sEcad–STAT3/NFκB axis in inflammatory breast cancer progression

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Introduction

- Inflammatory breast cancer (IBC) is a rare yet highly aggressive subtype, accounting for 2–5% of breast cancer deaths. It is marked by rapid progression and a strong tendency to metastasize, particularly to the brain. Despite its severity, the molecular basis of IBC aggressiveness remains poorly understood.
- We found that elevated serum soluble E-cadherin (sEcad; 80 kDa fragment) predicts poor survival and increased brain metastasis risk in IBC patients, while sEcad overexpression drives brain metastasis in xenograft models.
- RNA-seq revealed activation of the STAT3 and NFκB pathways in sEcad-overexpressing cells. Additionally, PDIA4 was identified as a novel sEcad-interacting partner by LC–MS–based proteomic analysis.
- These findings underscore the role of sEcad in IBC progression; however, the exact molecular mechanisms driving its metastatic behavior remain unknown.
- **Hypothesis:** *PDIA4 mediates sEcad-driven brain metastasis in IBC by activating the STAT3/NFκB signaling axis*.

Results

In Silico analysis predicts a favorable interaction between sEcad and PDIA4

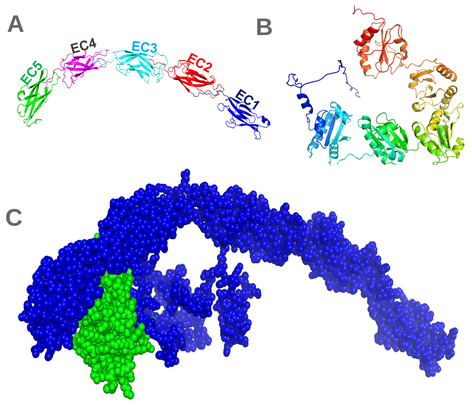


Fig. 1. The endogenous co-immunoprecipitation between sEcad (ID: AF-F1PAA9-F1-v6) (A) and PDIA4 (ID: AF-P13667-F1-v6) (B), previously established by our lab, was further supported by deep learning–assisted GSO-based molecular docking (C), which predicted a stable complex with the top-ranked pose (Swarm 110, Score 38.55) showing the strongest binding affinity.

GROMACS Molecular Dynamics simulation predicts a stable sEcad–PDIA4 interaction

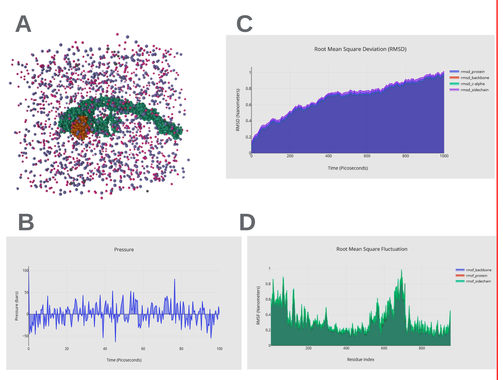


Fig. 2. GROMACS molecular dynamics simulation of sEcad–PDIA4 complex showing system solvation (A), pressure equilibration (B), structural stability via RMSD (C), and residue flexibility through RMSF analysis (D).

PDIA4 Knockdown in sEcad-overexpressing IBC cells reverses STAT3/NF-κB activation and oncogenic phenotypes

