Recent Developments in the Transmission of Human Life

Bone Marrow Derived Stem Cell- Extracellular Vesicles Shuttle miRNAs, LncRNAs, mRNAs and Chemokines Augmenting the Role of Stem Cells in Endometriosis

Prof. G. Krkun

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During the past few years non-coding RNAs (ncRNAs), previously thought as transcriptional junk, have become a research goldmine.

Extracellular vesicles, (EVs) have shown that EVs are considered a new mode of intercellular communication. EVs have been extensively investigated across many fields to improve the understanding of pathophysiological processes, as biomarkers of disease and as therapeutic targets for pharmacological intervention.

Graciela Krikun, PhD

Bone Marrow Derived Stem Cells produce Extracellular Vesicles (EVs) Shuttle miRNAs, LncRNAs, mRNAs, lipids and Chemokines and we posit that as such they augment the Role of Stem Cells in Endometriosis.

The paper below demonstrates that EVs cargo include cytokines, chemokines, messenger RNA (mRNA), growth factors, and so forth.

Olga Martinez-Arroyo et. al. Mesenchymal Stem Cell-Derived Extracellular Vesicles as Non-Coding RNA Therapeutic Vehicles in Autoimmune Diseases. Pharmaceutics. 2022 14(4): 733.

Faculty Disclosure

I have no potential conflict of interest to declare.
Thank you Alan Weinhouse for helping fund these experiments.

Extracellular vesicles (EVs) are lipid bound vesicles secreted by cells into the extracellular space. The three main subtypes of EVs are microvesicles (MVs), plasma derived vesicles, and apoptotic bodies, which are differentiated based upon their biogenesis. EVs are a heterogeneous group of cell-derived membranous structures. They are present in biological fluids and are involved in multiple physiological and pathological processes.

Importantly, EVs shuttle mRNAs, miRNAs, long non-coding RNAs (lncRNAs), lipids and chemokines.
Effects of EVS have been shown to be a mechanism of cell-to-cell communication. Cell communication represents a dynamic mechanism regulated by the release of factors able to influence cell fate, function and plasticity (cells of a given type can grow into a different type cell type).

EVs

Many diverse names have been used to refer to these vesicles released by healthy or diseased cells including ectosomes (vesicles that bud directly from the plasma membrane and are shed to the extracellular space), microparticles, and EVs from apoptotic bodies. In order to bring harmonization to the field, researchers are now encouraged to use the term extracellular vesicles (EVs) as a generic term for all secreted vesicles.

Exosomes (EVs)	Microvesicles	Apoptotic Bodies	Plasma membrane
Origin	Endocytic pathway	Apoptosis	EVs are shed from the plasma membrane
Size	40-120 nm	50-1,000 nm	500-2,000 nm
Function	Intercellular communication, delivery of nucleic acids, proteins and lipids which can be physiological or pathological.	Apo EVs formation has two key proposed functions: (a) aiding apoptotic cell clearance and (b) means of intercellular communication, both of which have implications in immune regulation.	Cell-to-cell communication, delivery of nucleic acids, proteins and lipids which can be physiological or pathological.
Markers	CD81, CD63, CD9, Alix and Tsg101 a protein derived from the tumor susceptibility gene	CD81, CD63, CD9, CD45	Phosphatidylserine CD81, CD63, CD9

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EV markers: CD81, CD63, CD9, Alix, TSG

CD81: (TAPA-1) cell-surface protein a molecule involved in signal transduction and cell adhesion in the immune system morphology, activation, proliferation, and differentiation.
CD63: is mainly associated with membranes of intracellular vesicles.
The protein participates in a variety of cellular processes, like cell activation, adhesion, differentiation and tumor invasion.
CD9 is a cell surface glycoprotein that is known to complex with integrins and other transmembrane 4 superfamily proteins.
CD45: is a type I transmembrane molecule found on the surface of all nucleated hematopoietic cells and their precursors cells (except erythrocytes) that assists in cell activation; expressed in lymphomas, B-cell chronic lymphocytic leukemia, hairy cell leukemia, and acute nonlymphocytic leukemia.
Alix: is a cytosolic protein in mammalian cells that was originally identified on the basis of its association with pro-apoptotic signaling. More recent evidence has established that Alix has a hand in regulating other cellular mechanisms, including endocytic membrane trafficking and cell adhesion.
TSG: is a tumor suppressor gene inflammation-associated secreted protein that has been implicated as having important and diverse tissue protective and anti-inflammatory properties, e.g. mediating many of the immunomodulatory and beneficial activities of mesenchymal stem/stromal cells.

