CMKLR1 as a potential target of atherosclerotic cardiovascular disease in PLWH

665

Ronald McMillan[1], Laventa M. Obare[1], Victoria R. Stephens[1], Kisyua Nthenge[1], Lindsey K. Stolze[2], Quanhu Sheng[2], Samuel Bailin[1], Xiuqi Zhang[1], Annet Kirabo[1], John R. Koethe[1], Yan Ru Su[1], Tarek Absi[1], Davey Smith[3], Sara Gianella[3], Celestine N. Wanjalla[1].

[1]Division of Infectious Diseases, Department of Medicine Vanderbilt University Medical Center, Department of Biostatistics, Vanderbilt University Medical Center[2] [3]University of California

BACKGROUND

- People living with HIV (PLWH) have a ~2-fold higher risk of atherosclerotic cardiovascular disease compared with people without HIV (PWoH).
- Persistent immune activation and adipose dysfunction are thought to contribute, but the underlying vascular mechanisms remain incompletely defined.
- Chemerin (RARRES2) is an adipokine that regulates immune activation, adipose biology, and vascular signaling via CMKLR1 and CCRL2.
- We hypothesize that in PLWH, immune activation accelerates atherosclerosis by upregulating CMKLR1 on smooth muscle cells, amplifying chemerin signaling and promoting phenotypic switching and matrix remodeling.

METHODS

Adipose tissue from 59 PWH in the HATIM cohort was used to isolate stromal vascular fractions, with chemerin transcript expression quantified. Aortic samples were obtained from PLWH (Last Gift, UCSD) and PWoH (ACACIA, VUMC) under standardized post-mortem or surgical protocols and promptly processed. Single-cell RNA sequencing was conducted to evaluate CMKLR1 expression by HIV status, with differential gene expression analyzed in aortic smooth muscle cells.

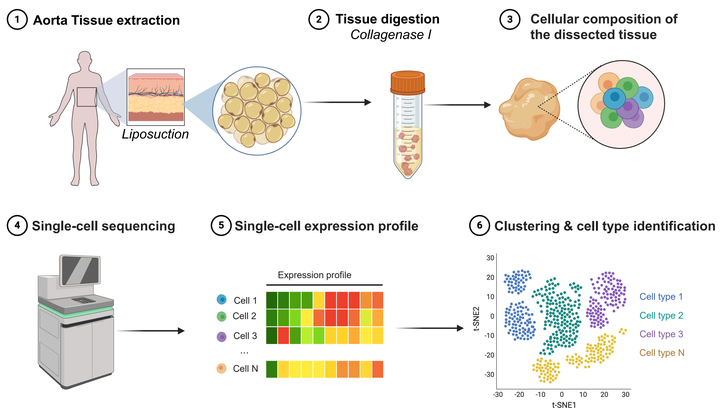


Figure 1. Methods Summary

Characteristic	HATIM (PWH)	Last Gift (PLWH, UCSD)
Total (N)	59	18
Diabetes Status	20 Non-diabetic 19 Prediabetic 20 Diabetic	Not reported
Median Age (years)	47.67	63.5
Male (%)	72%	89%
Race, Black (%)	38%	94%
Tissue Source	Adipose (SVF)	Aortic tissue
Sampling Context	Research cohort	≤6h post-mortem

Table 1. Demographics. SVF = Stromal Vascular Fraction, HIV, Adipose Tissue Immunology, and Metabolism

CMKLR1 expression is elevated in aortic smooth muscle cells of PLWH, alongside transcriptomic differences in genes involved in extracellular matrix organization.

RESULTS

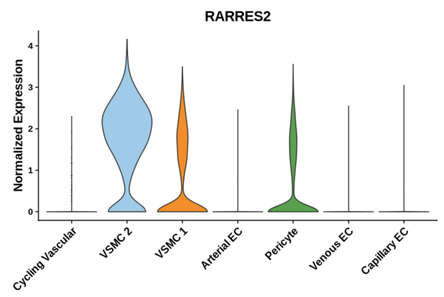


Figure 2. Chemerin is expressed in vascular smooth muscle cells and pericytes present in the stromal vascular fraction.