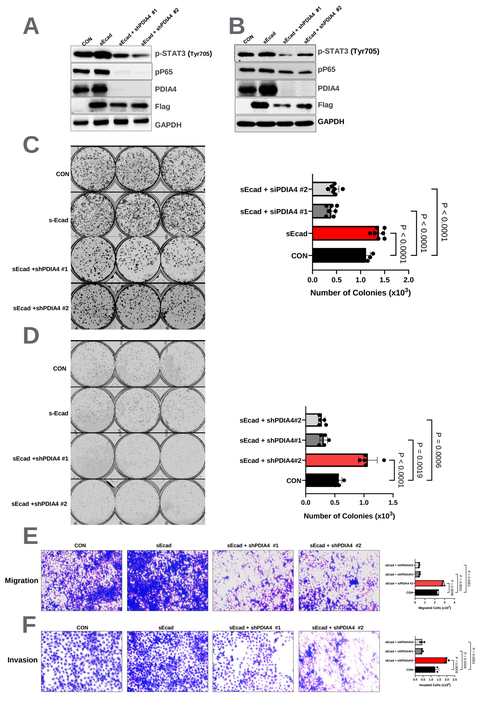


Fig. 3. Western blot analysis shows sEcad overexpression activates pSTAT3 and pNF-κB signaling in SUM149 cells (A) and MDA-IBC cells (B), while PDIA4 knockdown reverses this effect. Colony formation assay (C, D) further confirms that sEcad overexpression increases colony number, while PDIA4 knockdown reverses the process in these IBC cells. PDIA4 knockdown in sEcad-overexpressing cells also reduces migration and invasion in SUM149 Cells (E, F).

STAT3 and NF-κB inhibitors suppress the proliferation of sEcad-high cells

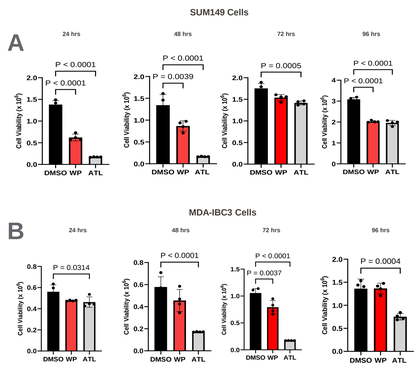


Fig. 4. WP1066 and ATL significantly reduced the proliferation of both sEcad-high SUM149 and MDA-IBC3 cells in a time-dependent manner at 24, 48, 72, and 96 hours.

Inhibition of STAT3 and NF-κB signaling diminishes oncogenic traits of sEcad-overexpressing IBC cells

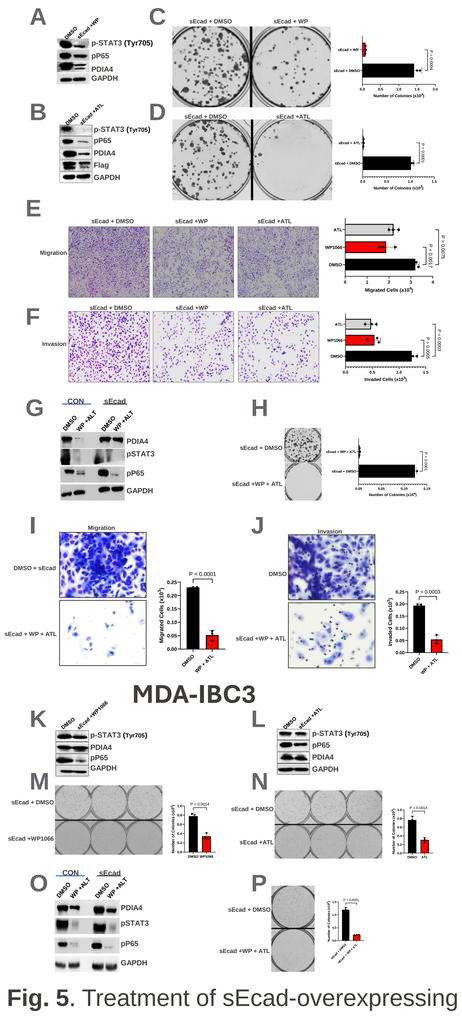


Fig. 5. Treatment of sEcad-overexpressing SUM149 cells with either STAT3 or NFκB pathway inhibitors significantly reduced the expression of target proteins (A, B), as well as colony formation (C, D) and migration and invasion (E, F). Combined treatment of these cells produced a comparable reduction to that observed with individual treatments (G, H, I, J). Consistent findings were also obtained in MDA-IBC3 cells (K, L, M, N, O, P).

