# Extracellular vesicle secretion: tissue-specificity and the impact of health and disease

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New Results

**Comment on this paper** 

Skeletal muscle tissue secretes more extracellular vesicles than white adipose tissue and myofibers are a major source ex vivo but not in vivo

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This article is a preprint and has not been certified by peer review [what does this mean?].

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#### **CSU Collaborators**

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# Outline

#### 1) Extracellular Vesicles in Health and Disease

- 2) Tissue-specific EV secretion *ex vivo*.
- 3) A dual fluorescent reporter mice to track cell typespecific EVs.

# What is an Extracellular Vesicle?



Holm et al. 2018, Trends in Neurosciences



Kalluri and LeBleu 2020, Science



# Exosome communication between organs/tissues/cells



Martinez and Andriantsitohaina 2017, Circulation Research

#### T2DM increases circulating EV abundance in humans.



Noren Hooten and Evans, 2020, AJP-Cell

Skeletal Muscle



Aswad et al. 2014, Diabetologia



Flaherty et al. 2019, Science

SkM and WAT tissues both secrete EVs that appear to respond to overnutrition and contribute to the etiology of metabolic disease...

1) Do SkM and WAT secrete a similar quantity of EVs?

2) Does contraction contribute to SkM tissue *EV* secretion ex vivo?

3) Can we track SkM myofiber-derived EVs in vivo using fluorescent reporter mice?

# Outline

1) Extracellular Vesicles in Health and Disease

### 2) <u>Tissue-specific EV secretion *ex vivo*.</u>

3) A dual fluorescent reporter mice to track cell typespecific EVs.

#### SkM expresses more machinery for EV biogenesis than WAT in humans

	IHC in Cell Types				RNA-Seq in Tissues				Π		
	Adipose Tissue	SkM			White Adipose					IHC + RNA	
Protein/Gene	Adipocyte	Myocyte	Greater		Tissue	SkM Tissue		Greater			
Arrdc1	0	3	SkM		8.4	9.1		SkM		Yes	Microvesicle Secretion
Arrdc4	1	3	SkM		9.2	10.1		SkM		Yes	Where vesicle Secretion
Atp13a2	0	2	SkM		9.6	7.5		Adipose		No	
Cd34	0	0	Equal		61.9	39.7		Adipose		No	
Chmp2a	1	1	Equal		40.7	75.7		SkM		No	
Cops5	3	3	Equal		20.2	64		SkM		No	
Hgs	0	2	SkM		26.8	36.3		SkM		Yes	Endosome Sorting
Myo5b	0	0	Equal		2	0.6		Adipose		No	8
Pdcd6ip	0	2	SkM		39.1	29.5		Adipose		No	A / 1
Prkn	0	2	SkM		6.8	41.8		SkM		Yes	Autophagy
Rab7a	2	2	Equal		61.5	146.3		SkM		No	
Rab11a	0	1	SkM		43.3	27.3		Adipose		No	
Rab27a	0	0	Equal		11.4	5.1		Adipose		No	
Sdc1	0	0	Equal		0.8	0.5		Adipose		No	
Sdc4	2	2	Equal		15.9	25.5		SkM		No	
Sdcbp	0	0	Equal		106.2	22.5		Adipose		No	
Smpd3	0	0	Equal		3.5	1.8		Adipose		No	
Snf8	0	1	SkM		22.5	40.7		SkM		Yes	ESCRT-II Component
Stam	1	0	Adipose		13.5	26		SkM		No	1
Steap3	2	1	Adipose		8.1	10.9		SkM		No	
Tsg101	0	2	SkM		24.7	44.5		SkM		Yes	ESCRT-I Component
Vps4a	1	1	Equal		20.2	81.6		SkM		No	r
Vps4b	0	0	Equal		16.8	9.9		Adipose		No	
		SkM	9			SkM		13		Targets	
		Equal	12			WAT		10		6	
		WAT	0								

0 = absent, 1 = low,

Expression reported as aggregate of HPA, GTEx and FANTOM5 datasets.

