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HIV-1 Vpr activates the DNA damage response in renal tubule epithelial cells

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Summary

HIV associated nephropathy (HIVAN) is a major cause of HIV related morbidity and mortality. Pathogenesis involves direct infection of the glomerular and tubular epithelial cells leading to characteristic pathology. Recently, we have shown that HIV-1 Vpr causes hypertrophy, hyperploidy, and apoptosis. Here we report that Vpr activates the DNA damage response resulting in the observed renal phenotype. Renal sections from the HIVAN transgenic mouse model and human biopsies both show an abundant DNA damage response.

Keywords

HIV Associated Nephropathy; Vpr; DNA Damage; gamma H2AX; Renal Epithelial Cell

Research Letter

HIVAN is an important cause of end stage renal disease (ESRD) despite remarkable advances in HIV therapy. More than 8,000 cases of ESRD were attributed to HIVAN in the United States between 1995 and 2004 with over 90% of cases affecting individuals with West-African ancestry [1]. The prevalence in HIV-infected blacks worldwide, as determined by renal biopsy, is between 3–12% suggesting a global prevalence of 1–3 million cases [2,3]. Other studies measuring microalbuminuria, a marker of kidney disease, found prevalence as high as 40% in HIV-1 infected Africans, suggesting an even greater potential epidemic of HIV related renal disease [4,5].

Transgenic murine models, where the HIV genome is expressed as a provirus in renal tissue, recapitulate human HIVAN and have been useful tools to study disease pathogenesis. Single HIV gene and gene deletion transgenic models have indicated pathogenic roles for HIV-1 Nef and Vpr [6,7,8]. Nef causes podocyte dedifferentiation and aberrant proliferation [9,10]. Vpr expression in RTECs *in vitro* causes impaired cell division, hypertrophy, and a dysregulated cell cycle profile including a large accumulation in the G₂ phase [11]. However, the mechanism by which Vpr induces these changes is unknown. In lymphocytes,

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P.R. performed the immunohistochemistry and contributed to study design; J.C. performed RTEC culture and immunofluorescence; A.S. and V.P. contributed to the immunoprecipitation experiments V.D. was responsible for the collection, processing, and analysis of human tissue; P.K. and M.K. contributed equally to study design and critical review.

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Vpr-induced G₂ arrest is a well-described process that is mediated by Vpr's direct interaction with DDB Cullin 5A Interacting Factor 1 (DCAF1), an E3 ubiquitin ligase substrate specificity module [12,13,14,15,16]. It has been proposed that Vpr usurps the E3 complex to possibly degrade an unknown target. The loss of this target precipitates a cascade of events ultimately resulting in G₂ arrest. However, the Vpr-DCAF1 interaction had not been assessed in renal cells or tissue and its relevance in HIVAN is unknown. To determine whether Vpr binds DCAF1 in kidney cells, immunoprecipitation was performed using an HA-tagged Vpr expressed in the HK2 cell line which is phenotypically similar to primary RTECs [17]. RTECs were transduced by pseudotyped lentivirus with wildtype Vpr or an inactive Vpr mutant with an altered DCAF1 binding motif, Vpr Q65R [13]. DCAF1 was detectable in the lysate of RTECs and bound only to wildtype Vpr, not the inactive Q65R mutant. (Fig. 1A) However, both wildtype Vpr and the mutant Q65R were detectable at equal levels in the immunoprecipitate. Given that Vpr's activity correlated with DCAF1 binding in both lymphocytes and RTECs, we hypothesized a similar mechanism may be responsible for both pathologic phenotypes.

Vpr-induced G₂ arrest has also been linked to DNA damage pathway activation [18]. One aspect of this pathway is Histone 2A.X (H2AX) phosphorylation resulting in phospho-H2AX or synonymously γ H2AX. γ H2AX coordinates the formation of characteristic foci that recruit DNA repair proteins. To determine if activation of the DNA damage response pathway is involved in HIVAN pathology, γ H2AX levels were assessed in Vpr expressing RTECs. HK2 cells were again transduced by pseudotyped lentivirus to express either HIV-1 Vpr and a GFP reporter gene via an intervening IRES or only the GFP reporter. Four days after transduction, RTECs expressing Vpr were hypertrophied with enlarged nuclei as has been described [11]. These Vpr expressing cells also displayed greater γ H2AX immunofluorescence than control cells and the focal staining pattern was consistent with typical DNA damage repair foci (Fig. 1B). To quantify the differences observed by γ H2AX immunofluorescence, flow cytometry was performed. The fluorescent intensity of Vpr expressing RTECs at 4 days after transduction was greater than that of control GFP transduced cells (Fig. 1C). Further investigation found that other HIV proteins (Nef, Tat, Vif, and Vpu) and the phenotypically inactive Vpr Q65R did not cause H2AX phosphorylation in HK2 cells (unpublished work).

