Therapeutic Inhibition of the sEcad–PDIA4 Axis Suppresses Brain Metastasis in Inflammatory Breast Cancer

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Introduction

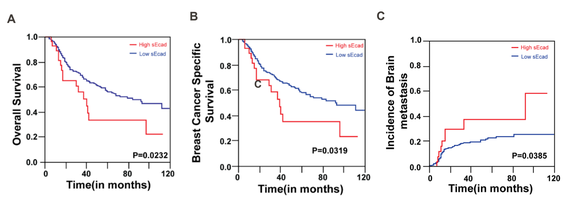
- Inflammatory breast cancer (IBC) is a highly aggressive form of breast cancer with a rapid onset and a strong propensity to spread to distant organs. Although considered rare, IBC accounts for 10% of breast cancer-related deaths owing to its rapid proliferation and strong propensity to metastasize. The molecular mechanisms underlying the aggressiveness and metastatic propensity of IBC remain elusive.
- E-cadherin is overexpressed in IBC tumors and tumor emboli. We and others reported that E-cadherin plays an oncogenic function in IBC tumor models.
- soluble E-cadherin (sEcad) is an 80-kDa proteolytic fragment of full-length E-cadherin. In our IBC cohort of patients, we show that serum levels of sEcad is significantly correlated with increased risk of brain metastasis and death.
- However, whether and how sEcad drives brain metastasis in IBC is unknown.

Hypothesis

sEcad promotes IBC brain metastasis via PDIA4 activation

Background

Higher sEcad expression correlates with poor survival outcome and increased brain metastasis risk



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Multivariate Cox regression

Covariate	Level	HR (95% CI)	P-value
sEcad	≤1.27 (90th percentile)	1.0	
	>1.27	2.0 (1.1-3.8)	**0.02**
Stage	IIIb	1.0	
	IIIc	2.4 (1.4-4.3)	**0.003**
	Iv	1.1 (0.6-2.2)	0.8
HR/Her2	HR+/Her2-	1.0	
	HR+/Her2+	0.5 (0.3-1.2)	0.1
	HR-/Her2+	0.4 (0.2-0.8)	**0.02**
	HR-/Her2-	2.5 (1.6-4.1)	**0.0002**
Adj XRT	No	1.0	
	Yes	0.3 (0.1-0.5)	**<.0001**

Fig. 1. (A) Higher serum sEcad level is associated with reduced overall survival; (B) Higher serum sEcad level is correlated with reduced breast cancer specific survival; (C) Higher serum sEcad is associated with increased risk of brain metastasis; (D) Higher serum sEcad level is independently correlated with poor overall survival in IBC patients (N= 348 patients)

Results

sEcad promotes cell growth, migration, invasion, and anoikis resistance in IBC models

