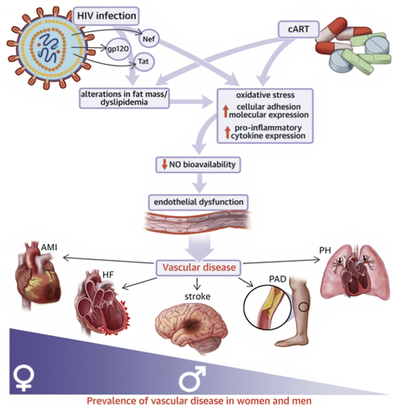
HIV-Encoded Proteins Induce Pulmonary Arterial Hypertension via Increases in Podocan-Like Protein 1

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Background



Contribution of HIV infection and cART to HIV-associated CVD

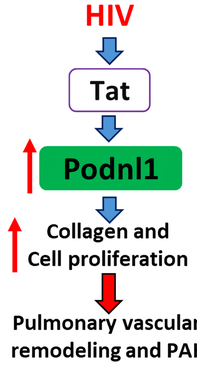
- Globally, over 39 million people are living with infection caused by HIV.
- Thanks to the advent of cART, HIV has shifted from an opportunistic, fatal disease to a non-infectious, chronic illness.
- People with HIV (PWH) have a greater life expectancy; however, they are exposed to higher risks of CVD including pulmonary arterial hypertension (PAH).
- HIV infection is an established risk factor for PAH.
- Prevalence of PAH is 8-14 times higher in PWH versus HIV- individuals.

Question

What are the underlying molecular mechanisms of HIV-associated PAH?

Hypothesis

HIV-derived proteins, which remain in circulation despite cART and well-controlled viremia, contribute to the development of PAH.



Mouse models of HIV

1) HIV-1 Tg26 transgenic mice (C57Bl/6 Bkgd)
- replication-deficient proviral HIV DNA randomly integrates into the host genome and ubiquitously express 7 of the 9 HIV viral proteins

2) BMT from Tg26 to WT mice
- to investigate the contribution of immune cell derived viral proteins to PAH

3) Chronic exposure to HIV protein Tat
- to study the specific roles of viral protein in the progression of PAH

Results I.

HIV-derived proteins induce cardiopulmonary complications in mouse models of HIV

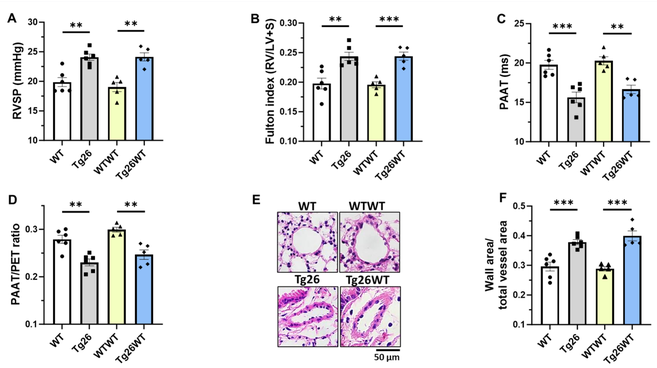


Figure 1. Expression of HIV-related proteins in Tg26 mice or in hematopoietic cells only promotes pulmonary vascular diseases. Changes in RVSP (A), RV/LV+S (B), PAAT (C) and in PAAT/PET (D). Representative images of lung sections of mice (E). Changes in ratio of wall area to total vessel area in the lung sections of mice (F). Data are mean ± SEM, n=5-6. **P<0.01; ***P<0.001.

Results II.

Podocan-like protein 1 (Podnl1) is the most significantly upregulated gene in the lungs of intact Tg26 and BMT mice

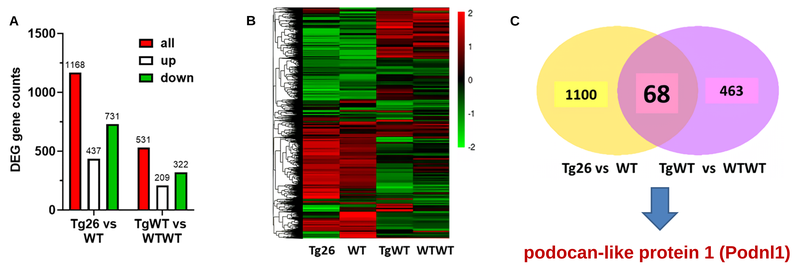


Figure 2. Bulk RNA sequencing of lungs of male and female intact Tg26 and BMT mice. Numbers of DEGs (A). Heat map (B). Venn diagram showing DEG distributions (C). n=3

Higher levels of Podnl1 are expressed in lungs of mouse model of HIV and PWH on cART

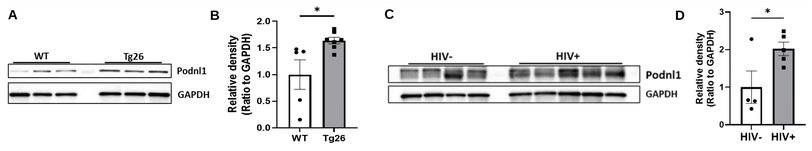


Figure 3. Protein levels of Podnl1 is increased in total lung homogenates of Tg26 mice and in lung specimens from HIV+ individuals on cART. Representative immunoblots of Podnl1 (A, C). Changes in protein levels of Podnl1 (B, D). Data are mean ± SEM, n=4-6. *P<0.05.

