Revealing SNAI2 as a Pseudo-Primed Substrate of GSK3

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Abstract

❖ SNAI2, a labile protein, functions as a **pseudo-primed substrate**, that is **phosphorylated by glycogen synthase kinase 3 (GSK3)** and **targeted to degradation**

Introduction

- **EMT (Epithelial-Mesenchymal Transition)** is **crucial** in various biological processes, including **neural crest migration**, **wound healing**, **proliferation**, and notably, **cancer metastasis**[1,2]
- SNAI2 is an **EMT transcription factor (TF)** with a **repressor activity**[3]
- SNAI2 is crucial for the **initiation of neural crest EMT** by **repressing cadherin6B**[3]
  - ➢ **Neural crest cells** undergo **EMT** during **delamination** and **migrate** to various regions of the body to **differentiate** into **diverse cell types**[4]
  - ➢ The **EMT** in **cancer cells that facilitates metastasis** also **aligns** with the **gene-regulatory module of neural crest EMT**[5-6]
- SNAI2 can be phosphorylated by GSK3 (Glycogen synthase kinase-3)
  - ➢ Promotes SNAI2 to undergo **ubiquitin-dependent proteasomal degradation**[7]
  - ➢ **Important regulation** of SNAI2 expression levels during EMT[7]
- **GSK3 substrates** can be either **primed (pre-phosphorylated)** or **non-primed (non-phosphorylated)**[8]
- However, the **mechanism** by which **SNAI2 is recognized by GSK3** is still **uncleared**

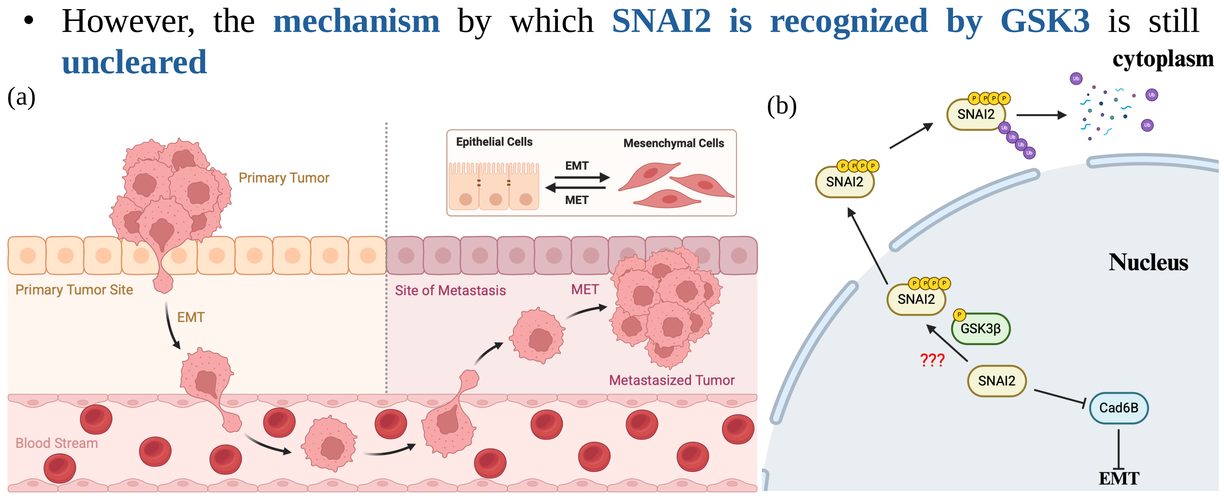

Fig.1 (a) Diagrammatic Representation of the Process of Epithelial-mesenchymal Transition (EMT), Mesenchymal-epithelial Transition (MET) and Cancer Metastasis. **(b)** Pathways of SNAI2 GSK3-ubiquitin-dependent proteasome degradation.

