A Breast Cancer MET Reporter Cell Line Model for Drug Discovery and Development

Metewo Selase Enuameh, PhD, Sangeeta Kumari, MS, Robert Newman, PhD, Weiguo Shu, PhD
ATCC Cell Systems, Gaithersburg, MD 20877, USA

Abstract

World-wide, metastasis continues to be the leading cause of death in cancer patients.1 Although epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) have been implicated in the incidence of cancer metastasis and drug resistance, their impact in cancer progression and patient survival is not fully understood.2 This is partly due to the lack of suitable in vitro models. Thus, to facilitate the study of the utility of the MET concept in therapeutic development, we have utilized some of the basic biology of EMT/MET to create a novel advanced in vitro model for use in both basic research and discovery of new anti-EMT drugs.

In breast cancer, vimentin (VIM) intermediate filament (IF) proteins are generally upregulated during EMT and down-regulated during MET.3 Here, we employed CRISPRi/Cas9 gene-editing technology to generate a VIM RFP (red fluorescent protein) reporter in the MDA-MB-231 (ATCC® HTB-26™) breast adenocarcinoma cell line. The VIM RFP C-terminal fusion gene at the endogenous VIM locus enables end-point or real-time tracking of the MET status as cells transition from the mesenchymal to epithelial phenotype under distinct conditions. We have validated the cell line at the nucleic acid (genomic and mRNA) and protein levels as well as in cell-based assays. Bio-functional evaluation of the MDA-MB-231 VIM RFP (ATCC® HTB-26MET™) cell line shows sensitivity to metastatic breast cancer drugs axitinib (tyrosine kinase inhibitor) and U0126 (MEK1/2 inhibitor) via the inhibition of the intrinsic signaling pathways which impact EMT. These effects provide the basis for the use of this cell line in high-throughput screening (HTS) applications such as the discovery of new anti-EMT drugs for metastatic breast cancer. Furthermore, the MDA-MB-231 VIM RFP reporter cell line is also a convenient and sensitive model for studying the mechanisms of metastasis and for basic science research.

Methods and results

Design of CRISPRi/Cas9 Reagents to Generate VIM RFP Fusion in the Human Breast Cancer Cell Line, MDA-MB-231

VIM RFP Fusion Was Confirmed at the DNA and mRNA Levels

Methods


References

Conclusion

We have generated a vimentin-RFP fusion, MET reporter cell line via CRISPRi/Cas9 gene editing. The MDA-MB-231 VIM RFP reporter cell line has similar growth kinetics as the parental cell line. MDA-MB-231 VIM RFP progresses from mesenchymal to epithelial phenotype upon U0126 stimulation for 3 days, resulting in a weak VIM RFP signal due to downregulated vimentin expression.

There is decreased invasion capacity and sensitivity to axitinib and U0126 inhibition following MET. MDA-MB-231 VIM RFP can be used in HTS applications for the identification of new anti-EMT drugs for breast cancer. MDA-MB-231 VIM RFP can also be used to study the basic mechanisms of metastasis.

U0126 MET-Directed MDA-MB-231 VIM RFP Cells Have Decreased Invasion Capacity

Small Molecule EMT Inhibitors Induce MET Transition in MDA-MB-231 VIM RFP Cells

Figure 1. Identification of single guide RNA (sgRNA) target site on the VIM genomic locus. A sgRNA was designed and built in guide Cas9 to land and cut near the VIM stop codon, facilitated the knock-in (KI) of the VIM-RFP donor template at the VIM locus upon co-transfection.

Figure 2. (A) Sanger sequencing results for the donor left homology arm (LHA) sgRNA construct RFP-PTJ (top). (B) Sequence of VIM RFP transgenic across CDA1 VIM RFP insertion for the isolated clone. The yellow line is the peptide sequence linking the VIM gene to the RFP sequence. The red asterisked lines in the chromatogram indicates the regions where the basic peptide (yellow line) merges with the VIM exon and the RFP sequence.

Figure 3. Morphology and Growth Rate of the MDA-MB-231 VIM RFP Cell Line and Parental Cell Line Are Similar

Figure 4. (A) MDA-MB-231 and MDA-MB-231 VIM RFP cells were incubated for 3 days in Cell-Elut Medium (ATCC®-30-2017® containing 0.1% methylene blue, 9% fetal bovine serum, 1% penicillin/streptomycin) and supplemented with either 10 ng U0126 (MET) or an equivalent volume of DMSO (as a MET control). Western blotting analysis of cell samples show decrease in VIM and VIM RFP in MDA-MB-231 VIM RFP cells captured by using a high-content imaging system. As shown, treatment of MDA-MB-231 VIM RFP with U0126, induced MET and resulted in decreased VIM RFP expression (left and top right). (B) The decreased VIM RFP expression upon MET induction was quantified by using the system software (top right); *p<0.05 compared with the “-MET” control. Additionally, a decrease in total vimentin (WT VIM & VIM RFP) expression (green; middle top and bottom) was observed by immunocytochemistry with a VIM antibody-fluorescent protein conjugate. The nuclei of cells were counterstained with a nuclei stain (blue). The right panels are an overview of the VIM RFP and VIM expression data.

Figure 5. After a 3 day incubation with (+MET) or without (-MET) U0126, MDA-MB-231 VIM RFP 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. MDA-MB-231 VIM RFP cells show decreased invasion capacity. The number of invading “+MET” nuclear and RFP cells are normalized to the “-MET” control. The similar number of RFP positive and nuclear counterstained cells depict the utility of the VIM RFP reporter cell line for evaluation in small molecule assays. Nuclear data represent mean ± SD. *p<0.05 comparison with the “-MET” control.

Figure 6. Treatment with actinocin (tyrosine kinase inhibitor) and U0126 (MEK1/2 inhibitor) facilitates MET via the inhibition of the intrinsic signaling pathways impacting EMT. In both cases, the drug was incubated for 3 days at a final concentration of 1µM U0126 and 2µM actinocin, respectively. MDA-MB-231 VIM RFP cells were processed for determination of cell viability and decreased VIM RFP expression. A dose-response plot of the data set enabled the determination of IC50 for actinocin and U0126. Error bars indicate the standard deviation over 6 wells.