miRNA as Bystander Effect Factor

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Abstract:

miRNA are 21-23 mer RNA molecules which are essential for organism development and cell functions. They regulate gene expression by binding to the 3'UTR of mRNA, inducing either mRNA degradation or mRNA silencing. The most characteristic properties of miRNA are their multi-targeting potential (one miRNA may target many genes). This high information content of miRNAs makes them very important factors in cell reprogramming. Since these are small molecules which can potentially pass through gap junctions, it is logical to consider their role in cell to cell communication. We hypothesized that miRNA transfer between cells is likely to occur under stress conditions. To test this hypothesis we developed a system designed to show 1) that there is transfer of nonfunctional or functional miRNA between normal human fibroblasts and 2) this transfer is stress dependent. For this, we generated stable cell clones of normal human fibroblast expressing GFP and lamin A specific miRNA. To detect miRNA transfer we co-culture these cells with non-miRNA expressing cells and determined the expression levels of lamin in all cells by quantitative fluorescent microscopy. The results show that lamin expression was reduced in the non-miRNA expressing cells only in condition where

all cells were irradiated. This effect was not detected in non-irradiated cells. These results suggest that functional miRNA may have been exchanged between the cells. To physically detect the transferred miRNA in the neighbor cells we used cell sorting where we measured the presence of the lamin specific miRNA in the non-miRNA expressing cells. Quantitative PCR of the sorted cells was inconclusive due to leak of miRNA expressing cells in the flow channel of the non-expressing cells. To overcome this problem, we designed and produced a microfluidic chip which allows sorting of live cells labeled with either live dyes or GFP. Manually operated, it can sort very small number of cells (1 to 100), reaches frequencies of 10 cells per minute, and automation can increase this number into the kHz regime. Sorting occurs through hydrodynamic switching, with very low hydrodynamic shear stresses, preserving the pre-sorted cell status. The chip is made from hard plastic material (PMMA) into which microchannels are directly milled with hydraulic diameter of 70um. A syringe pump provides a sheath flow that deflects the cells into one of the two collection reservoirs, depending on the state of two fast solenoid valves. The cells are maintained in an isotonic buffer throughout the sorting process and can be used immediately after the sorting. Experiments for determination of miRNA transfer between cells using this device are in progress.