TMEM165: Novel Golgi Antiporter involved in Breast Cancer Progression

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ABSTRACT: The TMEM165 gene encodes for a multiple pass membrane protein localized in the Golgi that has been linked to congenital disorders of glycosylation. The TMEM165 protein is a putative ion transporter that regulates H+/Ca++/Mn++ homeostasis and pH in the Golgi. Previously, we identified TMEM165 as a potential biomarker for breast carcinoma in a glycoproteomic study using late-stage invasive ductal carcinoma tissues with patient-matched adjacent normal tissues. The TMEM165 protein was not detected in non-malignant matched breast tissues and was detected in invasive ductal breast carcinoma tissues by mass spectrometry. Our hypothesis is that the TMEM165 protein confers a growth advantage to breast cancer. In this study we have investigated the expression of TMEM165 in early stage invasive ductal carcinoma and ductal carcinoma in situ cases. We created a CRISPR/Cas9 knockout of TMEM165 in the human invasive breast cancer cell line MDA MB231. Our results indicate that removal of TMEM165 in these cells results in a significant reduction of cell migration, tumor growth, and tumor vascularization in vivo. Furthermore, we find that TMEM165 expression alters the glycosylation of breast cancer cells and these changes promote the invasion and growth of breast cancer by altering the expression levels of key glycoproteins involved in regulation of the epithelial to mesenchymal transition such as E-cadherin.
Previous studies identified that UPF0016 family contain two highly conserved consensus motifs E-ϕ-G-D-[KR]-[TS] predicted to be involved in the ion transport function of UPF0016 members. We created point mutations in these two highly conserved motifs to test their contribution in promoting cell migration, invasion, cell-cell adhesion and Golgi glycosylation function. In order to measure the changes in \([\text{Ca}^2+]\) inside Golgi, cells were stained with red BODIPY-TR-CERAMIDE Golgi marker and green fluorescent Fluo4-AM was pre-loaded into cells. Thus, the calcium flux was measured in the colocalized regions allowing us to visualize the changes in calcium levels in human breast cancer cells with TMEM165KO and TMEM165KO rescued with wildtype or mutant forms of TMEM165. This study has demonstrated that a single amino acid mutation of TMEM165 in breast cancer cells can reduce the invasive phenotype similar to a complete TMEM165KO.