Summer 8-10-2023

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Summer **U**ndergraduate **R**esearch **P**rogram
2023

EHD2 Promotes Store-Operated Calcium Entry (SOCE) and Cellular Migration in Ovarian Cancer Cells

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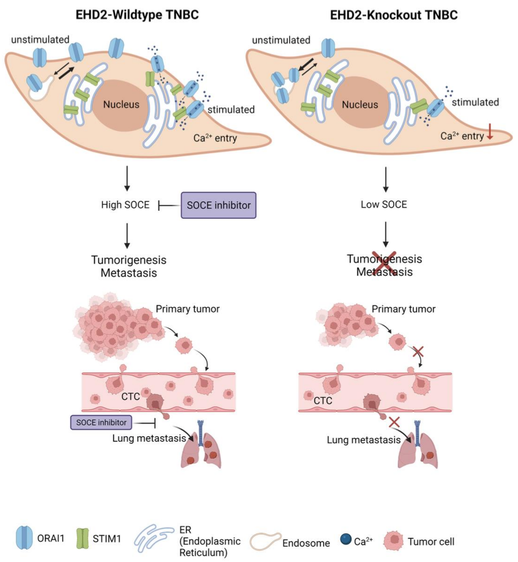
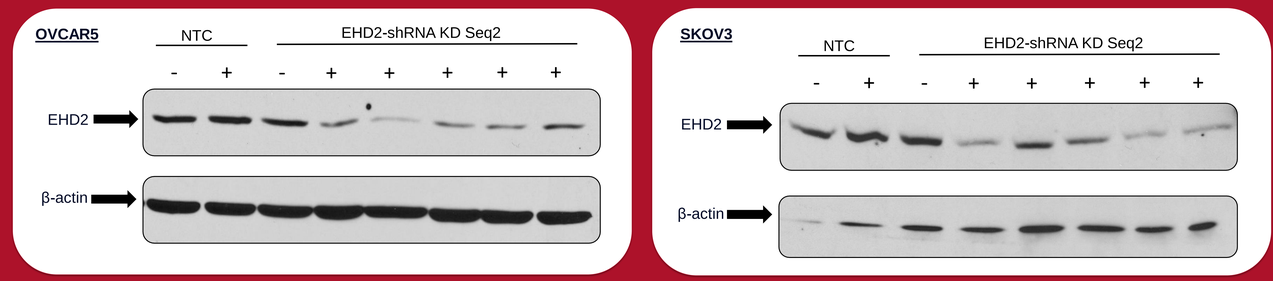
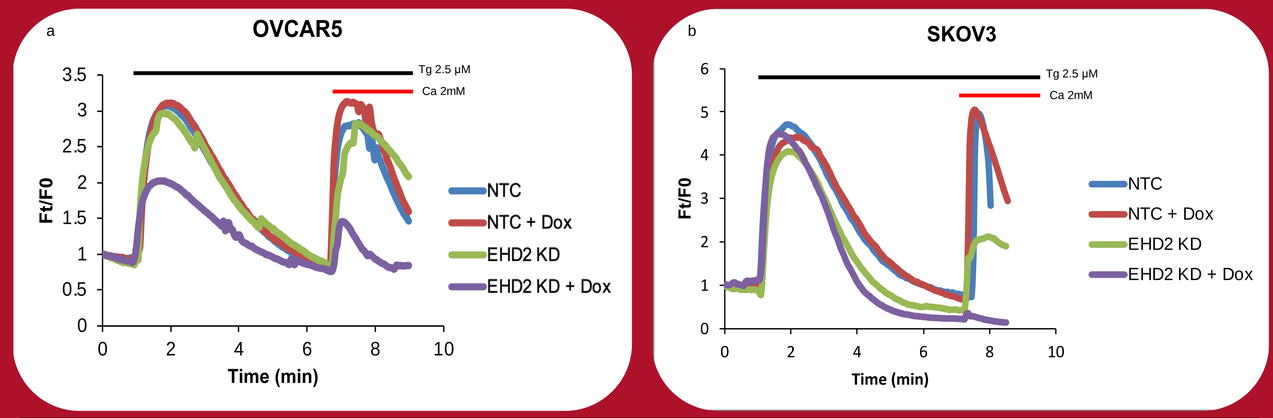
BACKGROUND: Ovarian cancer (OC) ranks as the 5th most common cause of cancer deaths of women, reflecting late diagnoses and lack of targeted therapies. EHD2, a member of the Eps15 homology (EH) domain containing (EHD) proteins family, regulates cell surface expression of Orai1, the mediator of store-operated calcium entry (SOCE) in breast cancer. Disrupting the EHD2-Orai1 axis in OC could provide novel targeted therapies against metastatic disease.