2 =medium and 3 =high

#### Nanoparticle Tracking Analysis



(Multispectral Advanced Nanoparticle Tracking Analysis)



#### Cuvette-based System



# SkM secretes more EVs than WAT ex vivo



Estrada et al., *BiorxiV* 

#### Does Exercise Increase Circulating EV Abundance?





# <u>Treadmill (6 km/h @ 1.5% incline + 3 km/h every 3 mins until exhaustion)</u>



30 min at 55%, 20 min at 70%, and 10 min at 80% of VO<sub>2</sub>max





Whitham et al. 2018, Cell Metabolism

#### Does Exercise Increase Circulating EV Abundance?





Brahmer et al., *JEV* 2019

https://www.youtube.com/watch?v=7AOyyZzieeQ

#### Do SkM fiber-derived EVs reach the blood during exercise?



SkM EVs may not reach plasma at rest or during exercise...

Brahmer et al. 2019, JEV

### Spontaneous Contraction Does Not Contribute to SkM EV Secretion *Ex Vivo*



Estrada et al., *BiorxiV* 

### EV Secretion Differs Between SkM Tissues Ex Vivo



<u>See also Nie et al. 2019,</u> <u>Experimental Physiology</u>

Estrada et al., *BiorxiV* 

# Summary

1) SkM tissue secretes more EVs than WAT in mice.

2) Differences in EV secretion between SkM and WAT cannot be explained by spontaneous SkM contraction.

3) SkM tissue EV secretion appears to be determined, at least in part, by intrinsic metabolic capacity.

# **Do SkM EVs reach the circulation** *in vivo*?

# Outline

- 1) Extracellular Vesicles in Health and Disease
- 2) Tissue-specific EV secretion *ex vivo*.
- 3) <u>A dual fluorescent reporter mice to track cell</u> <u>type-specific EVs.</u>

# EVs Everywhere!



How can we study EVs secreted from specific tissues and cell types *in vivo*?



# "Color coding" EVs secreted by specific cells *in vivo*

**<u>Approach</u>**: Cre mediated expression of mT and mG in vivo



# tdTomato-labeled EVs are taken up by naïve cells *in vivo*

Α



Crewe et al. 2018, Cell



# Labeling lung epithelium using the mT/mG mouse reveals cellular origin of BALF-derived EVs





Pua et al. 2019, Cell Reports

### Studying Endogenously Fluorescent EVs from Mouse Plasma and Tissues



Blood via cardiac puncture collected into EDTA tubes

#### Horiba ViewSizer



#### **Flow Cytometer**



## SkM from mT mice secrete mT<sup>+</sup> EVs *ex vivo*



# SkM and WAT from mT mice secrete fluorescently labeled EVs

**Relative Event Frequency (% Total)** 



Estrada et al., *BiorxiV* 

#### SkM-mG/mT mice secrete eGFP<sup>+</sup> EVs *ex vivo*



### SkM-mG/mT mice secrete eGFP<sup>+</sup> EVs *ex vivo*



Estrada et al., *BiorxiV* 

# Spectral flow cytometry reveals very few SkM myofiber-derived EVs in plasma



# Can immunocapture be used to detect endogenously fluorescent EVs?

Estrada et al., *BiorxiV* 



Kalluri and LeBleu 2020, Science

# Immunocapture detection of EVs



#### Wild Type Experiment

mT/mG Experiment



SkM EVs from mT/mG mice isolated with SEC...

### Immunocapture detection of mT/mG EVs ex vivo

#### **CD9** capture antibody



~57% of CD9-captured particles were positive for mT (tdTomato).

~20% of CD9-captured particles were positive for mG (eGFP).

<u>Mean Fluorescence Intensity</u> mCD63 Antibody = 523 Endogenous tdTomato = 497 Endogenous eGFP = 602

## Summary – Part 2

1) SkM and WAT tissues secrete endogenously fluorescent EVs *ex vivo*.

- 2) SkM myofiber-derived EVs are scarce (absent?) in the plasma of chow-fed mice.
- 3) Immunocapture appears to be a viable approach for detecting and quantifying endogenously fluorescent EVs. Will this reveal SkM EVs in plasma...?

# **Future Directions**

1) How does overnutrition alter the secretion of SkM myofiber-derived EVs?

2) How many SkM myofiber-derived EVs ever reach the blood?

3) How do non-vascular cell derived EVs cross the vascular endothelium?

# Thank you for your time!