SCAMP3 knockout decreases cell proliferation, migration, and invasion capacity of inflammatory breast cancer cells

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Abstract

Inflammatory Breast Cancer (IBC) is one of the most lethal forms of breast cancer (BC), where patients have a 43% increased risk of death compared to non-IBC advanced BC. The secretory carrier membrane protein 3 (SCAMP3) is an endosome-associated protein that has been related to poor prognosis of glioma and hepatocellular carcinoma patients. Recent studies have shown that knockdown of SCAMP3 decreases cell proliferation, motility, and growth of cancer cells. We previously identified increased SCAMP3 in EGFR overexpressing SUM-149 IBC cells, tissues, and in the IBC, metastasis promoting structure, the tumor emboli. Thus, we aim to elucidate the role of SCAMP3 in the regulation of IBC proliferation. To investigate how SCAMP3 contributes to the IBC cellular response, we knockout (SC3-KO) the expression of SCAMP3 in IBC SUM-149 using the CRISPR/Cas9 technique and overexpressed (SC3-OE) SCAMP3 in non-tumorigenic epithelial cells, MCF-10A. Wild type (WT) and SC3-KO cells were used to assess cell proliferation at 24, 48, and 72 hours, migration, and invasion with and without 10ng/ml EGF stimulation. Our results demonstrate inhibition of SUM-149 SC3-KO cell proliferation, whereas MCF-10A SC3-OE showed an increase. The results demonstrate a decrease in SUM-149 SC3-KO invasion and migration capacity with and without EGF stimulation. Meanwhile MCF-10A SC3-OE reveals and increase in the migration capacity. Taken together, our data show that SCAMP3 plays a promoting role in the proliferation, migration, and invasion of IBC.

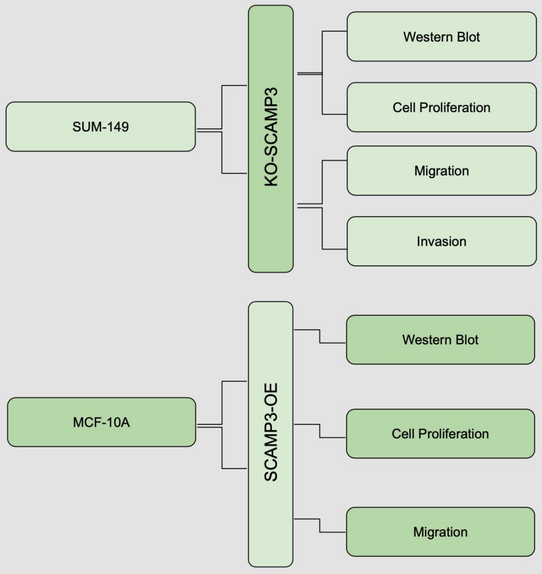
Introduction

- IBC accounts for 1-5% of breast cancers with a median disease-free survival of <2.5 years. *Van Uden DJ et. Al, 2015*
- IBC invades the vascular and lymphatic systems via formation of tumor emboli that results in the development of metastases. *Robertson FM, et. al, 2010.*
- There is no molecular marker for IBC.
- Secretory carrier membrane protein 3 (SCAMP3) is an integral membrane protein component of the eukaryotic cell surface recycling system. *Singleton DR et. al, 1997.*
- Previously, we found identified high SCAMP3 expression in IBC cells, tissues, and in the emboli structure. *Suarez-Arroyo IJ et. Al, 2016.*
- SCAMP3 functions as a protein carrier and is involved in receptor tyrosine kinase trafficking and recycling in endosomes. *Aoh QL et. al, 2009.*
- Overexpression of SCAMP3 has been identified as an indicator of poor prognosis and cancer survival. *Zhang X et. al, 2017; Li, C et. al, 2020*
- SCAMP3 is a potential biomarker for BC. *Györffy, B et. al, 2010.*

Aim

Elucidate the role of SCAMP3 in the regulation of IBC proliferation and cell motility.

Methods



Results

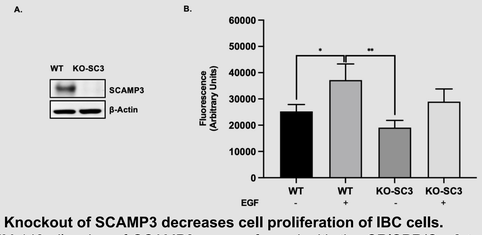


Fig.1: Knockout of SCAMP3 decreases cell proliferation of IBC cells.
A. SUM-149 silencing of SCAMP3 was performed with the CRISPR/Cas9 technique. Immunoblotting was performed to monitor SCAMP3 expression. β-Actin was used as a loading control. **B.** SUM-149 WT and KO-SC3 cells were stimulated with 10ng/mL of EGF for 30m before seeding, proliferation was assessed using CyQUANT® Proliferation Assay after 72h. **P≤0.05, **P ≤ 0.01.* Columns represent means ± SEM. (n=4).

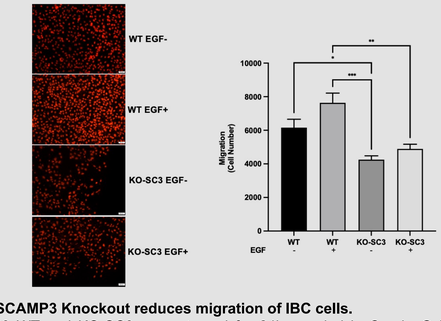


Fig. 2: SCAMP3 Knockout reduces migration of IBC cells.
SUM-149 WT and KO-SC3 were starved for 24h, seeded in Corning® FluoroBlok™ Cell Culture Inserts with or without 10ng/mL of EGF for 24h. Nucleus were stained with Propidium Iodide. Cell migration micrographs were obtained on an inverted fluorescence microscope using cellSens software. Pictures represent 14 micrographs/condition at a magnification of 200X. Scale=100µm. Cell migration was quantified by measuring the quantity of cells in the pores using ImageJ software. *P≤0.05, **P ≤ 0.01, ***P ≤ 0. 001.Columns represent means ± SEM. (n=4).

