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

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Credible leads to Incredible

Poster #1046

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 utility of the EMT 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.[3-6] Here, we employed CRISPR/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 inherent 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 CRISPR/Cas9 Reagents to Generate VIM RFP Fusion in the Human Breast Cancer Cell Line, MDA-MB-231

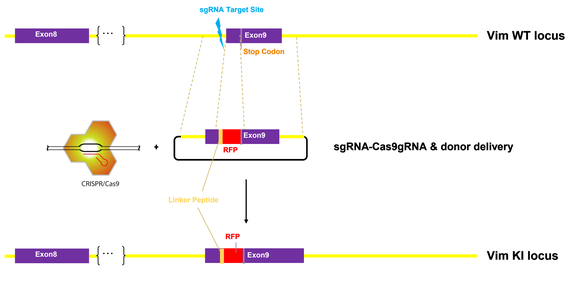

Figure 1. Identification of single guide RNA (sgRNA) target site at the VIM genomic locus. A sgRNA that was designed and built to guide Cas9 to bind and cut near the VIM stop codon, facilitated the knock-in (KI) of the VIM RFP donor template at the VIM locus upon co-transfection.

VIM RFP Fusion Was Confirmed at the DNA and mRNA Levels

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

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

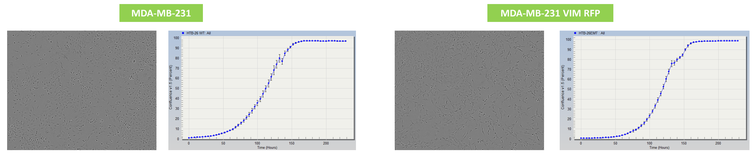

Figure 3. Growth rate of MDA-MB-231 VIM RFP cell line is within 10% of parental cell line.

Conclusion

- We have generated a vimentin-RFP fusion, MET reporter cell line via CRISPR/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.

MDA-MB-231 VIM RFP Shows Decreased Mesenchymal Marker Protein Expression After MET

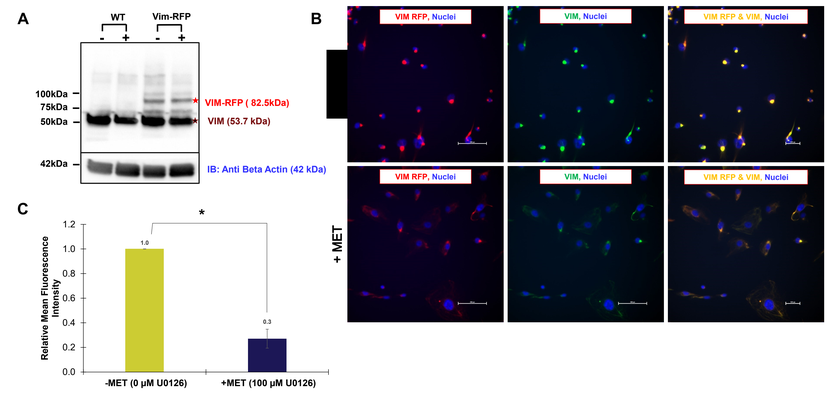

Figure 4. (A) MDA-MB-231 and MDA-MB-231 VIM RFP cells were incubated for 3 days in Eagle's Minimum Essential Medium (ATCC® 30-2003™) containing 0.01 mg/mL recombinant insulin and 10% Fetal Bovine Serum (ATCC® 30-2020™) and supplemented with either 100 µM U0126 (+ MET) or an equivalent volume of DMSO (as a no EMT control; – MET). Western blotting analysis of cell samples depict decrease in WT VIM and VIM RFP expression upon U0126 MET induction. (B) Images of "– MET" (top row) and "+ MET" (bottom row) MDA-MB-231 VIM RFP cells were 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 (red; top and bottom left). (C) The decreased VIM RFP expression upon MET induction was quantified by using the system software (n=16); *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 nuclear stain (blue). The right panels are an overlay of the VIM RFP and VIM expression data.

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

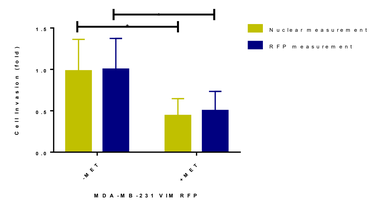

Figure 5. After a 3 day incubation with (+ MET) or without (– MET) U0126, MDA-MB-231-VIM RFP cells were monitored over a 24-hr period for invasion through an 8-µm pore filter of the basement membrane of the Corning™ BioCoat™ Tumor Invasion 24-well plate. MET-induced MDA-MB-231 VIM RFP cells show decreased invasive 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 expression to monitor invaded cells. Nuclear data represent mean±SD; n=5; RFP data represent mean ± SD; n=5; *p<0.05 compared with the "– MET" control.

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

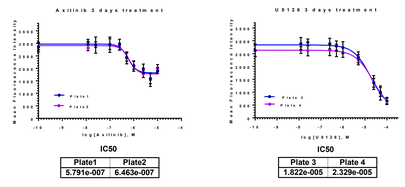

Figure 6. Treatment with axitinib (tyrosine kinase inhibitor) and U0126 (MEK1/2 inhibitor) facilitates MET via the inhibition of the inherent signaling pathways impacting EMT. In both cases, EMT was inhibited by the compounds. As shown, treatment of MDA-MB-231 VIM RFP with increasing concentrations of either drugs induced MET and resulted in decreased VIM RFP expression. A dose-response plot of the data set enabled the determination of an IC_{50} for axitinib and U0126. Error bars indicate the standard deviation over 6 wells.

References

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