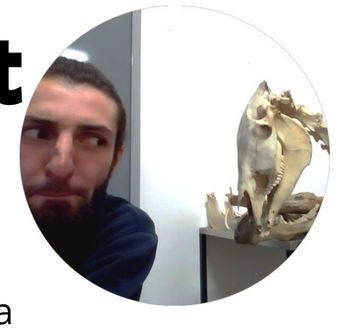
Game of Research Season Four, 4June 2021

Hacking EVs: how to load proteins of interest into MSC extracellular vesicles



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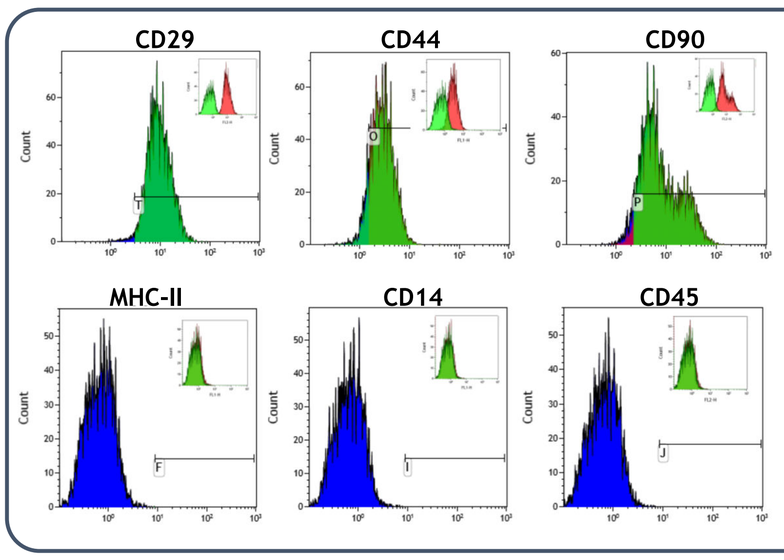
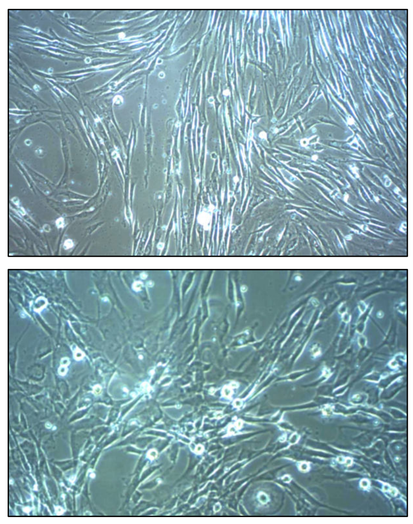
Over the last two decades the study of extracellular vesicles (EVs) has broadened our knowledge about intercellular communication, suggesting new ways through which cells exchange informations in physiological and pathological conditions. EVs are micro and nanoparticles bounded by a lipid bilayer that contain a large variety of biological molecules. They origin from parental cells that "package the cargo" into vesicles and release them in the extracellular space. EVs diffuse in biological fluids and carry out their functions in two principal ways: releasing their cargo into recipient cells by membrane fusion or interacting with surface receptors on target cells.

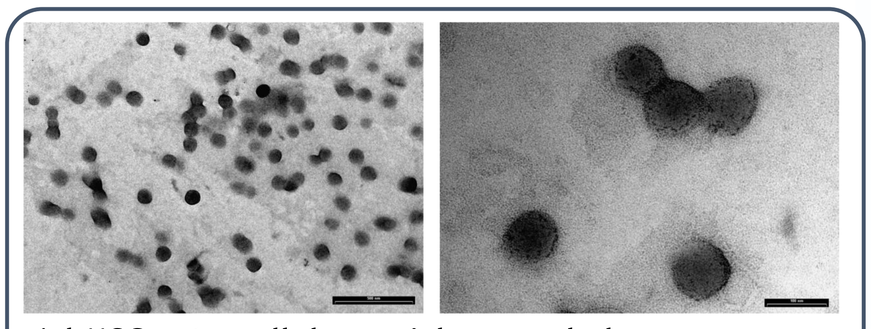
Mesenchymal stromal cells (MSC) are involved in homeostasis of connective tissues, providing support, nourishment and signals; the ever growing interest on their biology and clinical application is due to their effects on tissue regeneration, immunomodulation, inflammation and angiogenesis [1]. EVs represent an essential system through which MSCs carry out their role, making these cells an excellent model for the study of EV biology. Moreover, MSC-derived EVs are considered a possible platform for drug delivery, thanks to their innate pharmacokinetic properties [2].