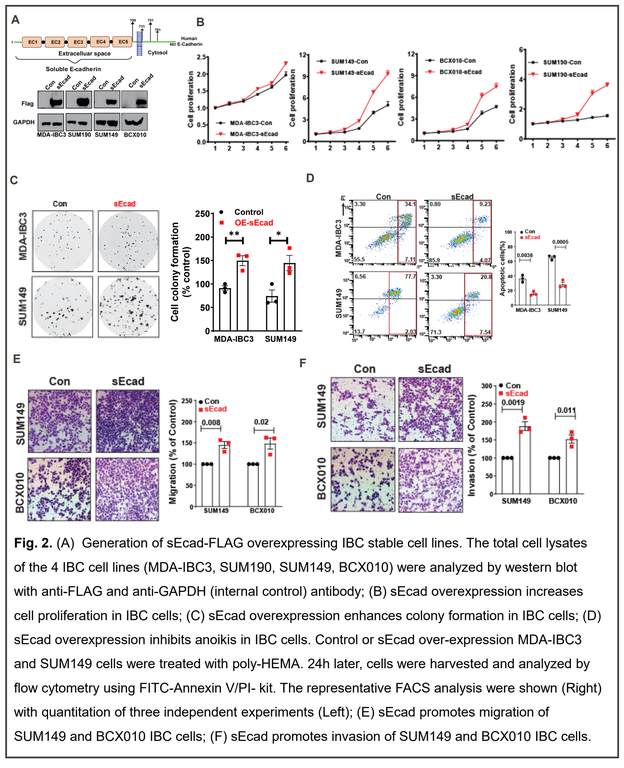


Fig. 2. (A) Generation of sEcad-FLAG overexpressing IBC stable cell lines. The total cell lysates of the 4 IBC cell lines (MDA-IBC3, SUM190, SUM149, BCX010) were analyzed by western blot with anti-FLAG and anti-GAPDH (internal control) antibody; (B) sEcad overexpression increases cell proliferation in IBC cells; (C) sEcad overexpression enhances colony formation in IBC cells; (D) sEcad overexpression inhibits anoikis in IBC cells. Control or sEcad over-expression MDA-IBC3 and SUM149 cells were treated with poly-HEMA. 24h later, cells were harvested and analyzed by flow cytometry using FITC-Annexin V/PI- kit. The representative FACS analysis were shown (Right) with quantitation of three independent experiments (Left); (E) sEcad promotes migration of SUM149 and BCX010 IBC cells; (F) sEcad promotes invasion of SUM149 and BCX010 IBC cells.

