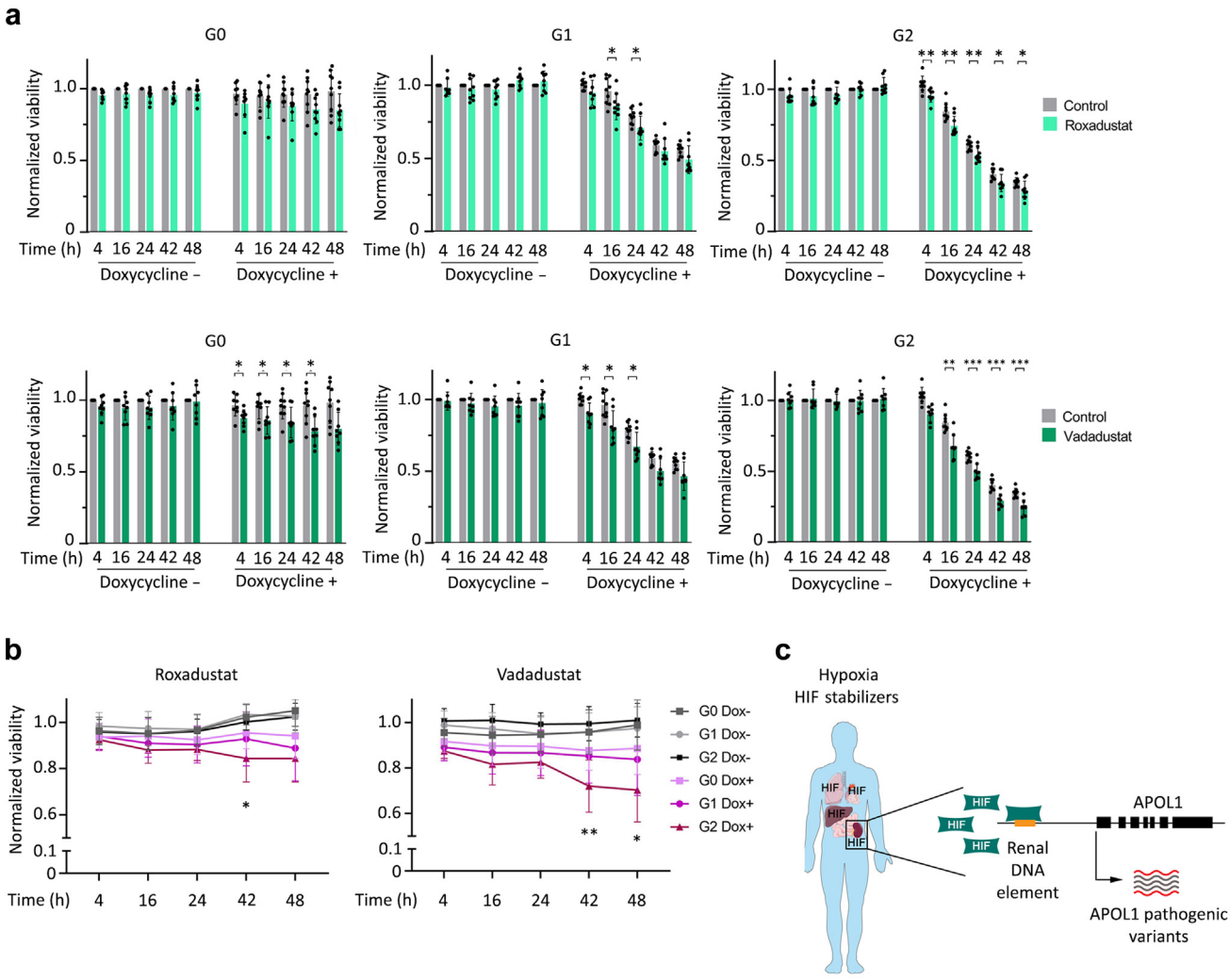

Figure 3 | Hypoxia-inducible factor (HIF) stabilization reduces cell viability in HEK293T cells overexpressing APOL1 risk variants. (**a**) Viability assay in HEK293T cells conditionally overexpressing APOL1 G0, G1, or G2 variants and treated with HIF stabilizers for the indicated time. Cells were treated without or with doxycycline (Dox) to induce expression of variants. An additional treatment was conducted with roxadustat (100 μM) or vadadustat (100 μM), as indicated. Data are presented as mean ± SD from 9 independent experiments, each conducted in technical triplicates. Student *t* test was performed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (**b**) Relative effects of the HIF stabilizer roxadustat or vadadustat on cell viability. Data were normalized on the respective control sample for each time point that was not treated with an HIF stabilizer. Statistical analysis was performed on the difference between effects determined for G0 and G1 or G2. Student *t* test was performed. $^{*}P < 0.05$, $^{**}P < 0.01$. (**c**) Schematic illustration of how HIF stabilization leads to increased expression of APOL1 pathogenic variants in kidneys.

reduced cell viability on concomitant HIF stabilization and expression of APOL1 risk variants could point to additive deleterious effects of both signaling pathways, possibly involving dysregulation of mitochondrial function.[9] Thus, further investigation of the interaction of HIF and APOL1 in renal cells is warranted.