After determining that Vpr expression results in DNA damage in RTECs, we assessed the *in vivo* relevance of this phenotype in the murine HIVAN model. The Tg26 mouse HIVAN model is a well-characterized transgenic line that recapitulates human HIVAN pathology [19]. Briefly, the transgene is HIV pNL43 with a deletion in the *gag* and *pol* genes. When bred to the FVB/N mouse background, a well characterized HIVAN like disease is observed including proteinuria, collapsing and sclerotic glomeruli, tubular microcystic dilation, and hypertrophic tubule cells with enlarged nuclei. Kidney sections from Tg26 heterozygote mice with 3+ proteinuria and their wildtype littermates were examined for evidence of DNA damage activation by immunohistochemistry for γ H2AX. In the HIVAN model, γ H2AX positive nuclei were abundant while in the control they were absent. (Fig 1D) Additionally, we observed that many enlarged hypertrophic tubule cells were also positive for γ H2AX (Figure 1D inset and arrow). This finding is consistent with the *in vitro* evidence suggesting DNA damage is a cause of hypertrophy and cell cycle change.

To extend these results to the human disease, HIVAN and control biopsies were obtained and assessed for γ H2AX nuclear staining. Formalin-fixed paraffin-embedded human renal core specimens of biopsy proven HIVAN were compared to normal core biopsies. Normal human biopsies were percutaneous renal biopsies performed for either isolated mild microhematuria or minimal proteinuria (< 1 g/day) in which no glomerular or other renal pathology was identified by the three modalities of light microscopy, immunofluorescence,

and electron microscopy. Control biopsies showed no evidence of DNA damage activation while HIVAN biopsies showed multiple γ H2AX positive tubular cell nuclei (Figure 1E). These results represent a new aspect of HIVAN pathogenesis, activation of the DNA damage response pathway.

In conclusion, we propose a HIVAN pathogenesis model where Vpr usurps an E3 ubiquitin ligase complex via binding to DCAF1. This interaction may permit off-target protein degradation culminating in activation of the DNA damage response. This response manifests as the H2AX phosphorylation, cell hypertrophy, and hyperploidy that is observed in the HIVAN kidney. Disruption of the Vpr DCAF1 interaction may be a therapeutic intervention to ameliorate HIVAN pathogenesis and preserve kidney function. In addition, positive staining for γ H2AX might be a useful marker of HIV associated renal disease.

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Figure 1.

A) RTECs were transduced with lentiviral vectors expressing an HA-tagged Vpr or the inactive mutant HA-Vpr Q65R. Cell lysates were harvested 4 days after transduction. Immunoprecipitation using anti-HA agarose beads was performed. Western blot for DCAF1, HA-Vpr, or HA-Vpr Q65R was performed on the input lysate and the immunoprecipitate. Comparison of lanes 3 and 4 show DCAF1 co-precipitates with the wildtype Vpr but not the inactive mutant. B) Immunofluorescence staining for γ H2AX of RTECs expressing Vpr or GFP control. Vpr expressing RTECs are enlarged with enlarged nuclei and have increased γ H2AX focal staining. C) Flow cytometry histogram measuring γ H2AX fluorescence for Vpr or GFP control expressing RTECs at 4 days post transduction. The average mean fluorescent intensities (MFI) from 5 independent flow cytometry experiments are shown offset right. Error bars indicate the standard deviation. Asterisk indicates statistical significance $p < 0.005$ compared to either control. D–E) Immunohistochemistry for γ H2AX on HIVAN murine model transgenic mice, littermate controls (D), human HIVAN biopsies and normal control biopsies (E). In total a minimum of 4 independent animal sections or human biopsies were examined. A representative field of each experiment is shown at 200x magnification. Increased γ H2AX staining is seen in hypertrophied and dilated tubules in the murine HIVAN or human HIVAN samples. Insets - 400x images of murine HIVAN or human HIVAN. A kidney tubule cell with γ H2AX positive nuclei is indicated with arrowhead.