Conclusions

- ✓We have shown that human bone marrow derived stem cells secrete and express EVs.
- ✓Vesicles from these cells express several miRNAs, with 100-5p being the highest expressed miRNA which inhibited decidualization of endometrial stromal cells.

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Hypothesis

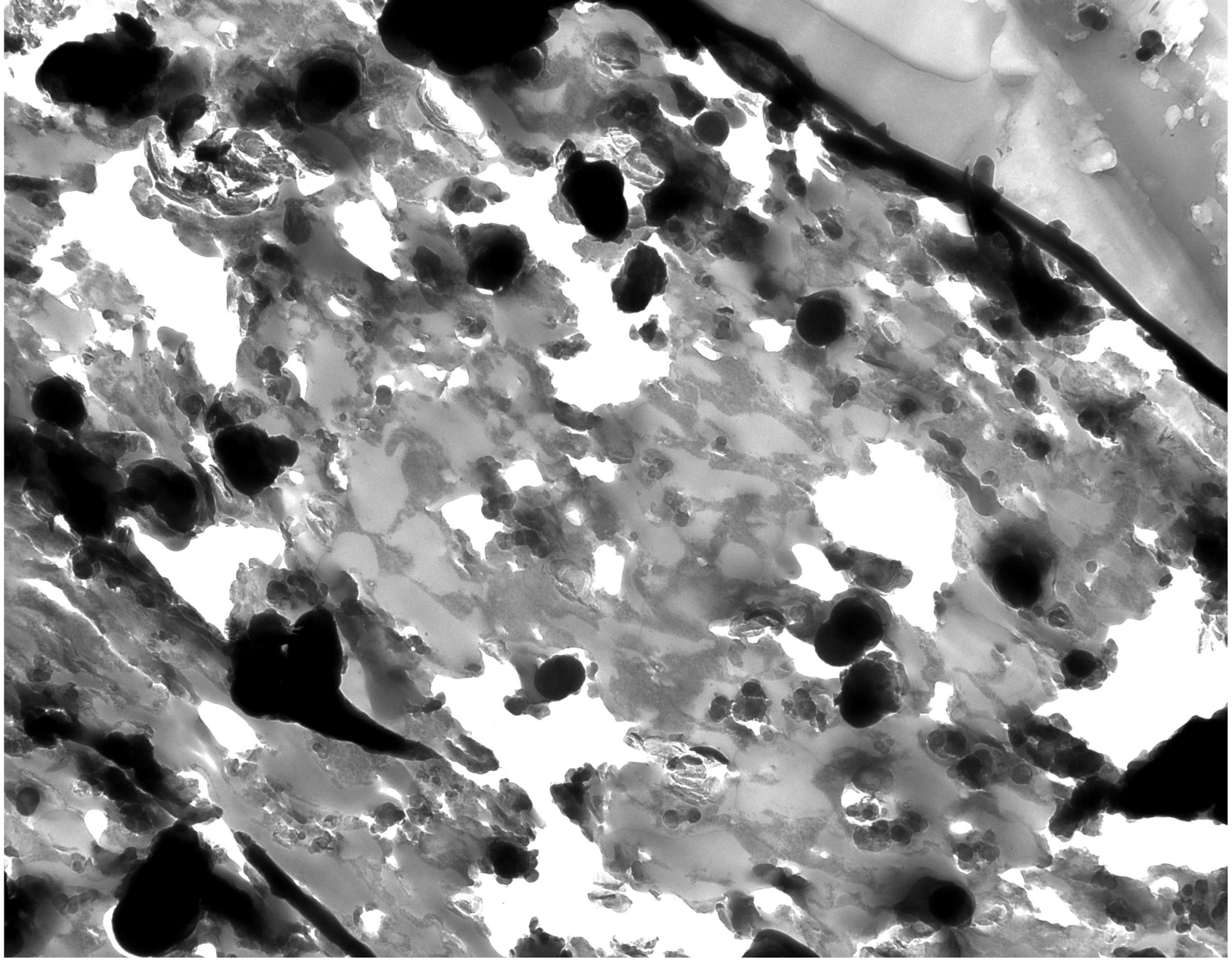
We propose that: while BMDS engraftment in endometriotic lesions may be low, their ability to shuttle EVs containing miRNAs, lncRNAs, mRNAs, chemokines, lipids and proteins amplifies BMDS effects in lesions resulting in endometriosis progression.