In the 733 biosamples data set, the renal cluster is the most prominent group displaying accessible chromatin at the *APOL1* enhancer.[14] However, when we screened other cell types in the database for chromatin activity at this *cis*-regulatory element, we noted that HepG2 cells, a hepatocellular carcinoma cell line, also show evidence for an active enhancer at this site. We used available HIF ChIP-sequencing and transcriptomic data and confirmed that *APOL1* is also regulated by HIF in HepG2 cells (Supplementary Figure S11).[18] The liver contributes substantially to circulating levels of APOL1, pointing to additional roles of the HIF pathway in regulating APOL1 homeostasis.

Deleterious effects of both HIF-α isoforms on glomerular disease have been described in mice. For example, HIF-2α triggers a rapid progressive glomerulonephritis via overexpression of the HIF target gene CXC chemokine receptor 4.[6] Systemic deletion of HIF-1α ameliorates the development of glomerulosclerosis in the Nphs1-hCD25 (NEP25) podocyte ablation model.[19] Because mice do not express APOL1, this indicates that HIF evokes a broader response of potential harmful transcripts. However, overexpression of APOL1 risk variants causes proteinuria in mice, supporting the genuine

effect of these variants on kidney function.[20] Although the exact role of HIF in human podocytes is not entirely known, it is expressed at relevant levels and therefore is capable of regulating *APOL1* expression *in vivo* in humans.[21] We also noticed some expression of HIF-2α in isolated primary tubular cells. HIF-2α has not been detected at relevant levels in tubular cells of the kidney in rodents and humans in immunohistochemistry experiments.[22,23] The reason for this discrepancy is currently unknown, but might be explained by different sensitivity of the assays used to detect HIF-2α or changes in the expression profile of the isolated cells. However, transcriptional regulation of *APOL1* appears to be entirely dependent on HIF-1α in tubular cells.

The observation that, in addition to hypoxia, a range of HIF stabilizers regulate *APOL1* expression in renal cells may be of great importance for anemic APOL1 nephropathy patients with residual kidney function. From our data, one might infer that the treatment of patients with HIF stabilizers to elevate erythropoietin levels may also increase *APOL1* expression and thereby impair residual kidney function. However, in our study, full activation of *APOL1* transcription is dependent on the additional inhibition of FIH-1, which is expressed in human kidneys and thus potentially able to limit *APOL1* expression *in vivo*.[24] A limitation of our study is that experiments involving risk variant G1-expressing primary cells were conducted in cells from only one individual.

The limited data from clinical trials produced no signal so far that indicates that time to dialysis start is reduced in patients on HIF stabilizers. However, in these trials, the number of patients carrying *APOL1* risk alleles might be too small to generate a significant signal (e.g., <10% of patients with African ancestry in the Anemia Studies in Chronic Kidney Disease: Erythropoiesis via a Novel Prolyl Hydroxylase Inhibitor Daprodustat–Non-Dialysis [ASCEND-ND] trial evaluating daprodustat).[25] We therefore suggest closely following up on glomerular filtration rate in patients carrying known *APOL1* risk variants who are on treatment with HIF stabilizers. More important, the finding that other stimuli, such as interferons, may potentiate HIF-induced expression of *APOL1* or *vice versa* and thereby affect cell survival further fuels concerns about the use of these substances in patients at risk.

DISCLOSURE

JS has received honoraria from Astellas Pharma and GSK. MS has received honoraria from Astellas Pharma. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Table S1. Oligonucleotides used in this study.

Supplementary Figure S1. Volcano blot of RNA-sequencing (seq) data from podocytes after dimethyloxalylglycine (DMOG) treatment.

Supplementary Figure S2. APOL1 RNA and protein expression in primary tubular cells (PTCs) on hypoxic stimuli.

Supplementary Figure S3. RNA and protein expression of APOL1 in primary tubular cells (PTCs) after hypoxia-inducible factor (HIF) knockdown.

Supplementary Figure S4. Cell-type–specific APOL1 RNA expression in scRNA experiments from kidneys of controls and patients with acute kidney injury (AKI).

Supplementary Figure S5. RNA and protein expression of APOL1 in primary tubular cells (PTCs) after hypoxia-inducible factor (HIF) stabilization and interferon γ treatment.

Supplementary Figure S6. Time course experiments of APOL1 expression using different prolyl hydroxylase domain (PHD) inhibitors in primary tubular cells (PTCs).

Supplementary Figure S7. APOL1 expression in dose-response experiments using different prolyl hydroxylase domain (PHD) inhibitors in primary tubular cells (PTCs).

Supplementary Figure S8. Time course experiments of APOL1 expression using different prolyl hydroxylase domain (PHD) inhibitors in podocytes.

Supplementary Figure S9. APOL1 RNA expression in primary tubular cells (PTCs) after hypoxia-inducible factor (HIF) stabilization and factor-inhibiting HIF-1 (FIH) inhibition.

Supplementary Figure S10. Characterization of APOL1 expression in APOL1 G0/G1/G2 inducible HEK293T cells.

Supplementary Figure S11. APOL1 expression and hypoxia-inducible factor (HIF) chromatin immunoprecipitation (CHIP) at the *APOL1* locus in HepG2 cells.

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