CRISPR/cas9-mediated Generation of an EMT Reporter Cell Line for Metastatic Breast Cancer Drug Discovery and Development

Metewe Selase Enuameh, PhD, Robert Newman, PhD, Weiguo Shu, PhD
ATCC Cell Systems, Gaithersburg, MD 20877, USA

Abstract

Among women, breast cancer continues to be the most common cancer, with metastasis being the leading cause of mortality in patients around the world. 1 Epithelial to mesenchymal transition (EMT)—the process by which epithelial cells shift to the mesenchymal state—has been implicated in many aspects of breast cancer, carcinogenesis, metastasis, and drug resistance. 2 However, despite the extensive accumulation of data on the association of EMT with cancer over the years, EMT has not been an active target for therapeutic development. This is due in part to the lack of appropriate in vitro models. Here, we have exploited some of the basic biology of EMT to create an advanced in vitro metastatic breast cancer reporter cell line model for use in basic research and the discovery of new EMT inhibitors.

Methods and Results

Design of CRISPR/Cas9 Reagents to Generate ECAD EmGFP Fusion in the Human Breast Ductal Carcinoma Cancer Cell Line, BT-474

Figure 1. Identification of single-guide RNA (sgRNA) target site of the ECAD genomic locus. A sgRNA that was designed and built to guide Cas9 to and cut near the ECAD stop codon was used to facilitate the knock-in (KI) of the ECAD EmGFP donor template at the ECAD locus upon co-transfection.

References


Figure 2. (A) Flanger sequencing results for the donor right homology arm EmGFP junction. (B) Sequence of ECAD EmGFP transport across DNA ECAD EmGFP junction for the isolated clone. The yellow line in the peptide sequence linking the ECAD gene to the EmGFP sequence. The red dashed line in the chromatogram indicates the regions where the linearizable yellow (line) merge with the ECAD arm or the EmGFP sequence.

Figure 3. Growth rate of BT-474 ECAD EmGFP cell linein within 0.5% of parental cell line.

Figure 4. (A) BT-474 ECAD EmGFP cells were incubated in DMEM medium containing 10% FBS and supplemented with either (B) EMT-inducing media supplement (+ EMT) or an equivalent volume of 1X DMEM (as a no EMT control). EMT for 3 days. Western blotting analysis of cell samples detect increase in W/T ECAD and EMT expression upon EMT induction. (B) Images of +/- EMT (top row) and +/- EMT (bottom row) BT-474 ECAD EmGFP cells were captured by using a high-content imaging system. Treatment of BT-474 ECAD EmGFP with 2X StemXVivo EMT inducing media supplement induced EMT and resulted in decreased ECAD EmGFP expression (green, top and bottom left). Additionally, a decrease in total ECAD (WT ECAD & ECAD EmGFP) expression (red, middle top and bottom) was observed by immunocytochemistry with an ECAD antibody-fluorescent protein conjugate. The nuclei of cells were counterstained with a nuclear stain (blue). The right panels are an overlay of the ECAD EmGFP and ECAD expression data (C) The decreased ECAD EmGFP expression upon EMT induction was quantified using the software system (n=16). *p<0.05 compared with the +/- EMT control.

Figure 5. After a 5-day incubation with (+ EMT) or without (- EMT) 2X StemXVivo EMT inducing media supplement, BT-474 ECAD EmGFP cells were monitored for 24 hr period in invasion through an 8-μm pore filter of the basement membrane of the Corning® BioCoat™ Matrigel Invasion plate. EMT-induced BT-474 ECAD EmGFP cells show increased invasion capacity. The number of invadopodia "+ EMT" nuclear counterstained cells are normalized to the "- EMT" control. Nuclear data represent mean ±SD. n=12; *p<0.05 compared with the "- EMT" control.

Summary

- We have generated an ECAD-EmGFP fusion, EMT reporter cell line via CRISPR/Cas9 genome-editing technology.
- The BT-474 ECAD EmGFP reporter cell line has similar growth kinetics as the parental cell line.
- The reporter cell line undergoes epithelial-to-mesenchymal phenotype change upon EMT stimulation for 5 days, resulting in a weak ECAD EmGFP signal due to downregulated ECAD expression.
- The BT-474 ECAD EmGFP exhibits increased invasion capacity following the induction of EMT.
- Given its sensitivity to U0126, the BT-474 ECAD EmGFP reporter cell line can be used in applications targeting the identification of new anti-EMT drugs for breast cancer and is a suitable and sensitive model for basic science research on the mechanisms of metastasis.