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Protein domains of APOL1 and its risk variants

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ABSTRACT

Increasing lines of evidence have demonstrated that the development of higher rates of non-diabetic glomerulosclerosis (GS) in African Americans can be attributed to two coding sequence variants (G1 and G2) in the *Apolipoprotein L1* (APOL) gene. Recent studies indicate that the gene products of these APOL1 risk variants have augmented toxicity to kidney cells. However, the biological characteristics of APOL1 and its risk variants are not well elucidated. The APOL1 protein can be divided into several functional domains, including signal peptide (SP), pore forming domain (PFD), membrane address domain (MAD), and SRA-interacting domain. To investigate the relative contribution of each domain to cell injury, we constructed a serial expression vectors to delete or express each domain. These vectors were transfected into the human embryonic kidney cell line 293T, and then compared the cytotoxicity. In addition, we conducted studies in which APOL1 wild type (G0) was co-transfected in combination with G1 or G2 to see whether G0 could counteract the toxicity of the risk variants. The results showed that deleting the SP did not abolish the toxicity of APOL1, though deletion of 26 amino acid residues of the mature peptide at the N-terminal partially decreased the toxicity. Deleting PFD or MAD or SRA-interacting domain abolished toxicity, while, overexpressing each domain alone could not cause toxicity to the host cells. Deletion of the G2 sites while retaining G1 sites in the risk state resulted in persistent toxicity. Either deletion or exchanging the BH3 domain in the PFD led to complete loss of the toxicity in this experimental platform. Adding G0 to either G1 or G2 did not attenuate the toxicity of the either moiety. These results indicate that the integrity of the mature APOL1 protein is indispensable for its toxicity. Our study not only reveals the contribution of each domain of the APOL1 protein to cell injury, but also highlights some potential suggested targets for drug design to prevent or treat APOL1-associated nephropathy.

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1. Introduction

Cumulative evidence over many years have demonstrated that African Americans (AAs) develop 4–5 fold higher rates of diverse forms of progressive kidney disease, including focal segmental glomerulosclerosis (FSGS), HIVAN, and hypertension-attributed end stage kidney disease (ESKD), compared with European Americans (EAs) (Tzur et al., 2010; Kopp et al., 2011; Quaggin and George, 2011; Genovese et al., 2013; Kasembeli et al., 2015). In several forms of nephropathy, this disparity reaches a greater than 10-fold difference. This overwhelming population health disparity is mainly attributed to two coding sequence variants (G1 and G2) in APOL1 gene (Genovese et al., 2010; Friedman et al., 2011; Foster et al., 2013); however, detailed underlying mechanisms are still poorly understood.

In previous studies, we observed that the APOL1 risk variants (Vs) G1 and G2 confer cytotoxicity to kidney cells such as podocytes (Lan et al., 2014, 2015). Consistent with these observations, Park et al. (2014) reported that podocyte specific expression of APOL1 in G1 and G2 transgenic mice developed severe albuminuria and glomerulosclerosis, while wild type mice expressing G0 APOL1 showed minimal renal abnormalities. Similarly, Olabisi et al. (2014), and Heneghan et al. (2014), observed that APOL1Vs caused toxicity to Zebrafish and *Xenopus Laevis* oocytes. The forgoing studies demonstrate that APOL1Vs confer toxicity to the kidney and its functional cells. However, knowledge of the biological function of APOL1 proteins, especially its risk variants, is not clearly understood.

According to the presumed function, the full length APOL1 wild type protein G0 can be divided into 4 domains: signal peptide (SP, 1-27 AA), pore forming domain (PFD, 60-237 AA), membrane address domain (MAD, 238-303 AA), and SRA-interacting domain (339–398) (Pérez-

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Morga et al., 2005; Lecordier et al., 2009). Therefore, we sought to advance our understanding of the potential mechanisms of cytotoxicity by determining the relative contribution of these domains to cytotoxicity.

2. Materials and methods

2.1. Cell culture

The 293T cell line was purchased from ATCC, and was cultured at 37 °C in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 × penicillin–streptomycin, and 1 mM L-glutamine.

2.2. Trypan blue staining

Cells were rinsed with PBS to remove the floating cells and cell debris, detached with Accutase (Innovative Cell Technologies, San Diego, CA), and then labeled with 0.2% Trypan blue and counted under a light microscope. The unstained cells (white cells) were counted as live cells, while the stained (blue) cells were counted as dead cells.

2.3. Propidium iodide (PI) and Hoechst staining

PI and Hoechst staining were performed as previously described (Vashistha et al., 2009; Lan et al., 2014). Briefly, after each treatment condition of interest, the culture medium was removed from the cells and fresh medium containing Hoechst 33342 (10 μg/ml) was added. Cells were subsequently incubated for 10 min at 37 °C. Then, PI solution was added and culture dishes were kept on ice for 7 min. Cell images were recorded with a ZEISS microscope (Carl Zeiss Micro Imaging GmbH, Jena, Germany) equipped with a digital imaging system.

