miR-125b is a negative regulator of extracellular vesicles biogenesis and distribution at the embryo-maternal interface

Maria M Guzewska[1], Elzbieta Karnas[2], Ewa K Zuba-Surma[2], Monika M Kaczmarek[1]

[1]*Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Olsztyn, Poland;*
[2]*Jagiellonian University, Krakow, Poland*

Extracellular vesicles (EVs) comprise a heterogeneous population of nanoparticles released by different cell types, which due to the advantage of small size can cross the cellular physical barriers playing a crucial role in intercellular communication. Several mechanisms of EVs biogenesis can take place, involving among other the endosomal sorting complex required for transport (ESCRT). Each of the ESCRT complexes (0-III) and accessory proteins (*e.g.*, Rab GTPases) have unique structures responsible for distinct biochemical functions. During stepwise biogenesis, EVs gain internal and external cargo (*e.g.*, RNAs, proteins lipids), which can be trafficked between cells as a means of intercellular communication. In this study we decided to take a deeper insight into the miRNA-governed biogenesis of EVs at the embryo maternal interface. We hypothesize that miR-125b, present in trophoblast cells and uterine EVs during early pregnancy, can: i) affect the expression of a ESCRTs responsible for multivesicular bodies formation (MVBs) and cargo sorting, and ii) change the ratio of subpopulation of released EVs, by switching off the ESCRT-dependent biogenesis pathway in trophoblast cells.

To test this hypothesis, we used porcine trophoblast cells collected at 16 day of pregnancy (DP). Cells were transfected with miR-125b to assess its inhibitory effect on expression of ESCRTs and accessory proteins as well as EVs subpopulations. In addition, the impact of miR-125b on the cellular localization of MVBs (VPS36/VPS37B proteins) and EVs (CD63 external marker) was investigated. Further, baculovirus expressing the fluorescently-tagged RABs was used to gain deeper insight into cellular localization of the population of endosomes/MVBs and $CD63^+$ EVs.

Our findings showed that after miR-125b delivery to 16DP trophoblast cells the abundance of two components of ESCRT complexes I and II (VPS37, $p<0.0001$; VPS36, $p=0.0033$) and associated proteins involved in the final step of EVs biogenesis and vesicular trafficking (VPS4B, $p<0.0001$; RAB8B, $p=0.0117$) is decreased. Using high sensitivity Apogee Flow Cytometer, we showed that after miR-125b delivery the majority of EVs released by trophoblast cells *in vitro* bare $CD81^+$>$CD63^+$>$CD9^+$ markers. Presence of MVBs and EVs in trophoblast cells was indicated after *in vitro* delivery of miR-125b. Significant negative correlation between VPS36 (ESCRT-II and MVBs marker) and CD36 (EVs marker) was observed only after miR-125b delivery ($r=-0.7589$, $p=0.0003$).

In conclusion, our data clearly showed that miR-125b can inhibit EVs biogenesis pathway in trophoblast cells collected at 16DP. As miR-125b expression increases in trophoblast cells after 16DP in pigs it seems likely that after first steps of implantation EVs biogenesis is decreased in trophoblast in favor of endometrium, showing an increasing secretory activity. These results are consistent with our earlier findings that after initiation of implantation embryo is mainly receiving information from endometrial EVs, bringing more evidence on highly controlled EVs-mediated communication between embryo and mother.

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