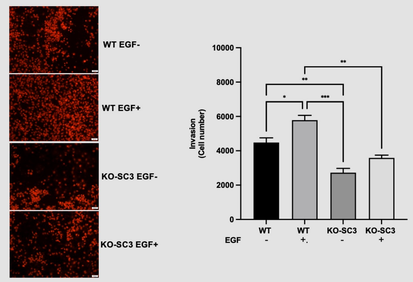


Fig. 3: SCAMP3 knockout reduces IBC cells invasion.
SUM-149 WT and KO-SC3 were starved for 24h, seeded in BD BioCoat Matrigel chamber with or without 10ng/mL of EGF for 24h. Nucleus were stained with Propidium Iodide. Cell migration micrographs were obtained with an inverted fluorescence microscope using cellSens software. Photos represent 14 micrographs/condition at a magnification of 200X. Scale=100µm. Cell migration was quantified by measuring the quantity of cells in the pores using ImageJ software. *P≤0.05, **P ≤ 0.01, ***P ≤ 0. 001.Columns represent means ± SEM. (n=3).

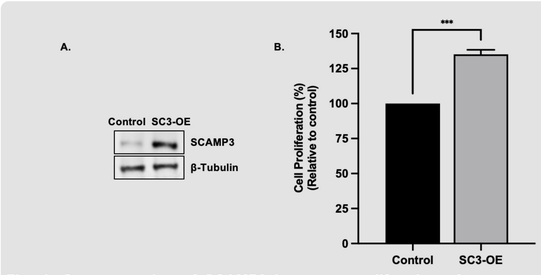


Fig. 4: Overexpression of SCAMP3 increases proliferation of non-tumorigenic mammary epithelial cells.
A. SCAMP3 was overexpressed in non-tumorigenic mammary epithelial cells MCF-10A using the SCAMP3 lentiviral vector from Vector Builder. Immunoblotting was performed to monitor SCAMP3 expression. β-Tubulin was used as a loading control. **B.** Proliferation of MCF-10A Control and SC3-OE cells was assessed using CyQUANT® Proliferation Assay after 24h. ***P ≤ 0. 001.Columns represent means ± SEM. (n=3).

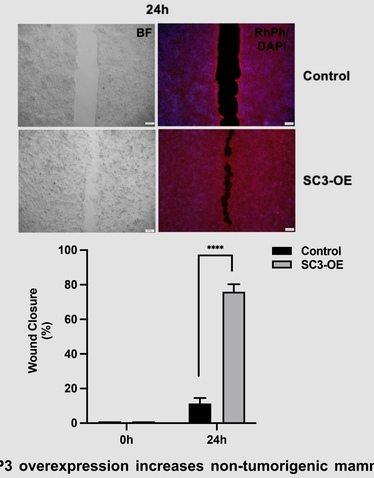


Fig. 5: SCAMP3 overexpression increases non-tumorigenic mammary epithelial cells migration capacity.
Control and SC3-OE were cultured on a two-well silicone inserts Ibidi® Plates for 24h. The insert was removed and leave with 2% HS culture media for 24h. Cell migration was quantified by measuring the distance between the edges of the wound on cellSens software for a total of three micrographs at a magnification of 40X. Scale=200µm. DAPI (blue) were used to stain the nucleus and Rhodamine Phalloidin (red) the actin cytoskeleton of cells. ****P ≤ 0. 0001.*Columns represent means ± SEM. (n=3).

Discussion

Secretory carrier membrane protein 3 (SCAMP3) is an integral membrane protein component and an endocytosis-associated protein that contributes to the regulation of Epidermal Growth Factor Receptor (EGFR) trafficking.

Previous studies have released the role of SCAMP3 as a tumor-related protein in hepatocellular carcinoma and glioblastoma multiforme. SCAMP3 expression was significantly correlated with vascular invasion, tumor stage and poor survival in hepatocellular carcinoma. A study assessed in BC demonstrated the expression of SCAMP3 in 86% of IBC tissues, lymphatic vessels, and tumor emboli cells.

In this study, we evaluated the relation of SCAMP3 expression with the inflammatory breast cancer progression. Our results demonstrated the inhibition of cell proliferation in SCAMP3 knockout cells. We observed the opposite when SCAMP3 is overexpressed. Additionally, the protein knockout decreased cell invasion and migration capacity of cells. Meanwhile the overexpressed cells showed a motile phenotype.

Our results validates that SCAMP3 expression plays a key role in promoting IBC progression and has the potential to be a prognostic biomarker for IBC. This work will increase our understanding of IBC and will open the opportunity to the development of new therapeutic strategies.

Conclusions

Knockout of SCAMP3 in IBC cells decreases:
- cell proliferation.
- migration capacity
- invasion capacity

Overexpression of SCAMP3 in non-tumorigenic mammary epithelial cells increases:
- cell proliferation.
- migration capacity

Taken together, our data show that SCAMP3 plays a promoting role in the proliferation, migration, and invasion of IBC.

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