(P15) Synthetic RNAs for sorting cells in an *in vitro* model of pancreatic reprogrammed cells

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In order to improve de quality life of Type 1 Diabetes patients, basic science is focusing in the develop Cell Therapies to reconstitute the critic pancreatic beta cell mass to restore the glycaemia control. Some strategies involve reprogramming or transdifferentiation from stem cells or differentiated cells to pancreatic beta-like cells, but until now they have to sort many technic impediments before to reach the clinic.

One of the main impediment is the heterogeneous cell population results following a reprogramming process, and it represents a high risk if these cells will be transplant into patients. Trying to avoid this problem, Miki et al., developed a new technique using synthetic RNAs to sorting cells reached a 99.7% of purification efficiency, overcoming the efficiency of some classic method. This technique depends on the activity of the endogenous microRNAs that function as lineage markers. The objective of our work was apply this technic in an *in vitro* model of pancreatic reprogramming cells to sorter microARN-Let7a positive cells. The microRNA-Let7a have been previously associated with insulin producing cells.

For this purpose, we test two cell lines, HEK293T as non-reprogramed cells and MIN6 acting like pancreatic beta cells post reprogramming. We validate the miR-Let7a in order to complement the miR-375 activity as pancreatic beta cells markers; so we synthesize as gBlocks the miR-Let7a-BIM-switch (containing the miR-Let7a complementary sequence and encoding the apoptosis inducer protein BIM), miR-Let7a-BFP-switch, miR-375-BFP-switch and we included mRNA-GFP as lipofection control. Constructions were synthetized by MegaScript® SP6 *in* vitro transcription kit. Both cell lines were transfected using Lipofectamine 2000® with different concentrations of mir-Let7a-Bim synthetic switch in order to probe their tolerance to the apoptotic protein, Bim. Apoptotic cells were visualized after 18 hrs by TUNEL assay under fluorescent microscope. After this time, we design an *in vitro* model simulating the post reprogramming enviroment with a co-cultured of HEK293T and MIN6 cells. The synthetic miR-Let7a-Bim was transfected with the miR-375-BFP. After 18hs apoptotic cells were counted under fluorescent microscope and 85% of HEK293T were dead, so we enriched insulin positive MIN6 cells in >80% from a heterogenic population in an *in* vitro model and without using any equipment like flow cytometry with cell sorter.

We confirmed that synthetic RNA switch are a novel tool for purification of living cell type applied in a post reprogramming enviroment based in their differential microRNA activity.