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Using CRISPRa to enduce EMT in Colo-357 cells

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1. Introduction

- Pancreatic ductal adenocarcinoma **(PDAC)** is a lethal and highly aggressive cancer with a low survival rate (Siegel et al., 2021).
- Key driver of PDAC is the activation of signalling pathway **TGF-β**, triggering **epithelial-to-mesenchymal transition (EMT)** causing stemness, tumorigenesis and metastasis.
- E-cadherin **(CDH1)** gene is an epithelial marker modulating cell-cell adhesion. During EMT, levels of CDH1 drop (Çoban et al., 2020).
- Transcription factor **SNAI1** induces EMT and plays a role in the silencing of the CDH1 gene by downregulating its expression.
- By inducing EMT in **Colo-357** (human PDAC) cells, we can determine CDH1 and SNAI1 levels — a critical event for metastasis and invasion in carcinomas.

2. Designing sgRNAs

- Synthetic guide RNAs **(sgRNAs)** are incorporated into the CRISPRa system to guide it to its targets (Wong et al., 2015), making possible the activation of specific genes in the genome.
  - Housekeeping gene Scramble **(Scr)** used as control.
  - Transcription factor **SNAI1** due to its GC% score.

3. Methods

- **Colo-357** cells stably expressing CRISPRa system are transfected with synthetic gRNAs Scr and SNAI1.

72-hour treatment of synthetic gRNAS

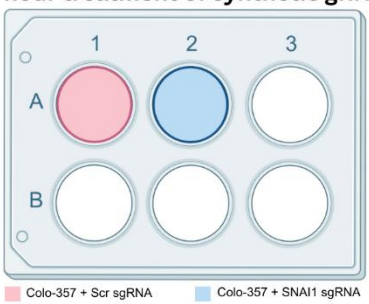

Fig. 1 Cells treated with Scr and SNAI1 sgRNAs in A1 and A2 wells respectively. Cells were left to incubate for 72-hours at 37ºC in a 5% CO2 incubator. Repeated for a total of 3 times to perform statistical analysis.

- After incubation, RNA was extracted with TRIzol® and Direct-zol™, and quantified with NanoDrop™ 2000.
- Reverse Transcription kit for the qPCR was used to determine levels of genes interested in (GAPDH as housekeeping, SNAI1, CDH1)
- Results were analysed using ΔΔCT method and Student's *t*-test was performed to determine significance of results.

4. Results

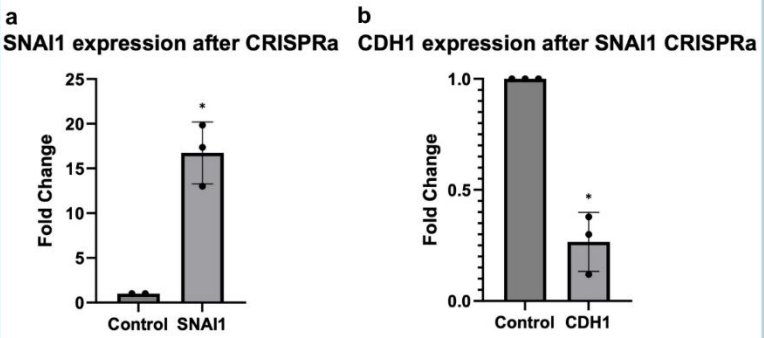

Fig. 2 Expression of SNAI1 and CDH1 after CRISPRa. **P*-value < 0.05. *P*- values were calculated using Student's *t*-test.

5. Conclusions

- When comparing cells transfected with Scr gRNA, the expression of SNAI1 in cells transfected with sgRNA SNAI1 is around ~16 times higher (significant).
- Lower CDH1 expression was found in cells transfected with SNAI1 gRNA.
- The phenotype of cells transfected with SNAI1 gRNA seem more **mesenchymal**, appearing spindle-shaped, indicating cells have undergone EMT.
- Results suggest that **activation of SNAI1** on its own is capable to **reducing CDH1 levels** and **driving EMT.**

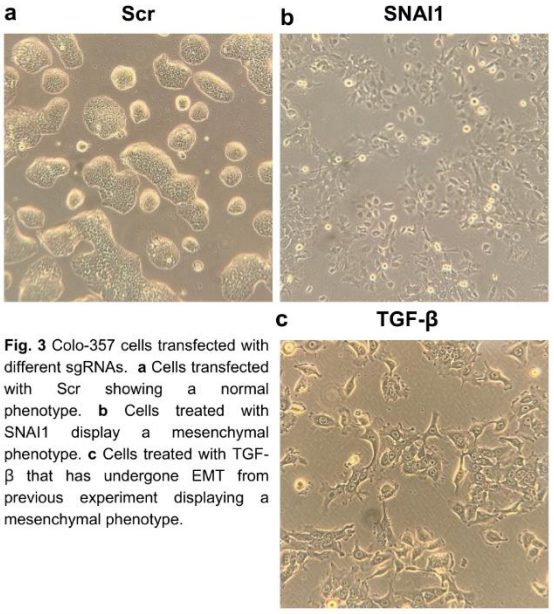

Fig. 3 Colo-357 cells transfected with different sgRNAs. **a** Cells transfected with Scr showing a normal phenotype. **b** Cells treated with SNAI1 display a mesenchymal phenotype. **c** Cells treated with TGF-β that has undergone EMT from previous experiment displaying a mesenchymal phenotype.

References

1. Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer Statistics, 2021. CA: a cancer journal for clinicians, 71(1), 7–33. https://doi.org/10.3322/caac.21654
2. Aydemİr Çoban, E., Tecİmel, D., KaŞikci, E., Bayrak, Ö. F., & Şahİn, F. (2020). E-cadherin might be a stage-dependent modulator in aggressiveness in pancreatic cancer cells. Turkish journal of biology = Turk biyoloji dergisi, 44(5), 230–237. https://doi.org/10.3906/biy-1912-60
3. Wong, N., Liu, W. & Wang, X. WU-CRISPR: characteristics of functional guide RNAs for the CRISPR/Cas9 system. Genome Biol 16, 218 (2015). https://doi.org/10.1186/s13059-015-0784-0

- Graphs were made using GraphPad.
- Illustration made using Biorender.