Take home message: *Chemerin gene expression is highly expressed in vascular smooth muscle cells of the SVF.*

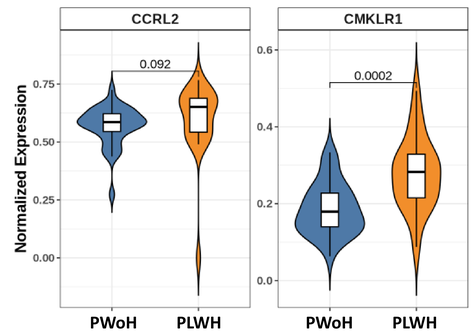


Figure 3. Subcutaneous adipose myeloid cells from PLWH show increased chemerin receptor expression relative to PWoH with diabetes .

Take home message: *In myeloid cells, CMKLR1, not CCRL2, is highly expressed in PLWH compared to PWoH.*

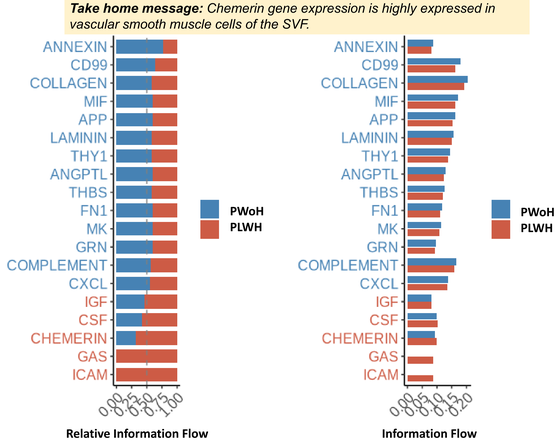


Figure 4. Enhanced chemerin-associated signaling from stromal cells to intermediate macrophages in adipose tissue of PLWH compared to PWoH.

Take home message: *Compared with PWoH, PLWH show higher chemerin and inflammatory signaling and lower structural support signals.*

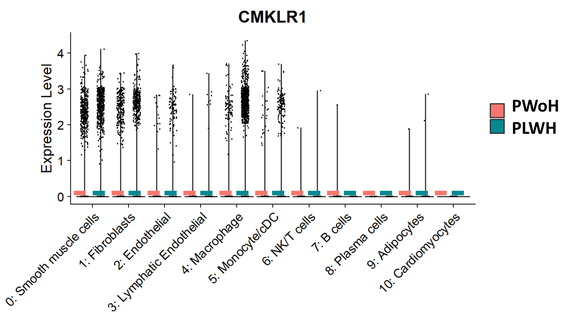


Figure 5: Chemerin receptor expression is also expressed in smooth muscle cells, fibroblasts, and macrophages present on the thoracic aorta of PLWH and PWoH.

Take home message: *In both PLWH and PWoH, CMKLR1 expression is detectable in SMCs, fibroblasts, and macrophages..*

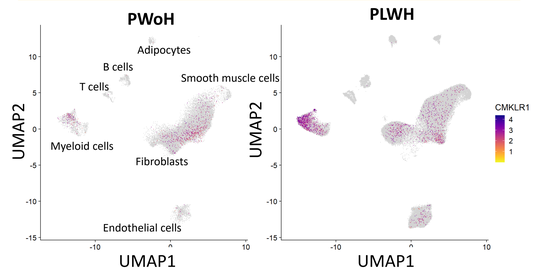


Figure 6. UMAP shows expression of CMKLR1 on myeloid cells and smooth muscle cells in the aorta by HIV status.

Take home message: *PLWH have higher expression levels of CMKLR1 in myeloid and smooth muscle cells, compared to PWoH.*

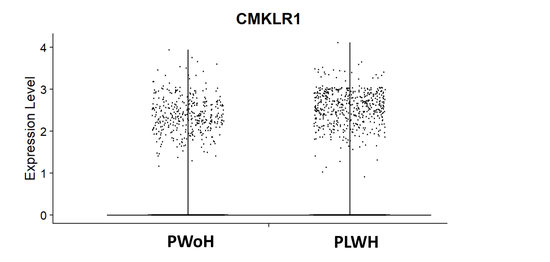


Figure 7. CMKLR1 is also elevated in smooth muscle cells in the aorta of PLWH.

