CRISPR/Cas9-engineered Immortalized Breast Epithelial MCF10A Reporter Line for EMT Studies and Anti-cancer Drug Discovery

Sangeeta Kumari, MS, Diana Douglas, BS, Luis G. Rodriguez, PhD, Robert Newman, PhD, Weiguo Shu, PhD
ATCC Cell Systems, Gaithersburg, MD 20877, USA

Abstract
Metastasis is responsible for most cancer-related deaths. One mechanism of metastasis involves epithelial-to-mesenchymal transition (EMT), a process characterized by the decrease in cell adhesion and increase in cell motility. Cells undergoing EMT often display downregulation of epithelial markers (such as E-cadherin), and upregulation of mesenchymal markers (such as vimentin). While metastasis, EMT has also been reported to be associated with other pathological conditions, such as acquired therapeutic drug resistance. Given the roles that EMT plays in the pathological processes, it is of increasing interest as a target for anti-cancer treatment and drug discovery. In vitro reporter models have proven to be a valuable tool for dissecting the signaling pathways that regulate the EMT process and for screening compounds targeting EMT. In previously developed EMT reporter lines, the reporter gene was driven by a truncated EMT marker gene promoter. Therefore, the establishment of a more physiologically relevant reporter cell model is critical for advancing our knowledge of EMT.

CRISPR/Cas9-engineering technology was utilized to generate an Ecad-EmGFP reporter model with immortalized breast epithelial MCF10A cells. The reporter gene, EmGFP, was tagged at the C-terminus of Ecad allowing for real-time monitoring of EMT progression in live cells. The targeted knock-in of the Ecad-EmGFP allele was verified at the genomic DNA, transcript (mRNA), and protein levels. Functional evaluation of the reporter cell line revealed that treatment of Ecad-EmGFP reporter cells with TGF-β resulted in increased motility (an indication of EMT), as demonstrated by a reduction in Ecad-EmGFP expression and an increase in VIM and fibronectin expression. Additional functional analyses revealed that the reporter cells possessed an enhanced migration capacity upon EMT induction with TGF-β. In summary, this MCF10A-ECAD-EmGFP reporter cell line serves as a physiologically relevant in vitro model for studying EMT cancer biology and anti-cancer drug discovery.

Results
I. Background Information

II. Generation of ECAD-EmGFP Knock-in Allele

III. Identification of ECAD-EmGFP Knock-in Allele

IV. Confirmation of ECAD-EmGFP Knock-in Allele

V. Growth Kinetics and Morphology of ECAD-EmGFP Cells

VI. ECAD-EmGFP Cells Undergo EMT Upon Induction

VII. Increased Motility of ECAD-EmGFP Cells Upon EMT Induction

Summary
We have successfully created an immortalized breast MCF10A ECAD-EmGFP EMT reporter cell line using CRISPR technology, in which a EmGFP gene was incorporated into the last exon of the endogenous ECAD gene.
MCF10A ECAD-EmGFP cells undergo EMT upon induction, enabling real-time monitoring of the dynamic EMT states in live cells.
MCF10A ECAD-EmGFP cell line is a valuable tool for studying EMT biology and use in screening compounds targeting EMT.

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