Results

sEcad promotes brain metastasis and reduces survival in mice

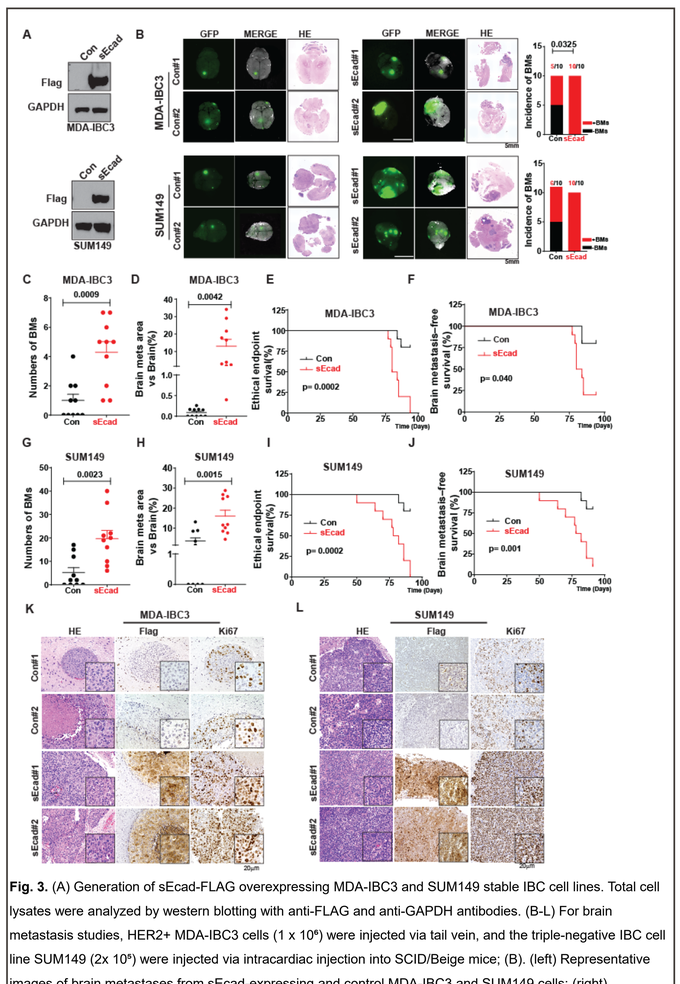


Fig. 3. (A) Generation of sEcad-FLAG overexpressing MDA-IBC3 and SUM149 stable IBC cell lines. Total cell lysates were analyzed by western blotting with anti-FLAG and anti-GAPDH antibodies. (B-L) For brain metastasis studies, HER2+ MDA-IBC3 cells (1 x 10^6) were injected via tail vein, and the triple-negative IBC cell line SUM149 (2x 10^5) were injected via intracardiac injection into SCID/Beige mice; (B). (left) Representative images of brain metastases from sEcad-expressing and control MDA-IBC3 and SUM149 cells; (right) Incidence of brain metastases in sEcad-overexpressing MDA-IBC3 and SUM149 cells compared with controls; (C, G) Numbers of brain metastases in sEcad-overexpressing group compared with controls.(D, H) Size of brain metastasis lesions in sEcad-overexpressing group compared with controls; (E, I) Overall survival rates in mice injected with sEcad-overexpressing or control IBC cells; (F, J) Brain metastasis-free survival rates in mice injected with sEcad-overexpressing or control IBC cells; (K, L) Hematoxylin-and-eosin and immunohistochemical staining of brain metastasis lesions from MDA-IBC3 and SUM149 control cells or sEcad-overexpressing cells. Flag indicates sEcad overexpression, whereas Ki67 indicates proliferation in brain metastasis lesions from the two mouse models of IBC brain metastasis.

sEcad induces reactive astrocytes

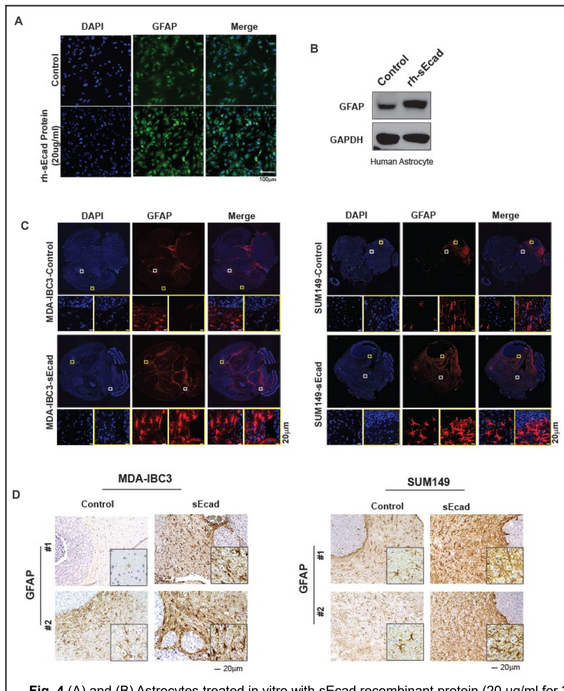


Fig. 4 (A) and (B) Astrocytes treated in vitro with sEcad recombinant protein (20 μg/ml for 36 h) show increased GFAP+ reactive astrocytes by immunofluorescence (IF) (A) and immunoblotting (B); (C) and (D) IF staining shows that metastatic lesions from sEcad-expressing tumors have significantly higher GFAP+ reactive astrocytes vs the control group. Data shown are from MDA-IBC3 tail-vein injection (C) or intracardiac injection (D) experiments; (E) and (F) Immunohistochemical stains of brain metastases generated from MDA-IBC3 (E) and SUM149 (F) control and sEcad-overexpressing cells confirm the upregulated expression of GFAP in the sEcad-overexpressing cells in mouse xenograft brain metastatic lesions.

Results

PDIA4 is a novel sEcad interacting partner

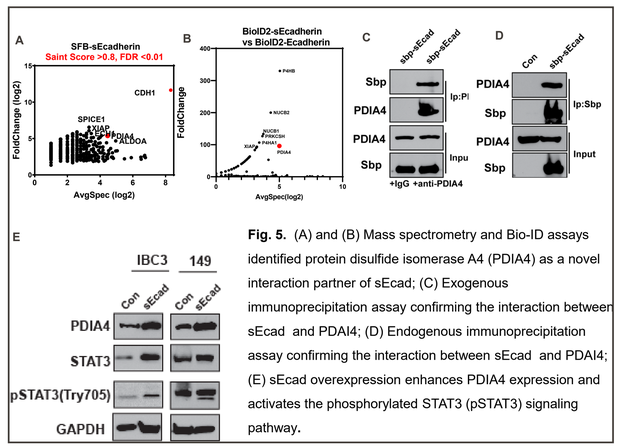


Fig. 5. (A) and (B) Mass spectrometry and Bio-ID assays identified protein disulfide isomerase A4 (PDIA4) as a novel interaction partner of sEcad; (C) Exogenous immunoprecipitation assay confirming the interaction between sEcad and PDAI4; (D) Endogenous immunoprecipitation assay confirming the interaction between sEcad and PDAI4; (E) sEcad overexpression enhances PDIA4 expression and activates the phosphorylated STAT3 (pSTAT3) signaling pathway.