Do hBMDS produce EVs?

So we isolated EVs from hBMDS. Using ultracentrifugation and extraction kit followed by EM, we can see EVs derived from the hBMDC.

Do hBMDS produce EVs?

So we isolated EVs from hBMDS.
First need to prove that we isolated EVs.
Using ultracentrifugation followed by EM, and nanoparticle detection software we can see EVs derived from the hBMDC.



1b.tif
365
Print Mag: 22900x @ 7.0 in
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500 nm
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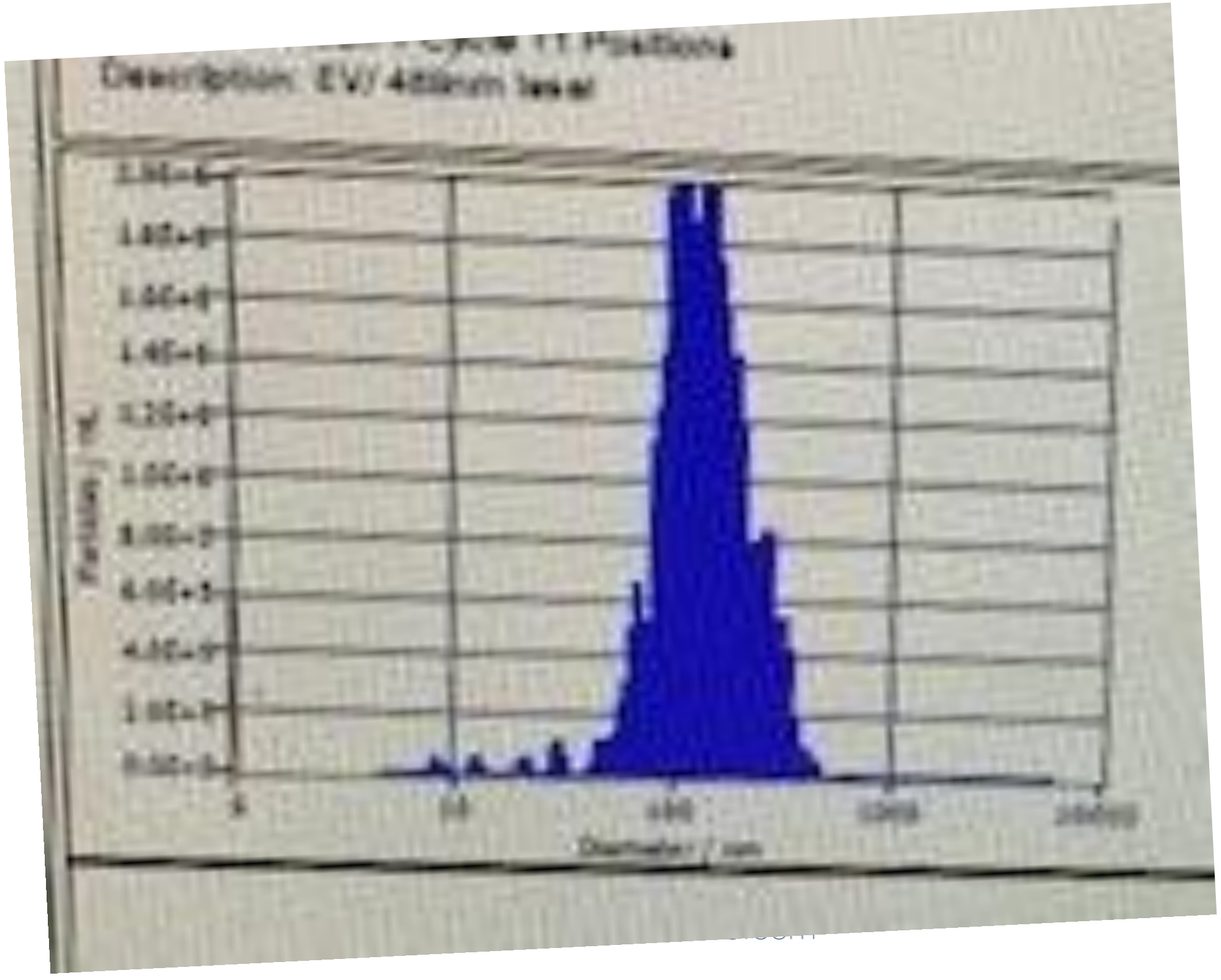
AMT Camera System

Nanoparticle tracking analysis, or NTA, allows for the determination of both particle size and concentration.
Zeta View Avg: 132.3 nm SD=5