Cardiopulmonary alterations and Podnl1 expression are more pronounced with aging

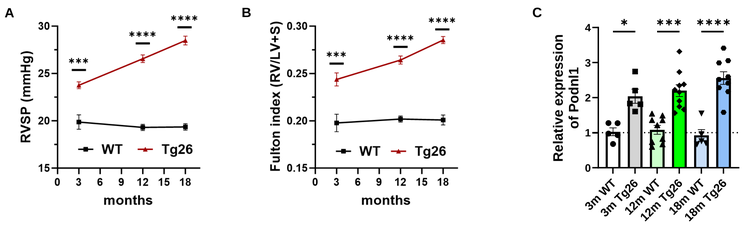


Figure 4. Aging exacerbates the HIV-induced increases in RVSP, further stimulates RV hypertrophy and elevates the expression of Podnl1 in Tg26 mice. Changes in RVSP (A), RV/LV+S (B), and relative expression of Podnl1 (C). Data are mean ± SEM, n=5-10. *P<0.05; ***p<0.001, ****p<0.0001.

Tat-treated mice exhibit pulmonary vascular remodeling and cardiopulmonary complications

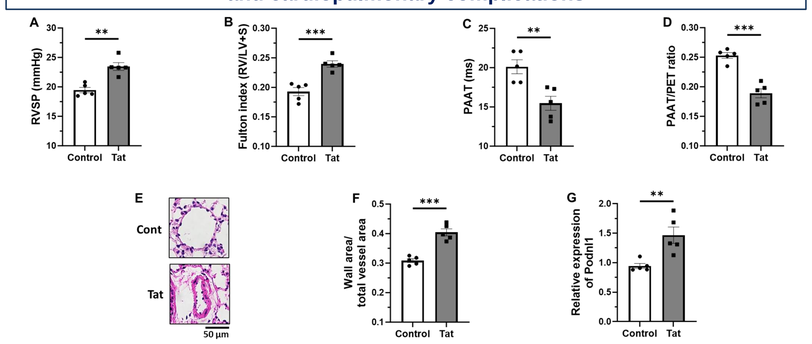


Figure 5. Chronic exposure of mice to HIV-derived Tat reproduces the cardiopulmonary complications of intact and BMT Tg26 mice and increases the Podnl1 expression. Changes in RVSP (A), RV/LV+S (B), PAAT (C) and PAAT/PET (D). Representative images of lung sections of mice (E). Changes in ratio of wall area to total vessel area in the lung sections of mice (F). Changes in relative expression of Podnl1 (G). Data are mean ± SEM n=5-6. **P<0.01; ***P<0.001 vs. control.

Results III.

Podnl1 overexpression leads to increased collagen synthesis and cell proliferation

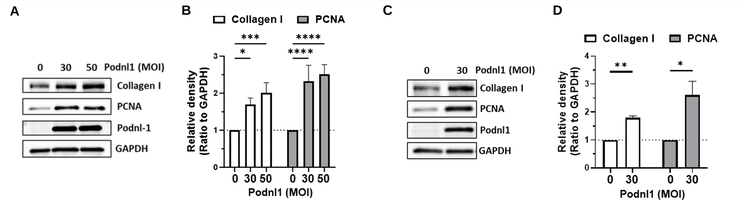


Figure 6. Collagen production and cell proliferation are elevated in Podnl1 overexpressing human pulmonary artery (PA) smooth muscle cells (PASMCs; A-B) and PA endothelial cells (PAEC; C-D). Representative immunoblots of collagen I, PCNA and Podnl1 (A, C). Changes in protein levels of collagen I and PCNA (B, D). Data are mean ± SEM, n=3. *P < 0.05, **P< 0.01, ***P< 0.001; ****P< 0.0001.

HIV-Tat upregulates Podnl1 expression and increases collagen synthesis and cell proliferation

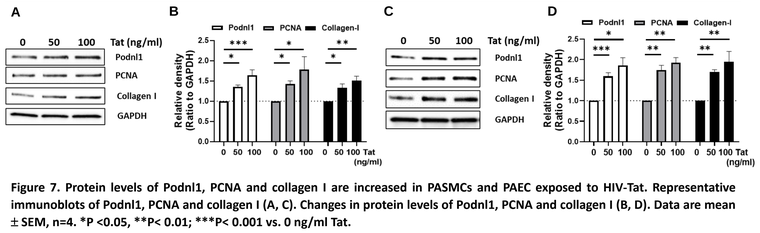


Figure 7. Protein levels of Podnl1, PCNA and collagen I are increased in PASMCs and PAEC exposed to HIV-Tat. Representative immunoblots of Podnl1, PCNA and collagen I (A, C). Changes in protein levels of Podnl1, PCNA and collagen I (B, D). Data are mean ± SEM, n=4. *P <0.05, **P< 0.01; ***P< 0.001 vs. 0 ng/ml Tat.

Summary and Conclusion

- Expression of HIV proteins in HIV-1 Tg26 mice promotes PAH.
- Bone marrow transplant (BMT) from Tg26 to wild-type (WT) mice fully recapitulate the cardiopulmonary phenotype of Tg26 mice, which supports a role for viral proteins derived from hematopoietic cell in PAH.
- Cardiopulmonary alterations are exacerbated with aging.
- RNA Seq analysis identified a new marker of PAH, Podnl1.
- Podnl1 is highly expressed in the lungs of intact Tg26 mice but more importantly in the lungs of PWH on cART.
- At least one HIV protein, Tat is involved in the progress of PAH.
- Overexpression of Podnl1 increases the levels of collagen I and PCNA, a marker of cell proliferation.
- HIV-Tat induces the increases in collagen I production and proliferation of PASMC and PAEC that can contribute to PAH.

Together, our data identify HIV-Tat and Podnl1 as major contributors to pulmonary vascular remodeling related to HIV infection. Inhibition of either Podnl1 or Tat represents potential new therapeutic avenues for the prevention of HIV-associated cardiopulmonary complications.

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