2.4. Plasmid preparation and transfection

Lentiviral vector LG12 was used as the backbone for all the expression vectors in this study, and CMV promoter was used to drive the expression of targeting gene. Plasmid was amplified in *Escherichia coli* strain DH5α, and was extracted by using QIAprep Spin Miniprep Kit (Qiagen, Valencia, CA), following the manufacturer's instructions. Host cell 293T was prepared on 35 mm culture dishes, and was transfected with plasmid (400 ng/dish) by using Effectene Transfection Reagent (Qiagen), following the manufacturer's instructions.

2.5. Statistical analyses

Data are presented as means ± standard deviation (SD) unless otherwise noted. All experiments were conducted and repeated at least three times, either in duplicate or triplicate for each assay. All data were evaluated statistically by analysis of variance (ANOVA), followed by Newman–Keuls multiple comparison tests using Prism 4.0, GraphPad software. In the case of single mean comparison, data were analyzed by Student t-test. P values < 0.05 were regarded as statistically significant.

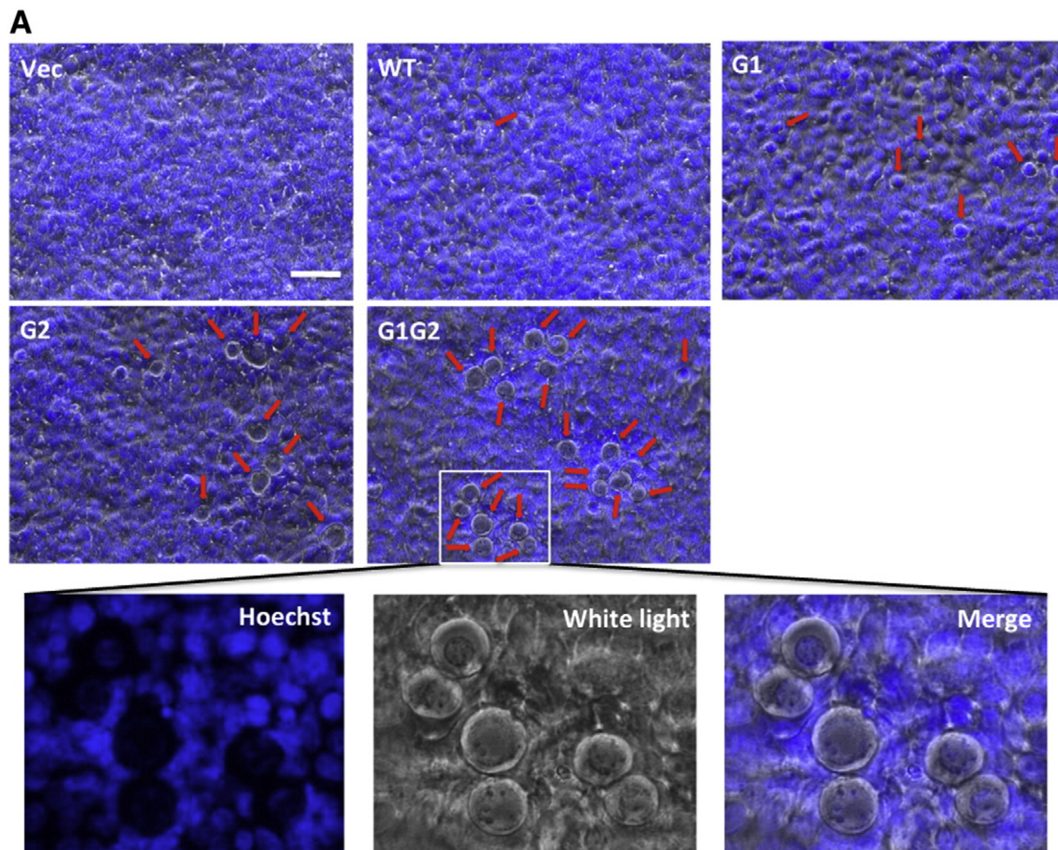

Fig. 1. Effect of APOL1G0 and variants on 293T cells. Kidney embryonic cell line 293T was transfected with LG12–APOL1G0 or Vs for 24 h. (A) Cells were stained with Hoechst 33342, and were then observed under phase contrast microscopy. Swollen cells were indicated with red arrows. Scale bar, 100 μm (A and B). Cells were subjected to PI staining, and the PI positive cells (white arrows) were counted under a fluorescent microscope. (C) Statistical calculation of the swollen cells in (A). (D) Statistical calculation of the PI positive cells in (B). (E) Cells were detached with Accutase and then subjected to Trypan blue staining, and the cell viabilities were calculated. *p < 0.05 when compared with vector; #p < 0.05 when compared with APOL1G0.

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