Median size (nm) AVERAGE 132 SD 5

Exosomes	Microvesicles	Apoptotic Bodies	Plasma membrane
Origin	Endocytic pathway		
Size	40-120 nm	50-1,000 nm	500-2,000 nm
Function	Intercellular communication	Facilitate phagocytosis	
Markers	CD81, CD63, CD9 flotillin	CD81, CD63, CD9 flotillin	CD81, CD63, CD9 flotillin
Contents	Proteins and nucleic acids (mRNA, miRNA, lncRNAs and chemokines)	Proteins and nucleic acids (mRNA, miRNA, lncRNAs and chemokines)	Nuclear fractions, cell organelles

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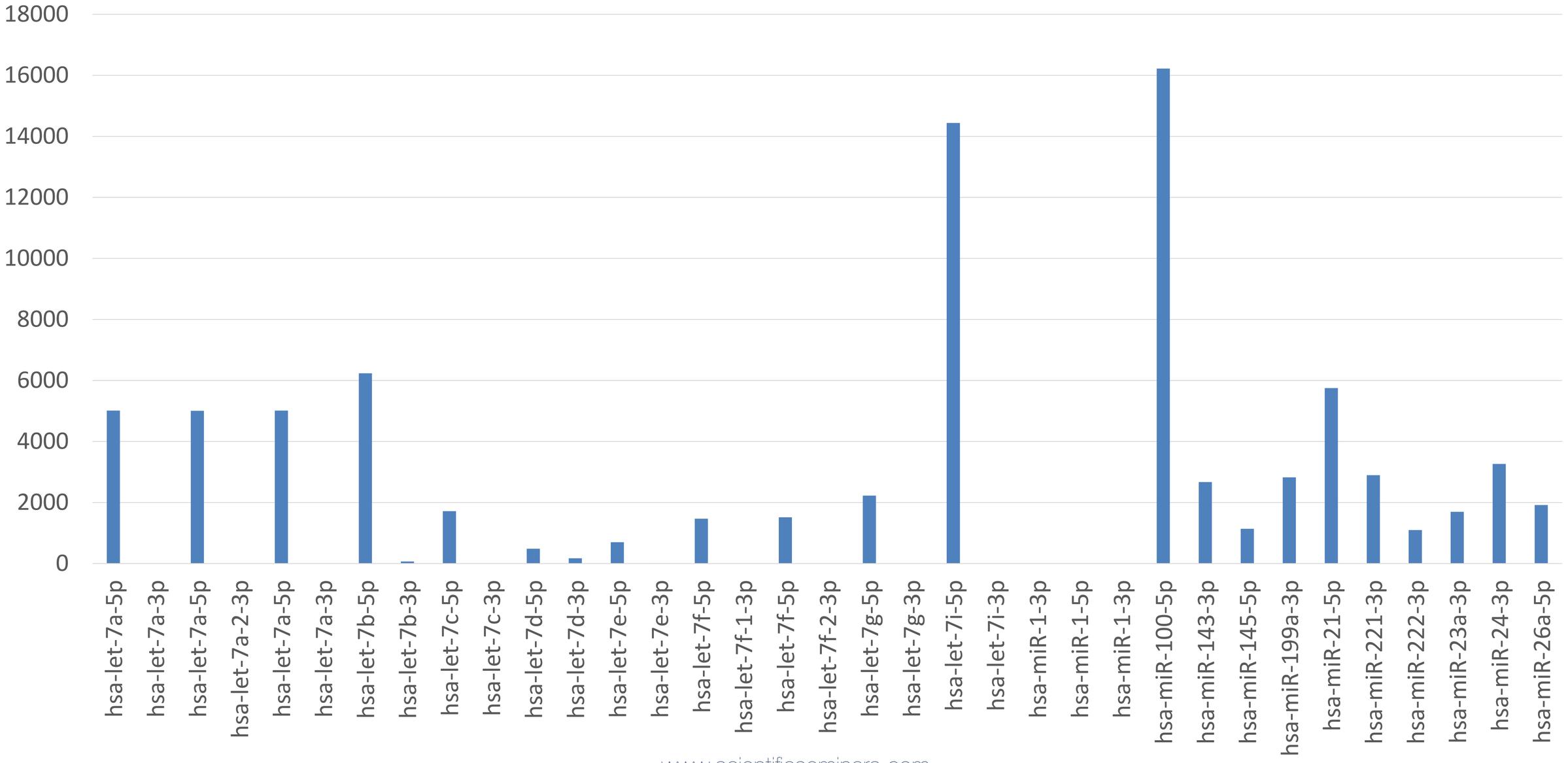
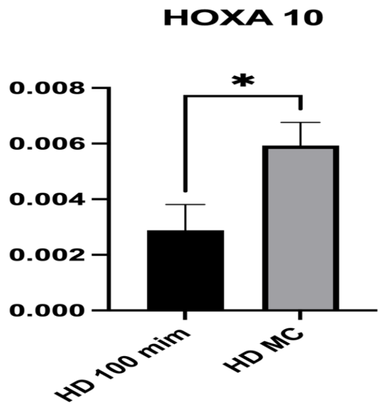
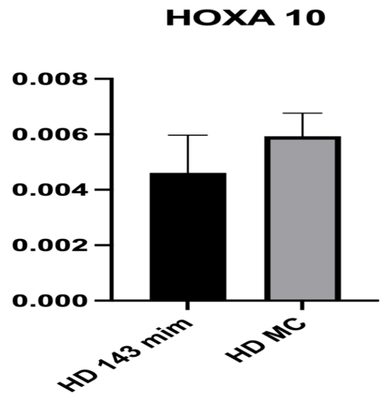
Total miRNA analysis for BMDS derived EVs display several miRNAs