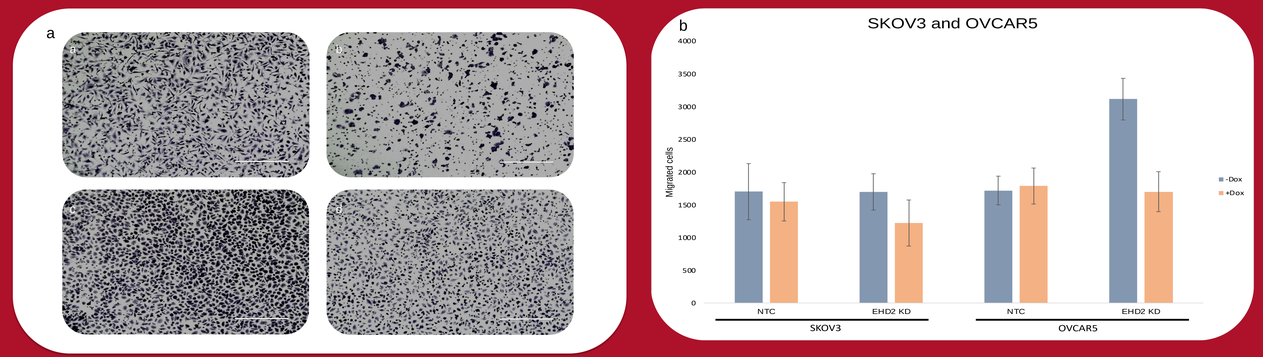
EHD2 Promotes SOCE and Migration in Ovarian Cancer Cells



Generation of EHD2 conditional KD ovarian cancer cell lines
Figure 1: Western blot analysis of EHD2 protein expression in **OVCAR5** and **SKOV3** cell lines treated with Doxycycline (Dox). The treatments included Nontargeting Control (NTC) cells with and without Doxycycline (minus Dox and plus 1000 ng/mL Dox, respectively). For EHD2 Knockdown (KD) Seq2 cells, the first two treatments were the same as NTC (minus dox and plus 1000 ng/mL Dox), followed by additional concentrations of 500, 250, 125, and 62.5 ng/mL Dox. Low protein expression for OVCAR5 was detected at plus 500 ng/mL Dox and SKOV3 at plus 1000 ng/mL Dox. Beta-actin served as the loading control for both cell lines



EHD2 KD inhibitas SOCE of Ovarian Cancer cells
Figure 2: **(a)(b)** SOCE measurement in SKOV3 and OVCAR5 cells treated with thapsigargin (Tg 2.5 µm) and calcium-free buffer (Ca 2mM). Thapsigargin was added at 15 cycles to deplete intracellular calcium stores to initiate SOCE. Changes in intracellular calcium levels were monitored using a fluorescent calcium indicator Fluo-4. Calcium buffer was added to measure the influx of calcium at 140 cycles. Both graphs showed correlation with the migration assays.



EHD2 KD inhibits migration of Ovarian Cancer cells
Figure 3: **(a)** The respective pictures are (a) SKOV3 NTC w/ Dox, (b) SKOV3 EHD2 KD w/ Dox, (c) OVCAR5 NTC w/ Dox, (d) OVCAR5 EHD2 KD w/ Dox. **(b)** ImageJ quantified the average migrated cells per field of view. The migrated cells represent the average number of migrated cells per field of view for each cell line, SKOV3 (the first two panels) and OVCAR5 (the last two panels). In both SKOV3 and OVCAR5, we see a reduction in EHD2 with the addition of Dox.

METHODS

- Cell Culture: OVCAR5 and SKOV3
- Treatment with Doxycycline to induce shRNA-based EHD2 Knockdown.
- Western Blot
- Migration Assay
- SOCE Assay

CONCLUSIONS

- EHD2 KD inhibits SOCE in SKOV3 and OVCAR5 Ovarian Cancer cells, suggesting that EHD2 may play a potential role in regulating calcium signaling pathways.
- EHD2 KD inhibits the migration of SKOV3 and OVCAR5 Ovarian Cancer cells. This indicates EHD2 may have a role in metastasis in Ovarian Cancer.
- Future studies of the impact of genetic alteration of Orai proteins and SOCE inhibitors on pro-metastatic behaviors of EHD2-overexpressing Ovarian Cancer can further our understanding and help discover new therapeutic approaches for Ovarian Cancer.

REFERENCES

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