Abstract

Background: Residual human breast tumor cells after conventional therapies are enriched in tumor initiating cells (TIC) characterized by CD44+/CD24-/low/lineage- with self-renewal capacities. Our gene expression analyses in those cells and in breast cancer cells propagated as mammospheres (MSs) reveal a tumorigenic signature which correlates with epithelial to mesenchymal transition (~500 genes) upregulated in claudin-low molecular subtype human breast tumors. SUM159 and BT549 cell lines have been identified as good models of this subtype. We recently demonstrated that targeting tumor initiating cells with siRNA/nanotherapy in triple negative breast cancer (TNBC) human breast xenografts implanted in mice could significantly reduce breast tumor burden.

Methods: We performed lentiviral siRNA knock-down of the tumorigenic signature in both cell lines (8 times) using high throughput mammosphere formation efficiency analysis (MFSE). Critical shRNAs were identified in primary breast tumor samples and targeted through a lentiviral shRNA screening platform.

Results: A subset of 15 genes were found to be fundamental for MS formation in both cell lines. Here we show the pathways important in MS formation in SUM159 (left) and BT549 (right).

Conclusions

This method of screening in both the BT549 and SUM159 cell lines allowed us to confirm the most significant genes and follow them up in-vivo xenograft experiments. Among the top candidates we found STAT3 (positive control), MLF2/NTN4 and RPL39L significantly reduce the MSFE of secondary MSs when compared to the scrambled control in BT549 cells. For SUM159 cells, we observed a decrease in secondary MSFE of secondary MSs when compared to the scrambled control (100%) in both cell lines. We conducted in vivo experiments to document the effect of MLF2 and RPL39L using siRNA packaged in multistage nanoparticles in triple negative human cancer xenografts (BCM-2665) derived from biopsies of human primary breast cancers transplanted in SCID Beige mice (n=3). Groups: (1) Scrambled control. (2) Scrambled siRNA against MLF2 packaged in nanoparticles (single injection i.v. on Day 1) (3) siRNA against RPL39L packaged in nanoparticles (single injection i.v. on Day 1). Animals were sacrificed on day 14 and MFSE was performed after the tumor tissue digestion. A dramatic decrease in MS formation was seen in those injected mice with siRNA against MLF2 (33%) and RPL39L (67%) compared to the scrambled control (100%). This clinically relevant delivery system will allow development of therapies that complement the current standard of care.

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