NOVEL CLONE SELECTION TECHNIQUE REVEALS HETEROGENEITY AMONG HEK293T CELLS ENGINEERED TO PRODUCE THERAPEUTIC EXTRACELLULAR VESICLES

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HEK293T cells have been engineered to produce extracellular vesicles (EVs) that deliver miR-199a-3p to CD44+ hepatocellular carcinoma cells. Restoration of this miRNA has been shown to slow cancer progression in-vitro.

Isolation and analysis of EVs from cell culture media containing selection agent revealed that the number of miRNA-199a-3p copies was less than the number of cells in culture suggesting that not all cells produce therapeutic EVs. Therefore, therapeutic EV production can be significantly increased by selecting the HEK293T clones that produce the most therapeutic EVs. While clone selection is traditionally accomplished by cell analysis techniques such as fluorescence activated cell sorting (FACS), detection of therapeutic EVs poses a unique challenge in that cellular expression of miRNA-199a-3p does not necessarily correlate to the amount of exosomal miRNA-199a-3p. In response to this challenge, a fibrous microwell array was developed to screen thousands of clones for therapeutic EV productivity (figure 1).

The fibrous microwell system is able to evaluate cell growth rate under fluid shear stress, EV productivity and EV characterization using fluorescently labeled antibodies or cationic lipoplex nanoparticles (detect presence of miRNA-199a-3p inside captured EVs produced by single clones). The most productive clones can be released from the microwells and grown in large scale cell culture to significantly increase therapeutic EV production.

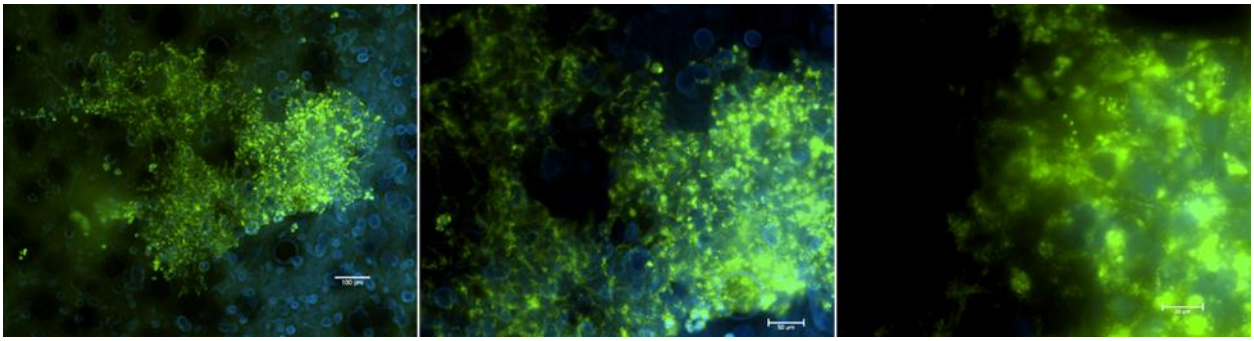

Figure 1: Three colonies of HEK293T cells stained with WGA (green). One of the colonies appears to contain more WGA stain than the other two. Increasing the magnification reveals that the green dots are mostly inside the cell; however, some of the green dots (presumed EVs) are also located outside of the cells. The blue circles in the 10x image (left) are not cells, but beading that occurred as a result of electrospinning with a fresh batch of PCL solution. Cells are denoted by the weak green stain outlining the cell membrane.

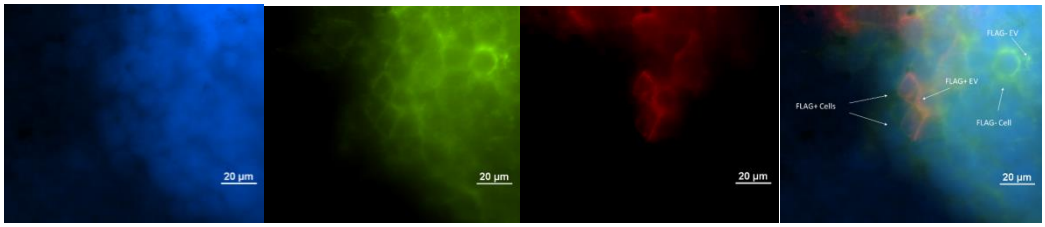

Figure 2: Polyclonal HEK293T colony demonstrates heterogeneity among individual cells. Cells secreting FLAG+ EVs (red) also express FLAG protein on the extracellular surface of their plasma membrane. Nuclei are stained blue, lipid bilayer is stained green