# Context-Dependent Tumor Suppressor Role of *RHOA* in Breast Cancer Through Cooperation with *CDH1* Loss

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## Introduction

*RHOA* is a small GTPase that regulates actin cytoskeleton dynamics, playing key roles in various solid tumors, such as in diffuse gastric cancer (DGC). DGC and invasive lobular carcinoma (ILC) of the breast share biologic and phenotypic features, including E-cadherin (*CDH1*) deficiency, and a characteristic single-cell invasive pattern due to impaired cell–cell adhesion. In breast cancer (BC), the prevalence, spectrum and functional impact of *RHOA* mutations remain to be determined. Here, we sought to characterize the *RHOA* mutational landscape in BC compared to gastric cancer (GC), assess its association with *CDH1* mutations, and investigate its functional impact in BC cell models.

## Methods and Materials

We conducted a re-analysis of targeted sequencing data from 8,939 BCs (n=4,182 primary, n=4,757 metastatic) and from 1,378 GC (n=1,044 primary; n=334 metastatic). *RHOA* mutational frequency, spectra and co-occurrence with other genomic alterations were assessed. The functional impact of *RHOA* alterations was investigated in the MDA-MB-134-VI ILC cells with RHOA knockdown (*RHOA*-KD) and inducible *CDH1* overexpression.

## Disclosure

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## Results

### *RHOA mutations are enriched in ILC and diffuse gastric cancers with distinct mutational spectra*

A B

**Fig 1. (A)** Histology of breast cancers and gastric cancers with *RHOA* mutations. (**B**) Lollipop plot depicting *RHOA* mutations in ILC (predominantly loss of function) and diffuse gastric cancer (predominantly gain of function).

### *Outcomes according to RHOA mutational status*

A B C

**Fig 2. O**verall survival in (**A**) primary and (**B**) metastatic ILC and in (**C**) diffuse gastric cancer patients according to *RHOA* mutational status

### *Distinct Co-mutation Patterns Associated with RHOA Mutations in ILC Breast and Diffuse Gastric Cancers*

A B

**Fig 3.** Volcano plots depicting differentially altered cancer genes in (**A**) ILC and (**B**) diffuse gastric cancer according to *RHOA* mutational status

### *Functional impact of RHOA loss in ILC cell models*

A B

**Fig 4.** *RHOA* loss enhances (**A**) cell invasion and (**B**) anoikis resistance in ILC cell models in a *CDH1*-dependent manner.

A

**Fig 5. (A)** Volcano plot showing differential pathway enrichment between MDA-MB-134-VI cells with *RHOA*-knock down (KD) vs shNT controls. Each point represents a gene set. Red points denote significantly enriched pathways

## Conclusions

- *RHOA* mutations are enriched in ILC and are associated with worse outcomes.
- Gain-of-function *RHOA* mutations prevail in GC, while loss-of-function *RHOA* mutations are predominant in BC.
- *RHOA* inactivation synergizes with *CDH1* loss, promoting oncogenic phenotypes in BC cell models.
- These findings support a context-dependent tumor suppressor role of *RHOA* in BC, particularly in ILC, through cooperation with *CDH1* deficiency

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