Objectives

❖ Elucidate the role of **a negatively charged region C-terminal** to the GSK3 phosphodegron to **mediate** the **phosphorylation** and **degradation** of SNAI2 by GSK3

❖ Generating **constructs encoding SNAI2 mutants** fused with **EGFP (emerald)**

Methods

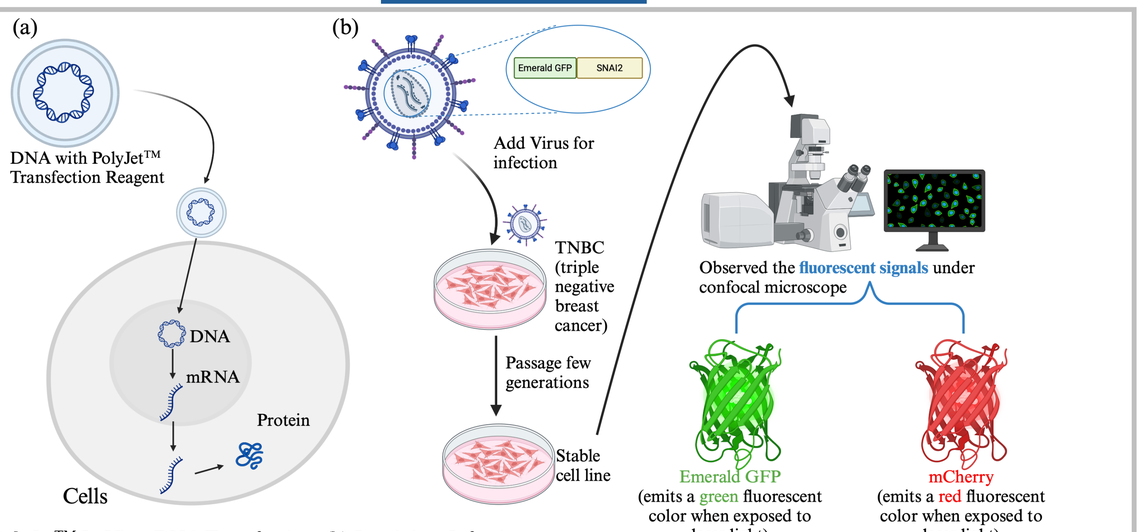

Fig.2 (a) PolyJet™ In Vitro DNA Transfection. (b) Lentivirus Infection.

Results

1. SNAI2 acts as a primed substrate of GSK3

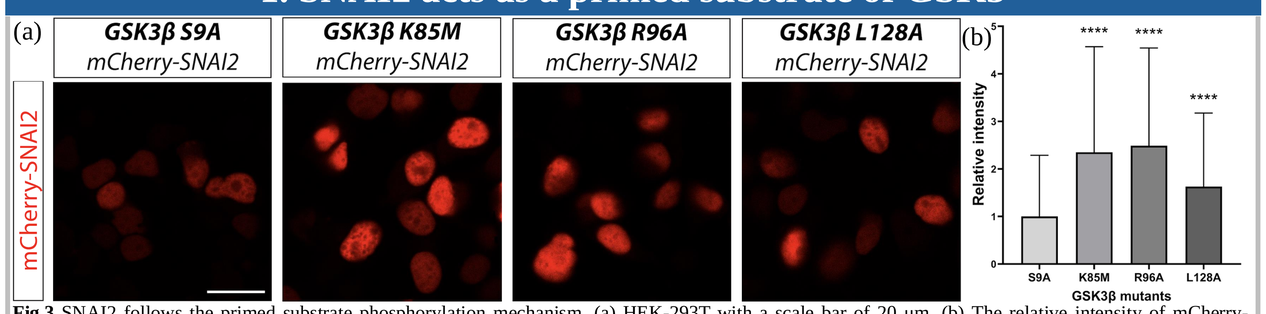

Fig.3 SNAI2 follows the primed substrate phosphorylation mechanism. (a) HEK-293T with a scale bar of 20 μm. (b) The relative intensity of mCherry-SNAI2. The p-values for quantification are labeled as follows: ns indicates $p > 0.05$; * indicates $p \leq 0.05$; ** indicates $p \leq 0.01$; *** indicates $p \leq 0.001$; **** indicates $p \leq 0.0001$.