Canine Mesenchymal Stromal cell characterization:
Flow cytometry of cultured Adipose derived MSC (left); positive markers (CD29, CD44, CD90) and negative markers (MHC-II, CD14, CD45).
Phase contrast micrographs of cultured Ad-MSC (right).

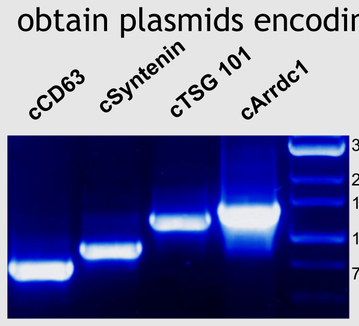


The **purpose of this work** is to deepen the knowledge on mechanisms that regulate EV biogenesis, with special focus on protein loading.
The **long term goal** is to develop a strong method to upload proteins of interest inside EVs from MSCs in order to use them as a tool in drug delivery. Through genetic engineering, we induced expression of fluorescent probe fusion proteins in MSCs to observe and measure the loading index of different EV related proteins.



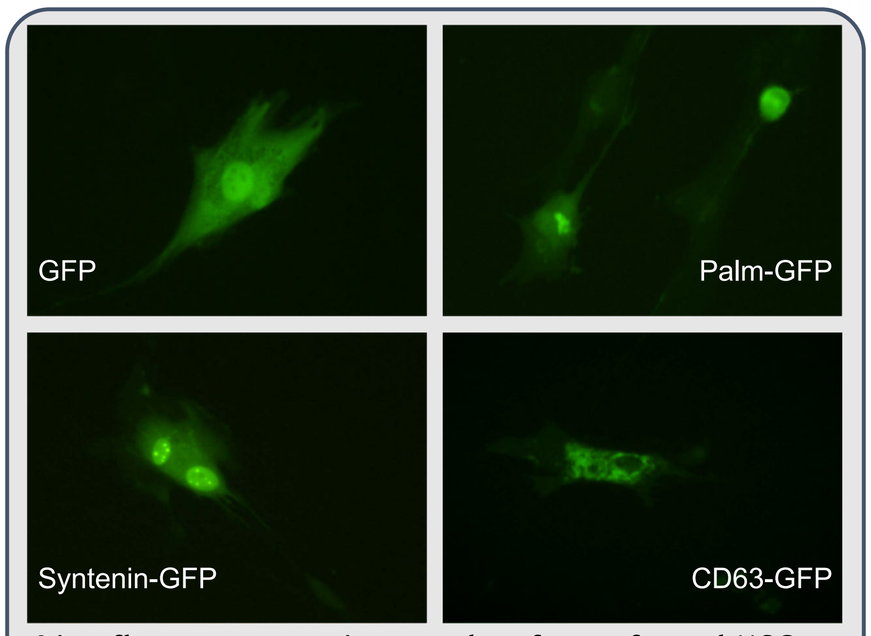
Ad-MSC extracellular vesicles morphology
Electron micrographs of isolated EV from cell culture supernatant of MSC, uranyl acetate staining. Scale bar 500 nm (left), 100 nm (right).

cDNAs encoding **EV related proteins** were isolated and cloned from canine total RNA and used to generate fluorescent probe fusion proteins; sequence encoding EV related proteins were cloned into green fluorescence protein (GFP) expression vector in order to obtain plasmids encoding for chimeric proteins.

