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

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Credible leads to Incredible

Poster #1045

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 tumorigenesis, 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.

During EMT, E-cadherin (ECAD) protein expression is downregulated in cancer cells in association with the loss of cell-to-cell adhesion and apico-basal polarity and changes to spindle-shaped morphology. By installing an emerald green fluorescent protein (EmGFP) tag on the C-terminus of the ECAD gene in the epithelial BT-474 (ATCC® HTB-20™) breast cancer cell line via CRISPR/Cas9 genome-editing technology, end-point or real-time EMT status of cells can be tracked under defined conditions. The EMT reporter cell line was verified at the nucleic acid (genomic and mRNA) and protein levels as well as in cell-based assays. Functional evaluation of the BT-474 ECAD EmGFP cell line can be monitored by both EmGFP expression and the invasive capacity of the cells. Our results demonstrate that these cells respond to EMT induction by showing decreased ECAD EmGFP expression along with an increased invasive capacity. Further, this EMT reporter cell line shows sensitivity to the MEK1/2 inhibitor U0126, thereby providing the basis for the use of this cell line in high-throughput screening (HTS) applications such as the identification of new anti-EMT drugs for metastatic breast cancer. Further, the BT-474 ECAD EmGFP reporter cell line is also a convenient and sensitive model for basic science research on the mechanisms of metastasis.

Methods and Results

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

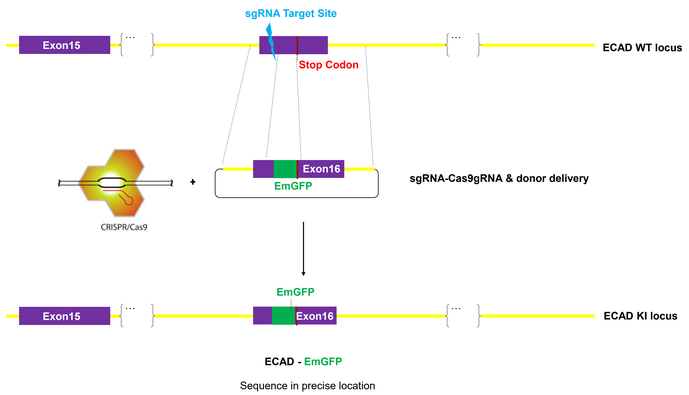


Figure 1. Identification of single guide RNA (sgRNA) target site at the ECAD genomic locus. A sgRNA that was designed and built to guide Cas9 to bind and cut near the ECAD stop codon was used to facilitate the knock-in (KI) of the ECAD EmGFP donor template at the ECAD locus upon co-transfection.

The ECAD EmGFP Fusion Was Confirmed at the DNA and mRNA Levels

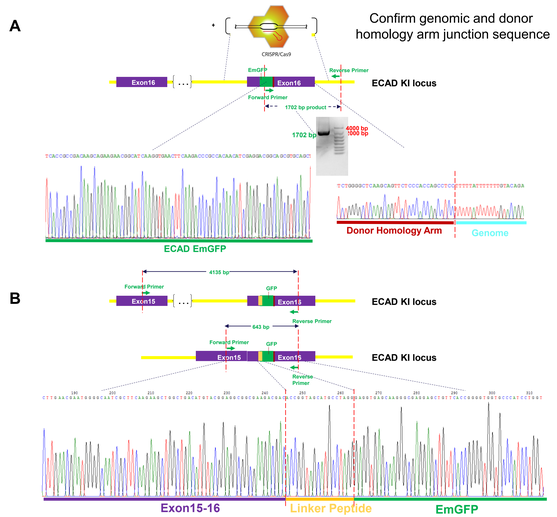
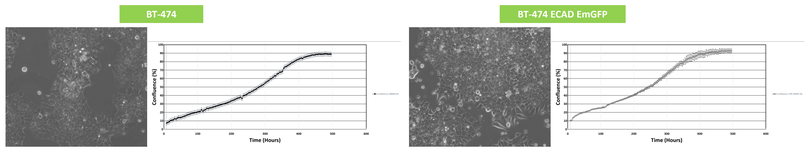


Figure 2. (A) Sanger sequencing results for the donor right homology arm-linker peptide-EmGFP junction. (B) Sequence of ECAD EmGFP transcript across cDNA ECAD EmGFP junction for the isolated clone. The yellow line is the peptide sequence linking the ECAD gene to the EmGFP sequence. The red dashed lines in the chromatogram indicate the regions where the linker peptide (yellow line) merges with the ECAD exon or the EmGFP sequence.