References

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- Although SNAI2 follows the **primed substrate phosphorylation mechanism**, it **does not contain any primed (pre-phosphorylated) sites** for GSK3 to **recognize**

2. Negatively charged DEEE amino acid sequence is found in SNAI2

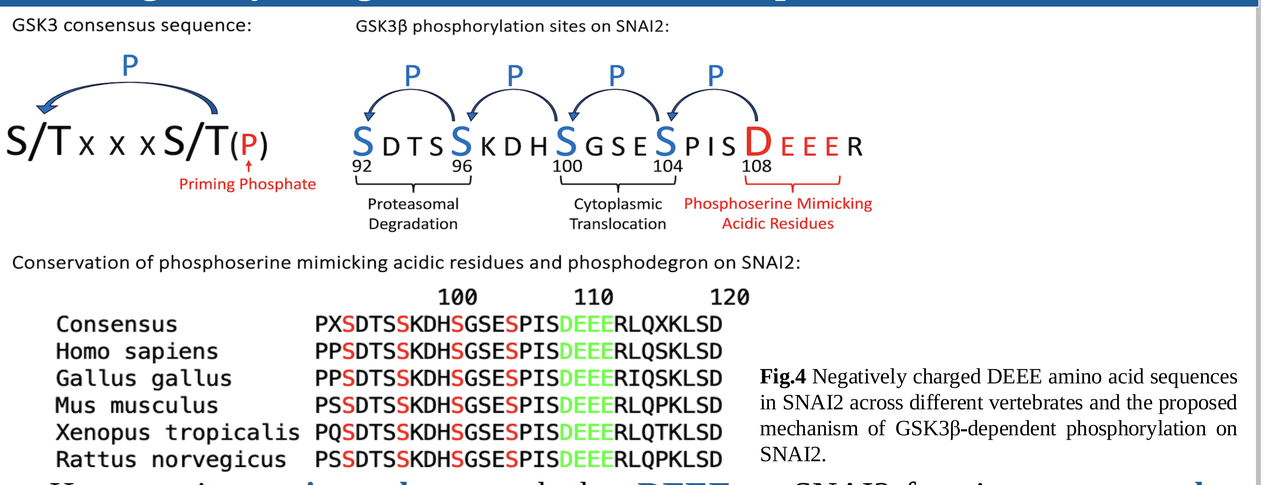

Fig.4 Negatively charged DEEE amino acid sequences in SNAI2 across different vertebrates and the proposed mechanism of GSK3β-dependent phosphorylation on SNAI2.

- However, it **remains unknown** whether **DEEE** on SNAI2 functions as a **pseudo-priming site** for GSK3 **phosphorylation** and **degradation**

3. Generating SNAI2 mutants and EGFP (emerald)-SNAI2 fusion protein

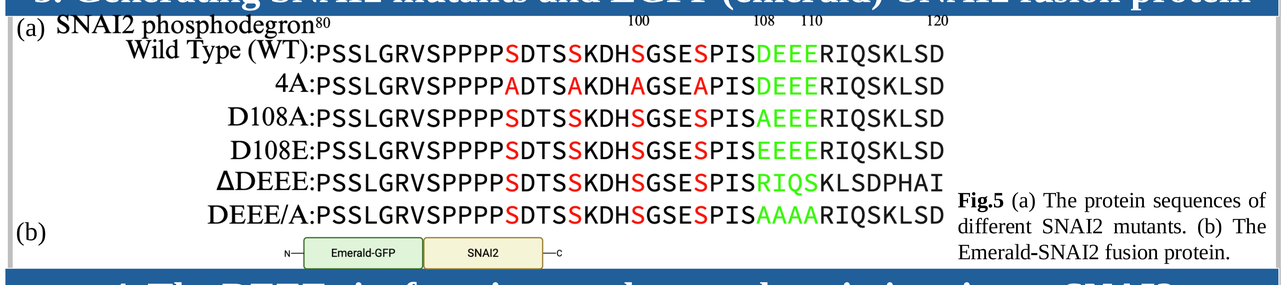

Fig.5 (a) The protein sequences of different SNAI2 mutants. (b) The Emerald-SNAI2 fusion protein.

