Characterizing the Long Non-Coding RNA Profile of Endometrial Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential Role in Treating Osteoarthritis

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INTRODUCTION: Endometrial tissue-derived mesenchymal stem cells (eMSC) are a dynamic population of cells that exhibit immense regenerative and immunomodulatory capacities, making them a strong candidate for stem cell-based therapies.[1,2] The therapeutic effects of eMSC are mainly mediated through their extracellular vesicles (EVs), which transport bioactive molecules that modulate cellular activity in target cells.[3,4] Pre-clinical models of MSC-derived EVs (MSC-EV) show their high therapeutic potential for treating osteoarthritis (OA), a condition with no current disease modifying pharmacotherapy.[5-7] The perivascular subgroup of eMSC, marked by CD146 expression, possesses EVs (CD146High eMSC-EVs) with an immunomodulatory miRNA profile that can functionally shift macrophages toward an anti-inflammatory M2 phenotype that may be advantageous in the OA patient.[8,9] Further research defining the bioactive components of CD146High eMSC-EVs is necessary to understand how they directly affect cellular pathways involved in cartilage homeostasis and inflammation which will help discern their therapeutic potential in OA. Specifically, long non-coding RNAs (lncRNA) are key components of EVs known to interact with miRNA, DNA, and proteins in target cells,[10] whereas in OA lncRNAs affect inflammation and degradation processes.[11-13] In our study, we aimed to characterize the lncRNA profile of CD146High and CD146Low eMSC-EVs and further assess their immunomodulatory and anabolic therapeutic function in OA. On this basis, we hypothesized that the CD146High eMSC-EVs lncRNA profile is enriched with anti-inflammatory and pro-anabolic cartilage effects when compared to the CD146Low eMSC-EVs lncRNA profile.

METHODS: Human endometrial tissue (n = 4) was collected after participants provided written informed consent to the CryoVida stem cell bank (Guadalajara, Mexico). The eMSC were magnetically sorted based on CD146 expression to yield the CD146High and CD146Low eMSC subpopulations. eMSC-EVs were isolated from both subpopulations using ultracentrifugation and CD63 magnetic immunoselection methods and characterized by nanosight and flow cytometry analyses. The lncRNA cargo in both groups was assessed by a pre-designed 92 lncRNA qPCR array. Functional assessment of eMSC-EVs was performed in chondrocyte/synoviocyte co-cultures exposed to pro-inflammatory cues. A predesigned 88 genes human osteoarthritis qPCR array was used to assess chondrocytes gene expression profile with and without eMSC-EVs treatment. Pro-angiogenic potential of eMSC-EVs was assessed in an angiogenesis scratch assay using human umbilical vein endothelial cells (HUVEC) exposed to pro-inflammatory cues with and without the addition of eMSC-EVs.

RESULTS: The eMSC-EV extraction protocol reported previously[8] revealed EVs with high purity (89.4%) and size less than 200 nm diameter. LncRNA profiling of the eMSC-EVs revealed 23 lncRNAs highly present in CD146High eMSC-EVs compared to 21 lncRNAs highly present in CD146Low eMSC-EVs (Fig 1A-B). Note that the results of the pathway analysis are displayed in the tables in Figure 1 A-B, with the predicted number of genes involved in each pathway listed along with the statistical significance of each pathway's predicted engagement. Pathway analysis revealed CD146High EVs lncRNAs predicted to be involved in cellular proliferation, osteochondral differentiation, and inflammation (Fig 1A). For the CD146Low EVs lncRNAs, pathways such as osteochondral differentiation, cell proliferation, and cell cycle were top hits (Fig 1B). After exposure to inflammation in the chondrocyte/synoviocyte co-culture assay, there were 29 upregulated genes in chondrocytes treated with CD146High eMSC-EVs and 23 upregulated after treatment with CD146Low eMSC-EVs (Fig 1C-D). Pathway analysis revealed some overlap between groups in involved pathways such as cartilage development and extracellular matrix organization. However, chondrocytes exposed to CD146High eMSC-EVs engaged these anabolic pathways with greater number and significance when compared to CD146Low eMSC-EV exposure. In the angiogenesis scratch assay, both the CD146High (83% reduction) and CD146Low (76%) eMSC-EVs reduced the wound gap more than the no-EV control (62%).

A. CD146High eMSC-EVs — 23 highly present lncRNAs

Top Reactome hits	Number of Genes	Pval
Cell Proliferation	53	0.145
Regulation of Stem Cell	50	0.343
Inflammation	49	0.304
Cell Death	49	0.453
Osteochondral differentiation	48	0.162
Cell Cycle	48	0.392
Immune Response	47	0.427
Has-mir-379 cluster	43	4.75E-08
EGFR1	30	0.137
mir-154	19	0.00059

B. CD146Low eMSC-EVs — 21 highly present lncRNAs

Top Reactome hits	Number of Genes	Pval
Osteochondral differentiation	40	0.0404
Cell Proliferation	38	0.271
Cell Cycle	36	0.375
Regulation of Stem Cell	36	0.454
Apoptosis	35	0.473
Inflammation	34	0.525
MYC	31	0.189
Has-mir-379 cluster	27	0.000403
EGR1	21	0.384
NKFB1	18	0.488
E2F1	17	0.58
TP53	13	0.606
mir-154	13	0.00922

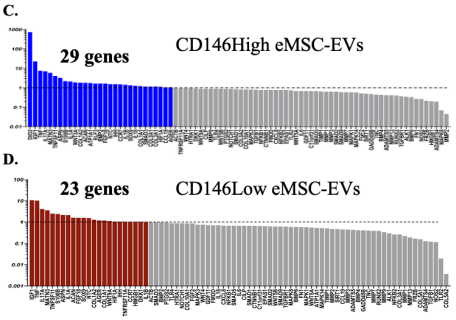

Figure 1. Pathway analysis of the lncRNA profile of CD146High (A) and CD146Low (B) eMSC-EVs. CD146High eMSC-EV (C) and CD146Low (D) effects on chondrocyte gene expression in a chondrocyte/synoviocyte co-culture assay.

DISCUSSION: Given the variety of anabolic and catabolic lncRNAs in the CD146High eMSC-EVs, further functional studies are necessary to define their synergistic effect on cartilage metabolism in OA. The strong pathway engagement of chondrocyte genes induced by CD146High eMSC-EVs exposure suggests their potential benefit over CD146Low eMSC-EVs in treating OA. Additionally, the angiogenesis scratch assay results suggest that CD146High eMSC-EVs demonstrated the greatest protection toward endothelial cell function in an inflammatory environment.

SIGNIFICANCE: We are the first to report the lncRNA profile of CD146High and CD146Low eMSC-EVs, which are strong candidates for OA therapy. LncRNAs engage numerous OA-related molecular pathways that function in cartilage metabolism and inflammation, such as NF-kB and TGF-b,[13-15] and therefore require further investigation to discern their full therapeutic potential in OA.

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