Take home message: *In the aorta, SMCs express CMKLR1 more in PLWH than PWoH.*

Reactome Pathway	p-value	q-value	Gene
Signal Transduction	0.000018	0.0045	[PDGFRB, NOTCH3, LAMA5, SMAD3, ACTN1, NTRK3, PDE4D, LRP5, ARHGEF10L, ECE1, THBS2, DHRS3, RHOB, VEGFA, SMAD7, SH3PXD2A, COL4A2, COL4A1, MYH11, ITGA5, ARHGEF7, PDK1]
Signaling by PDGF	0.000040	0.0045	[PDGFRB, COL4A2, COL4A1, THBS2]
Non-integrin membrane-ECM Interactions	0.000043	0.0045	[LAMA5, COL4A2, COL4A1, ACTN1]
Extracellular Matrix Organization	0.000058	0.0046	[LAMA5, SH3PXD2A, COL4A2, COL4A1, ACTN1, ELN, ITGA5]
Laminin Interactions	0.00013	0.0082	[LAMA5, COL4A2, COL4A1]
Signaling by Receptor Tyrosine Kinases	0.00037	0.019	[PDGFRB, LAMA5, COL4A2, COL4A1, NTRK3, ARHGEF7, THBS2, VEGFA]
Smooth Muscle Contraction	0.00041	0.019	[TPM2, MYH11, SORBS1]
Regulation of Gene Expression by Hypoxia-inducible Factor	0.00058	0.023	[HIF3A, VEGFA]
Anchoring Fibril Formation	0.0011	0.037	[COL4A2, COL4A1]
Degradation of the Extracellular Matrix	0.0012	0.037	[LAMA5, COL4A2, COL4A1, ELN]

Table 2. Enriched signaling and ECM-related pathways in PLWH SMCs are consistent with the observed elevated CMKLR1 expression.

CONCLUSIONS

- In aortic tissue, macrophages and smooth muscle cells were the predominant cell types expressing CMKLR1.
- Aortic smooth muscle cells from PLWH demonstrated significantly higher CMKLR1 expression compared with PWoH.
- Differential gene expression of aortic SMCs by HIV status showed upregulation of genes that were enriched for several pathways, including signal transduction and extracellular matrix organization
- Overall, the observed differences in CMKLR1 expression and SMC transcriptomes in PLWH warrant follow-up in larger cohorts and targeted mechanistic studies.

Acknowledgements

- K23 HL156759 (CNW), Doris Duke CSDA 2021193, Burroughs Wellcome Fund 1021480 (CNW), and the Gilead Research HIV Scholars Award. Last Gift P01 grant AI169609 (SWG). We also thank all the patients who volunteered to be involved in our studies.

References

Hidemizu Kunimoto, Kyosuke Kazama, Mizuho Takai, Mayuko Oda, Muneyoshi Okada, and Hideyuki Yamawaki, American Journal of Physiology-Heart and Circulatory Physiology 2015 309:5, H1017-H1028

Obare LM, Temu T, Mallal SA, Wanjalla CN. Inflammation in HIV and Its Impact on Atherosclerotic Cardiovascular Disease. Circ Res. 2024 May 24;134(11):1515-1545. PMID: 38781301

PLAIN LANGUAGE SUMMARY

People living with HIV are at higher risk of heart disease. Smooth muscle cells help keep arteries healthy and control blood flow, but changes in these cells may contribute to this risk. We found that a protein called CMKLR1, linked to inflammation and smooth muscle cell function, is increased in the main heart artery of people with HIV. This could be an important target for future research and treatments.

Contact Information:

Email: Ronald.mcmillan@vumc.org, LinkedIn: linkedin.com/in/mcmillanron