4. The DEEE site functions as the pseudo-priming site on SNAI2

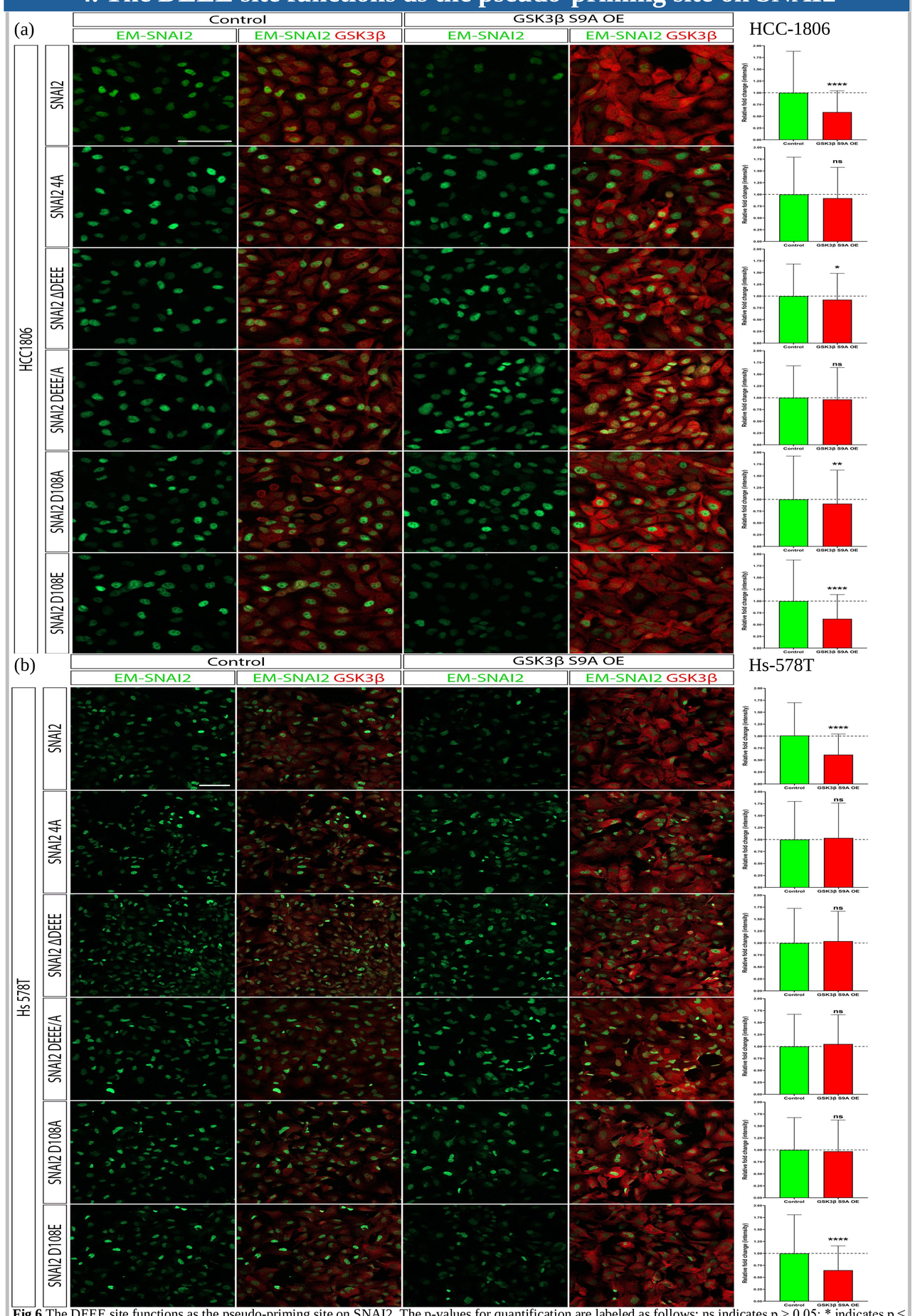

Fig.6 The DEEE site functions as the pseudo-priming site on SNAI2. The p-values for quantification are labeled as follows: ns indicates $p > 0.05$; * indicates $p \leq 0.05$; ** indicates $p \leq 0.01$; *** indicates $p \leq 0.001$; **** indicates $p \leq 0.0001$. (a) HCC-1806 with a scale bar of 50 μm. (b) Hs-578T with a scale bar of 100 μm.

Discussion

- **The DEEE sites can serve as the pseudo-priming site on SNAI2 for GSK3 to recognize**
- **Pre-phosphorylation by another kinase, including other components such as CK1, in the Wnt complex is not necessary for GSK3 to bind to SNAI2**

Future Direction:

- **The experiment will be repeated in the chick cranial neural crest cells for further validation**