Morphology and Growth Rate of the BT-474 ECAD EmGFP Cell Line and Parental Cell Line Are Similar



Doubling time = 136.1 hours

Doubling time = 136.8 hours

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

Small Molecule EMT Inhibitors Induce MET Transition in BT-474 ECAD EmGFP Cells

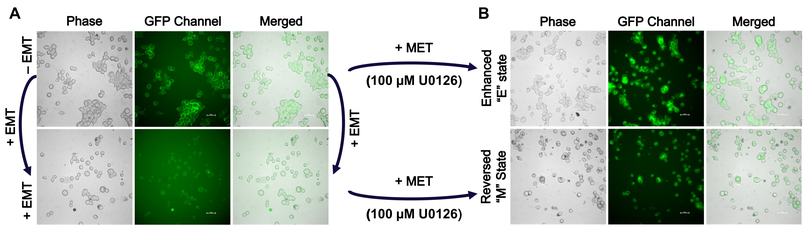


Figure 6. (A) BT-474 ECAD EmGFP cells were incubated in DMEM complete medium and supplemented with either 2X StemXVivo EMT inducing media supplement (+ EMT) or an equivalent volume of 1X DPBS (– EMT) for 3 days. Images of "– EMT" (top row) and "+ EMT" (bottom row) BT-474 ECAD EmGFP cells were captured 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). (B) Images here depict the condition where the "– EMT" and "+ EMT" experiments in 'A' were carried out in the presence 100 µM U0126 such that epithelial cells become more epithelial or EMT is reversed respectively, resulting in the respective increased ECAD EmGFP expression. The nuclei of cells were counterstained with a nuclear stain (blue). The right panels are an overlay of the phase contrast and GFP channel images.

BT-474 ECAD EmGFP Displays Decreased Epithelial Marker Protein Expression After EMT

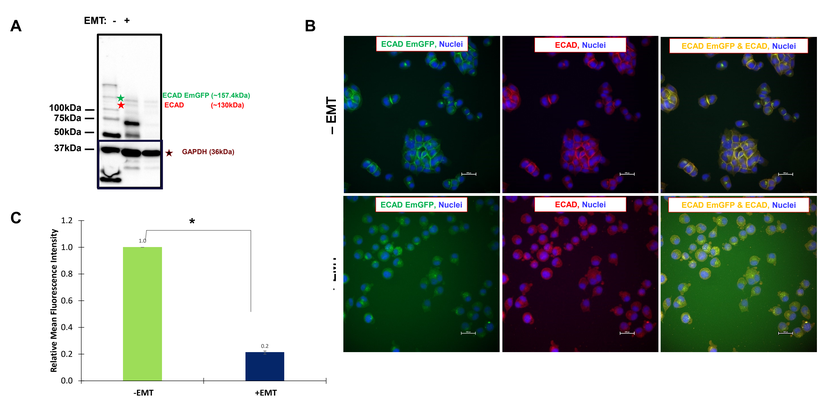


Figure 4. (A) BT-474 ECAD EmGFP cells were incubated in DMEM medium containing 10% FBS and supplemented with either 2X StemXVivo EMT inducing media supplement (+ EMT) or an equivalent volume of 1X DPBS (as a no EMT control; - EMT) for 3 days. Western blotting analysis of cell samples depict decrease in WT ECAD and ECAD EmGFP 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 system software (n=16); *p<0.05 compared with the "– EMT" control.

EMT Induced BT-474 ECAD EmGFP Cells Have Increased Invasion Capacities

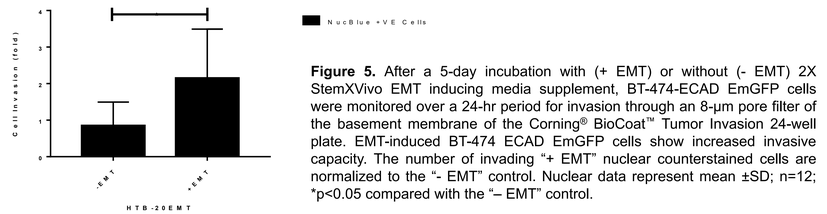


Figure 5. After a 5-day incubation with (+ EMT) or without (- EMT) 2X StemXVivo EMT inducing media supplement, BT-474-ECAD EmGFP cells were monitored over a 24-hr period for invasion through an 8-µm pore filter of the basement membrane of the Corning® BioCoat™ Tumor Invasion 24-well plate. EMT-induced BT-474 ECAD EmGFP cells show increased invasive capacity. The number of invading "+ 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.
- BT-474 ECAD EmGFP exhibits increased invasion capacity following the induction of EMT.
- Given it's 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.

References

1. Dizon DS, *et al*. Clinical Cancer Advances 2016: Annual Report on Progress Against Cancer From the American Society of Clinical Oncology. *J Clin Oncol* 34: 987-1011, 2016.
2. Hay ED, An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)* 154: 8-20, 1995.

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