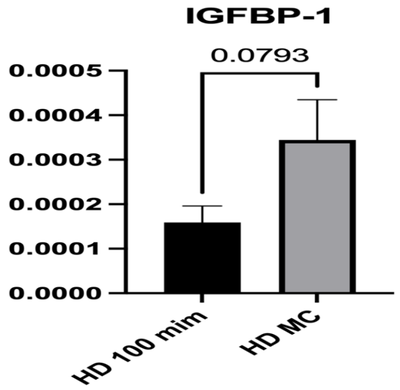


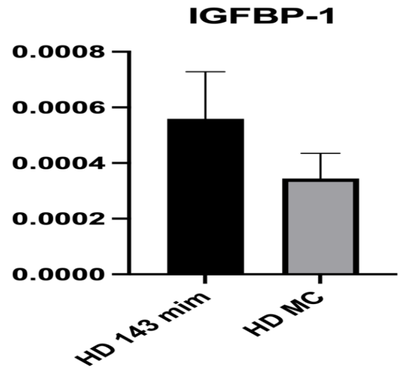
HESCs transfected with: miRNA 100-5p mimic, miR 143-3p mimic, and the mimic control

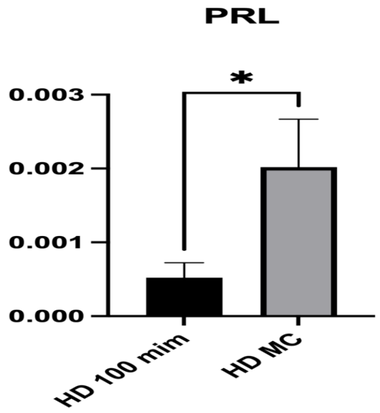
Results are from RT-PCR

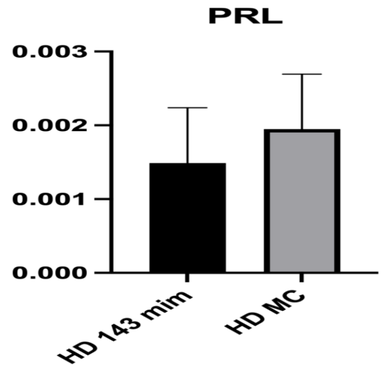












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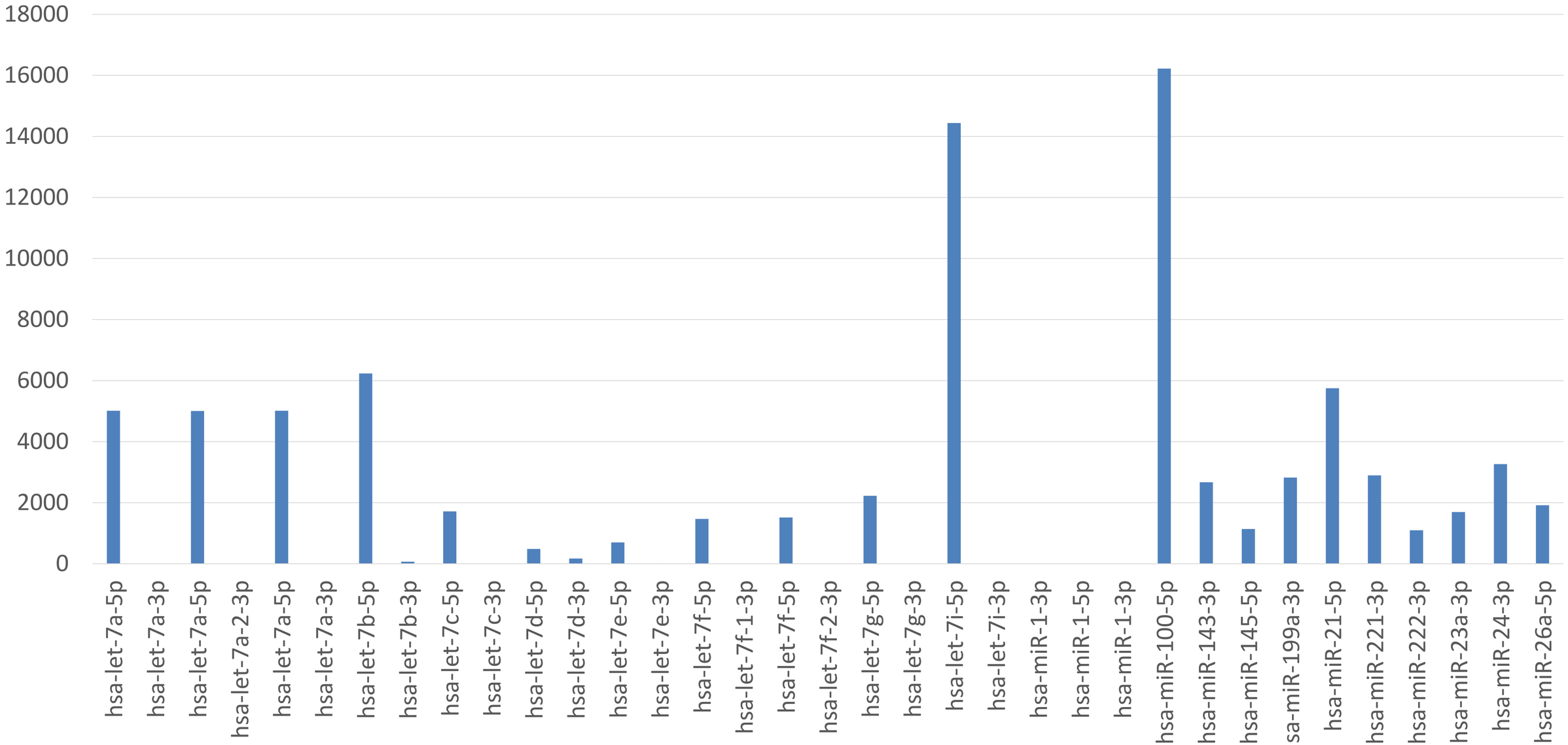
BMDS derived EVs display several miRNAs



In summary, Extracellular vesicles (EVS) have been demonstrated to be carriers of biomarkers and have been shown to be a mechanism of cell-to-cell communication.
Cell communication represents a dynamic mechanism regulated by the release of factors able to influence cell fate, function and plasticity.

Conclusions

Our initial studies in human bone marrow derived stem cells EVS are composed of microvesicles, apoptotic bodies and plasma derived exosomes containing mRNAs, miRNAs, long non-coding RNAs (lncRNAs), lipids and chemokines. Our initial studies in human bone marrow derived stem cells (hBMS) identified EVS by electron microscopy and nano particle analysis (Zeta view analysis). Additionally, several miRNAs were highly expressed by hBMS derived EVS. miRNA 100-5p was the highest expressed miRNA derived from hBMS.

hBMS identified EVS by electron microscopy and nano particle analysis (Zeta view analysis). Additionally, several miRNAs were highly expressed by hBMS derived EVS. miRNA 100-5p was the highest expressed miRNA derived from hBMS.

Summary cont…

Transfection with this highly expressed miRNA (100-5p) mimics resulted in blocking decidualization of human endometrial stromal cells. Specifically, transfection resulted in down regulation of IGFBP, prolactin, HOX-A 10.

Previous studies have shown an increase in EVS derived from endometrial cells, however no studies have been carried out regarding the role of EVs derived hBMS. The inhibitory function of decidualization by miRNA 100-5p would result in infertility related diseases. We propose to study such diseases and the role of highly expressed miRNAs in future studies.

THANK YOU
Yuping Zhao
Abdullah Cosar
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