PDIA4 knockdown suppresses sEcad-driven oncogenic phenotypes and reduces STAT3 activation

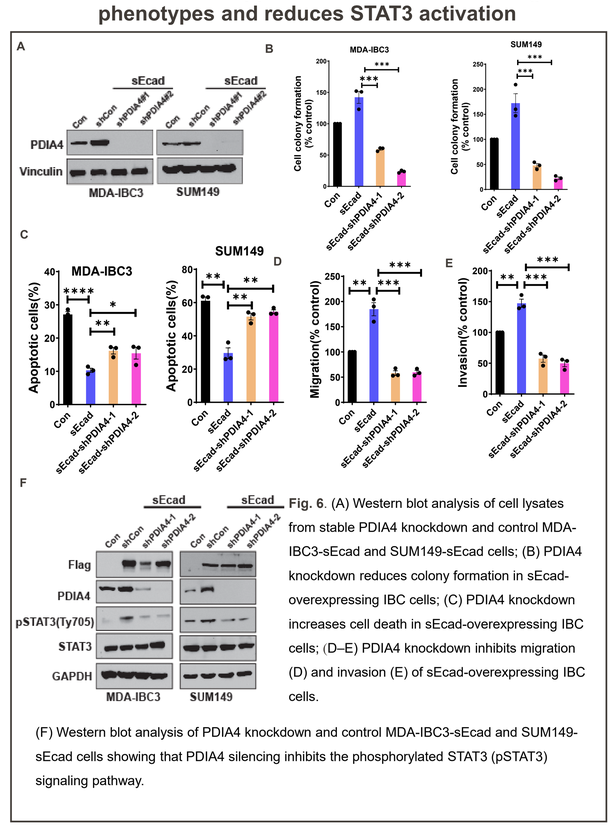


Fig. 6. (A) Western blot analysis of cell lysates from stable PDIA4 knockdown and control MDA-IBC3-sEcad and SUM149-sEcad cells; (B) PDIA4 knockdown reduces colony formation in sEcad-overexpressing IBC cells; (C) PDIA4 knockdown increases cell death in sEcad-overexpressing IBC cells; (D–E) PDIA4 knockdown inhibits migration (D) and invasion (E) of sEcad-overexpressing IBC cells.

(F) Western blot analysis of PDIA4 knockdown and control MDA-IBC3-sEcad and SUM149-sEcad cells showing that PDIA4 silencing inhibits the phosphorylated STAT3 (pSTAT3) signaling pathway.

PDIA4 inhibitor 35G8 suppresses sEcad-driven oncogenic phenotypes and reduces STAT3 activation