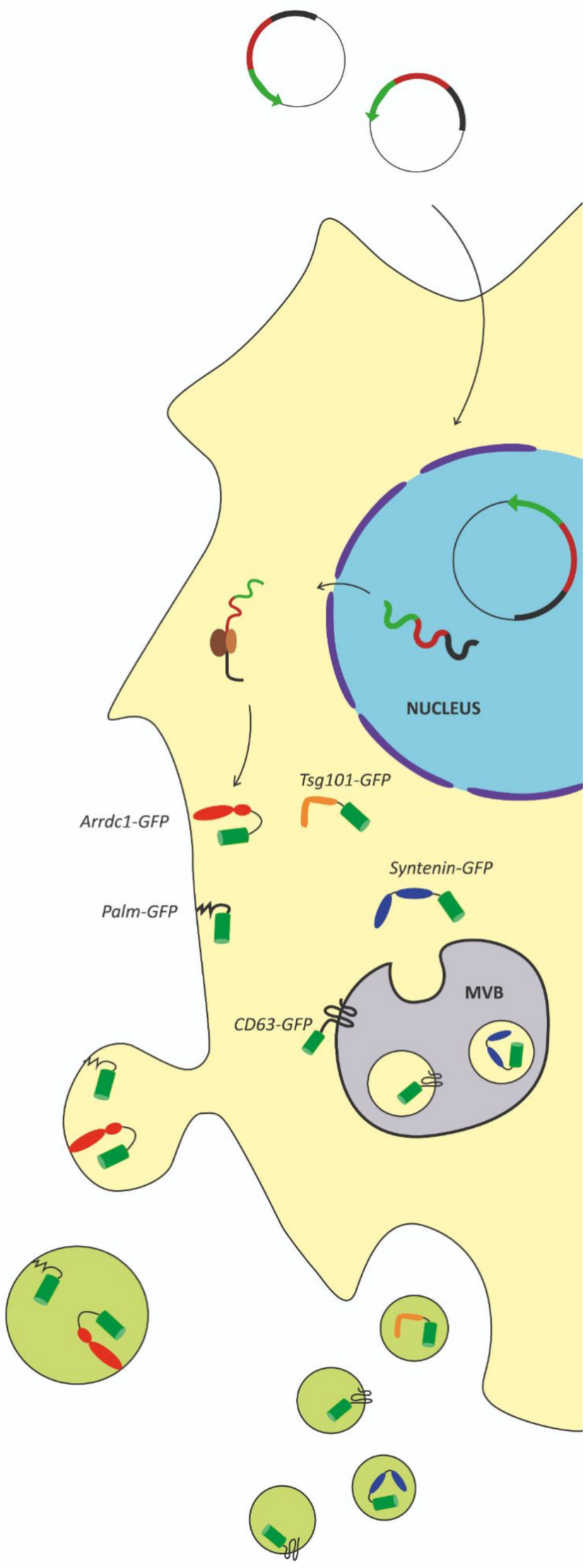


cCD63	740 bp
cSyntenin	921 bp
cTSG101	1255 bp
cArrdc1	1422 bp

Gel electrophoresis of PCR products obtain from canine cDNA amplification (left). Expected size of PCR products (right).



Live fluorescence micrographs of transfected MSC
Canine MSCs were transfected with plasmids to allow the expression of labeled proteins. Images of fluorescent living cells were acquired 48 hours post-transfection



Results: cDNA encoding EV related proteins (obtained from total RNA) show a size compatible with expected length, sequences were confirmed by Sanger sequencing.
Live fluorescence microscopy displayed different intracellular localization and distribution of reporter fusion proteins.

Discussion: preliminary results suggest the high complexity of the mechanism involved in EVs biogenesis. The intracellular distribution of reporter proteins will be investigated with confocal microscopy and immuno-gold. Loading efficiency of reporter protein into EVs will be verify and quantify through Western Blotting.

REFERENCES
[1] Fitzsimmons R. Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering, Stem Cells International, eCollection 2018. [2] Crivelli B. Mesenchymal stem/stromal cells extracellular vesicles: From active principle to next generation drug delivery system, Journal of Control Release, 262:104-117, 2017. [3] Corso G. Systematic characterization of extracellular vesicle sorting domains and quantification at the single molecule – single vesicle level by fluorescence correlation spectroscopy and single particle imaging, Journal of Extracellular Vesicles, eCollection 2019.