STAT3 and NF-κB inhibitors suppress brain metastasis formation *in vivo*

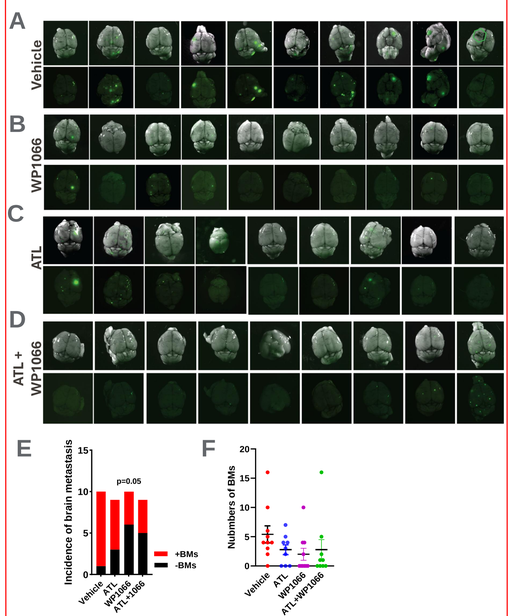


Fig. 6. SUM149 cells were injected intracardially into SCID/Beige mice, followed by treatment with vehicle, WP, ATL, or their combination starting one day post-injection. Representative brain images (A–D) show WP1066, ATL, and their combined treatment reduced the incidence (E), and number of brain metastases (F) compared with vehicle-treated controls.

ATL–PDIA4 docking supports inhibition of the PDIA4/STAT3/NFκB signaling axis

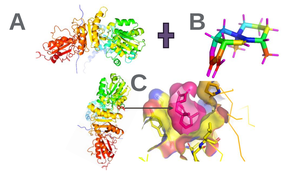


Fig. 7. PDIA4 (A) interacts with ATL (B), and deep learning–assisted docking (C) further supports the western blot findings by confirming a stable binding interface consistent with pathway inhibition. The top-ranked model showed the strongest binding energy (–5.26 kcal/mol) and an excellent RMSD (1.89 nm), indicating a stable PDIA4–ATL complex consistent with ATL-mediated inhibition of PDIA4/STAT3/NFκB signaling.

Docking analysis reveals WP1066–PDIA4 binding consistent with suppression of PDIA4/STAT3/NFκB signaling

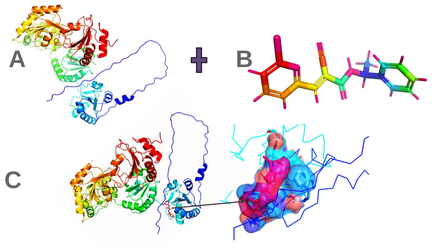


Fig. 8. PDIA4 (A) interacts with WP1066 (B), and deep learning–assisted docking (C) supports the western blot results by confirming a stable binding interface. The top-ranked model showed strong binding energy (–3.65 kcal/mol) and an excellent RMSD (1.39 nm), indicating a stable PDIA4–WP1066 complex consistent with WP1066-mediated inhibition of PDIA4/STAT3/NFκB signaling.

Methods

- sEcad was overexpressed in SUM149 and MDA-IBC3 cells via lentiviral transduction.
- Immunoblotting assessed STAT3/NFκB expression and phosphorylation.
- The effects of sEcad overexpression and stable PDIA4 knockdown were assed using functional assays (colony formation, migration, and invasion).
- STAT3 (WP1066) and NFκB (ATL) inhibitors were used to test pathway dependency.
- For in vivo efficacy, SUM149-sEcad cells were administered via intracardiac injection into SCID/Beige mice.
- Molecular docking (Glowworm Swarm Optimization) and deep learning simulations were used to model sEcad, PDIA4, STAT3, and inhibitor interactions.

Conclusions

- sEcad activates STAT3 and NF-κB signaling through PDIA4.
- In silico analyses (docking and molecular dynamics) confirm a stable and favorable binding between sEcad and PDIA4.
- PDIA4 knockdown or pharmacologic inhibition of these pathways reverses oncogenic behaviors, reducing migration and colony formation in vitro.
- Brain-permeable inhibitors of STAT3 and NF-κB reduce brain metastasis in IBC mouse models, identifying these pathways as key therapeutic vulnerabilities.

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