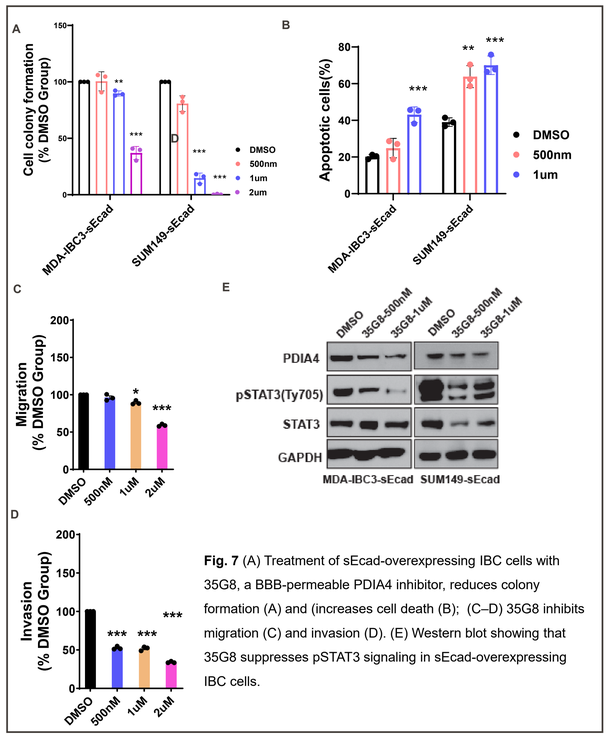


Fig. 7 (A) Treatment of sEcad-overexpressing IBC cells with 35G8, a BBB-permeable PDIA4 inhibitor, reduces colony formation (A) and (increases cell death (B); (C–D) 35G8 inhibits migration (C) and invasion (D). (E) Western blot showing that 35G8 suppresses pSTAT3 signaling in sEcad-overexpressing IBC cells.

Results

BBB-pearmeable 35G8 inhibits brain metastasis in mouse models of IBC

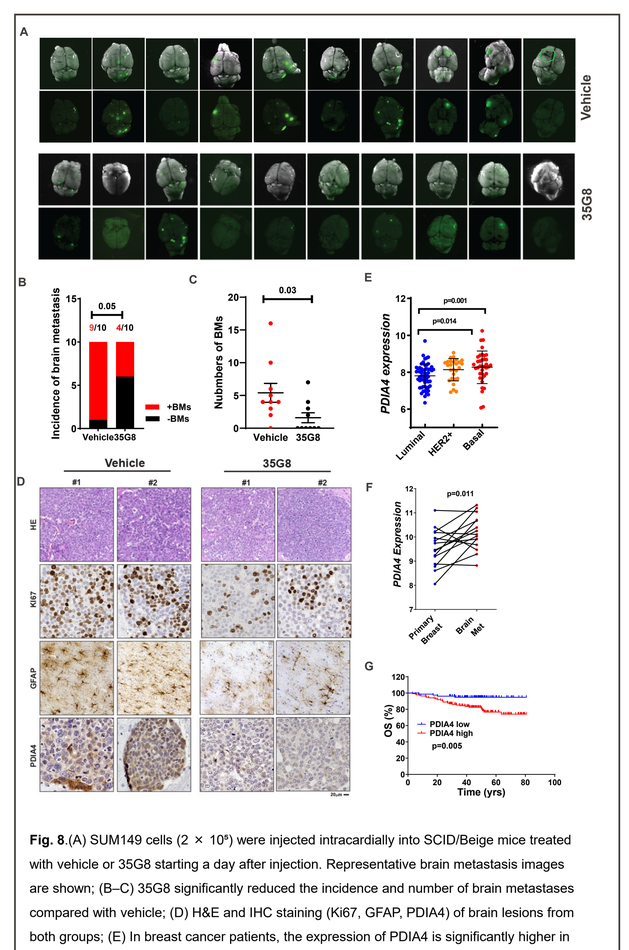


Fig. 8.(A) SUM149 cells (2 × 10^5) were injected intracardially into SCID/Beige mice treated with vehicle or 35G8 starting a day after injection. Representative brain metastasis images are shown; (B–C) 35G8 significantly reduced the incidence and number of brain metastases compared with vehicle; (D) H&E and IHC staining (Ki67, GFAP, PDIA4) of brain lesions from both groups; (E) In breast cancer patients, the expression of PDIA4 is significantly higher in more aggressive, basal-like breast cancer subtypes versus less aggressive luminal subtypes; (F) PDIA4 expression is higher in brain metastases than in the corresponding primary tumors; (G) High PDIA4 expression in breast cancer patient tumors is associated with poor overall survival (OS) compared to low PDIA4-expressing tumors (KM Plot).

Summary

- **High serum sEcad is an independent predictor of poor survival outcome in IBC patients.**
- **sEcad promotes invasion and anoikis resistance in vitro and drives brain metastasis growth and progression in IBC mouse models in vivo.**
- **PDIA4 inhibition with the BBB-permeable compound 35G8 decreases brain metastasis burden, supporting the sEcad–PDIA4 pathway as a therapeutic vulnerability.**

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