Proteomic profile of serum-derived extracellular vesicles from HIV+ Subjects

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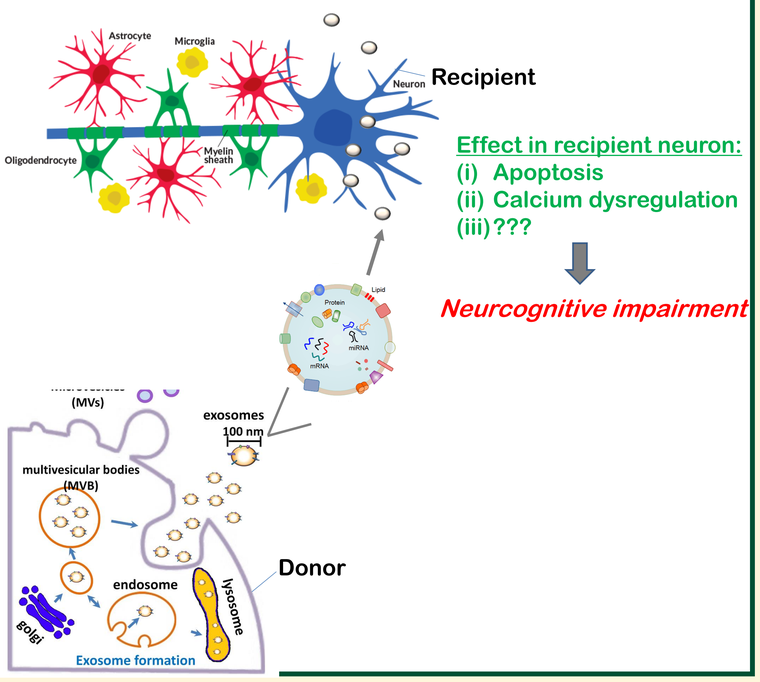
ABSTRACT

Despite viral suppression, HIV-infected patients develop neurocognitive impairments with most severe being HIV encephalitis (HIVE) and HIV-associated dementia (HAD). Incidence of HIVE and HAD are significantly reduced in the United States due to cART. However, less severe cognitive impairments such as asymptomatic neurocognitive impairment (ANI) and HIV-associated neurocognitive disorders (HAND) occur in aviremic HIV+ individuals. ANI and HAND negatively impact HIV+ patient's quality of life and should be investigated. Currently there are no therapeutics that directly address the cause of ANI, HAND, and even HAD in aviremic HIV+ subjects. Hematopoetic cells release three class of extracellular vesicles – (1) apoptotic bodies, (2) microvesicles, and (3) exosomes that function primarily as intercellular messengers inducing cellular responses in recipient cells. HIV-infected cells have been shown to release the HIV Negative factor (Nef) exosome. The role of these Nef+ exosomes (exNef) in HIV infection or pathogenesis is unknown. We theorize that exNef may play a role in the onset ANI and/or HAND. *Here we propose to develop the proteomic profile of exosomes as a diagnostic biomarker of HIV neurocognitive diseases status among aviremic HIV-infected non-drug users.* Preliminary findings suggests that regimen may influence exosome protein cargo. Further studies will be performed to confirm our hypothesis.

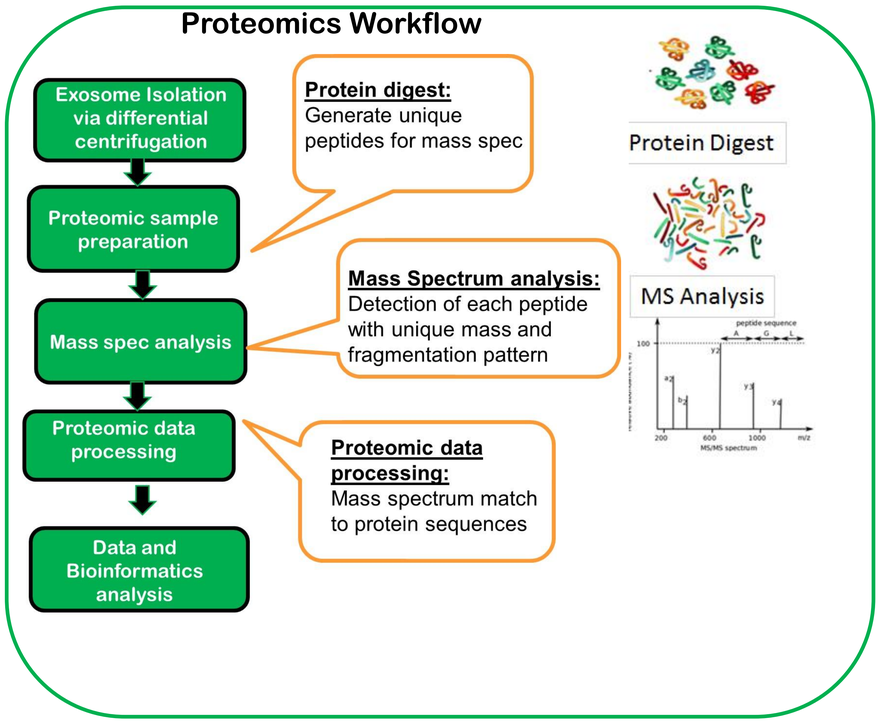
BACKGROUND

- Exosomes-mediated functional effects are based on their cargo. We predict that in HIV-infected.
- Preliminary data demonstrated that exNef impairs the blood-brain-barrier and may be neurotoxic.
- Despite viral suppression, HIV-infected patients develop neurocognitive impairments with most severe being HIV encephalitis (HIVE) and HIV-associated dementia (HAD). Incidence of HIVE and HAD are significantly reduced in the United States due to cART. However, less severe cognitive impairments such as asymptomatic neurocognitive impairment (ANI) and HIV-associated neurocognitive disorders (HAND) occur in aviremic HIV+ individuals. ANI and HAND negatively impact HIV+ patient's quality of life and should be investigated. Currently there are no therapeutics that directly address the cause of ANI, HAND, and even HAD in aviremic HIV+ subjects.
- Perform a cross-sectional study correlating proteomic profile of serum-derived exosome cargo from HIV+ non-drug users on different regimens and with different neurocognitive stages.

Model of exosome-mediated neuropathogenesis



MATERIALS AND METHODS



Cohort Demographics (N=20)	
Age (years)	29 – 59 (mean = 49)
Gender	Female - 35%, (7/20) Male - 65% 13/20)
Race	African-American (100%)
Medications:	
(i)Truvada	25%(5/20)
(ii) Non-Truvada	25%(5/20)
(iii) None	50% (10/10)

RESULTS

Proteins detected in exosome lysates

Group	Protein	Accession ID	Mascot Score* (SwissProt)	Residue number	Sequence Coverage(%)	Taxonomy	Sample origin
Truvada-containing regimen	PIGA (Phosphatidylinositol N-acetylglucosaminyl transferase subunit A)	P37287	62*	484	19	Homo sapien (human)	Patient # 1029967_BRH Cohort
	B4GT6 (Beta-1,4 galactosyl transferase)	Q9UBX8	43	382	22	Homo sapien (human)	
	RTF2 (Replication Termination Factor 2)	Q9BY42	42	306	32	Homo sapien (human)	
	AFDN-AS1 (AFDN Anti-sense RNA-1)	Q9Y6Z5	38	254	34	Homo sapien (human)	
	PK3CB (Phosphatidylinositol 4,5, bi-phosphate 3 kinase subunit beta)	Q8NHP7	37	306	32	Homo sapien (human)	
	Pol (Gag-Pol poly protein)	P03366	33	1447	9	Viruses (Human Immunodeficiency virus)	
	VL1	P50786	33	516	15	Viruses (Human Papilloma Virus)	

Group	Protein	Accession ID	Mascot Score* (SwissProt)	Residue number	Sequence Coverage(%)	Taxonomy	Sample origin
Non-Truvada-containing regimen	NR2E3 (Photoreceptor-specific nuclear receptor)	P37287	50	410	19	Homo sapien (human)	Patient# 1029964_BRH Cohort
	PIGA (Phosphatidylinositol N-acetylglucosaminyl transferase subunit A)	Q9UBX8	46	484	15	Homo sapien (human)	
	MGN2 (Mago Nashi)	Q96A72	43	148	41	Homo sapien (human)	
	MSD4 (Myb/SANT-like DNA-binding domain 4)	Q8NCY6	42	345	22	Homo sapien (human)	
	CC175 (Coiled-coil domain-comtaining 175)	POC221	39	793	13	Homo sapien (human)	
	ENV (Envelope glycoprotein GP1260)	P20888	32	855	11	Viruses (Human Immunodeficiency virus)	
	NEP I56A3 (Nuclear export protein)	Q20P38	33	121	40	Viruses (Influenza A virus)	

Group	Protein	Accession ID	Mascot Score* (SwissProt)	Residue number	Sequence Coverage(%)	Taxonomy	Sample Origin
No Medication	ORN (Oligoribonuclease)	Q9Y3B8	49	237	40	Homo sapien (human)	Patient # 1029972_BRH Cohort
	NCS1 (Neuronal Calcium Sensor)	P62166	38	190	36	Homo sapien (human)	
	ATR (Ataxia Telangiectasia and Rad3 related protein)	Q13535	38	2644	10	Homo sapien (human)	
	PCNP (Proteolytic signal-containing nuclear protein)	Q8WW12	37	178	37	Homo sapien (human)	
	EXD1 (piRNA biogenesis protein)	Q8NHP7	33	514	20	Homo sapien (human)	
	Vpr (Viral protein R)	P18100	30	87	37	Viruses (Human Immunodeficiency virus)	
	Vif (Virion Infectivity Factor)	Q9WC55	26	192	30	Viruses (Human Immunodeficiency virus)	

*Protein score is 10*Log(P), where P is the probability that the observed match is a random event, scores greater than 56 are significant(p<0.05)

Matrix Science – SwissProt data base

CONCLUSIONS

- Anti-viral regimen may influence protein content of exosomes.
- HIV proteins are detected despite viral suppression.

FUTURE DIRECTIONS

- Validate proteins via western blots.
- Further optimize exosome preparation and Mass spectrometry conditions.
- Bioinformatics

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