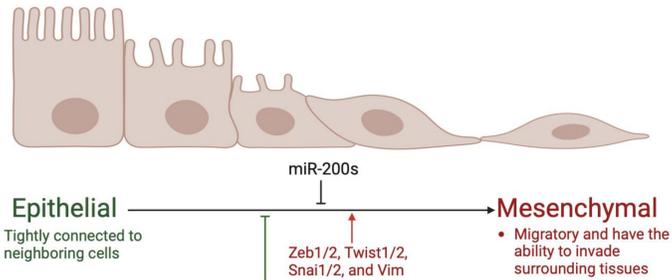
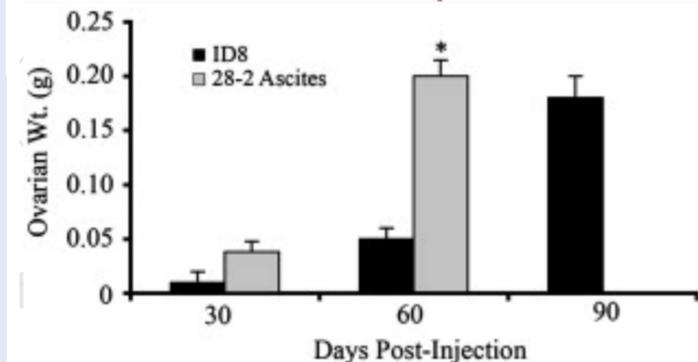
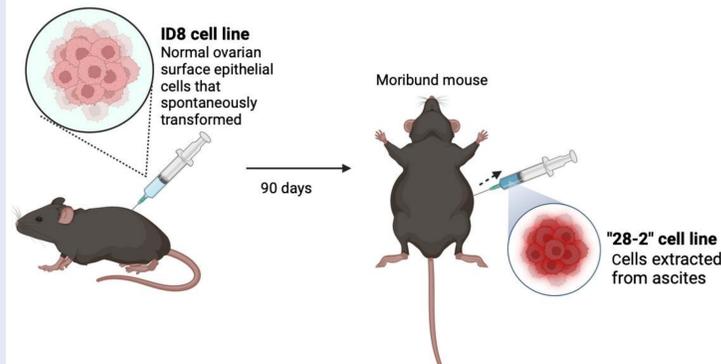


## Background: miR-200s

- Little is known about miR-200s in ovarian cancer.
- Expression studies of patients with epithelial ovarian cancer have shown that miR-200s are down-regulated in late-stage disease vs. early-stage tumours.
- In other types of cancers, miR-200s have been shown to induce apoptosis, inhibit proliferation, and prevent angiogenesis
- The miR-200 works to inhibit the epithelial to mesenchymal transition (EMT), which is critical for cancer progression
- miR-200 family is expressed in two clusters
  - Cluster 1 (**ba429**): miR-200a, miR-200b, miR-429
  - Cluster 2 (**c141**): miR-200c, miR-141
- By over-expressing one or more miR-200 family clusters in HGSOE cells, an epithelial phenotype may predominate to suppress cancer growth and metastasis.



## Background: ID8 and 28-2 cell lines



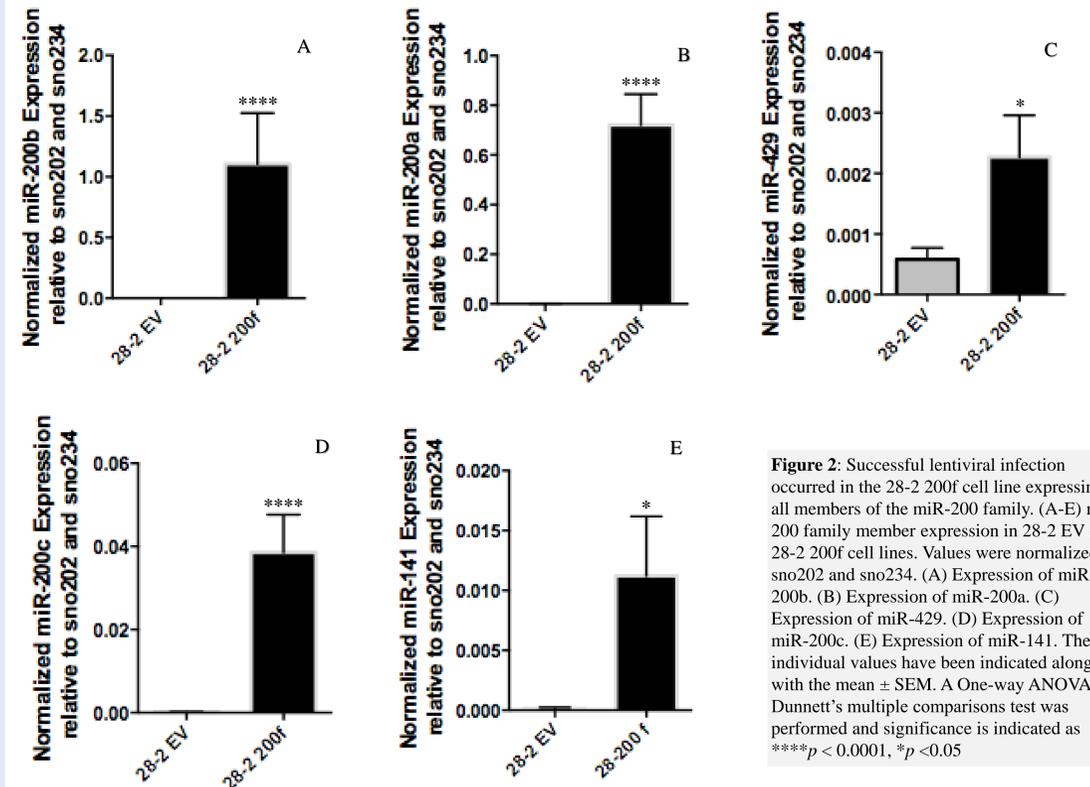
Greenaway et al., 2007

**Figure 1:** Weight changes in ovarian tissue in mice injected with ID8 cells or ascites fluid-derived cells (28-2). The tumor weight of mice injected with 28-2 ascites cells significantly increased by 60 days. Injection of ID8 did not result in significant morbidity and tumor growth until 90 days after injection. A 90-day time point was not reached for mice injected with ascites cells due to the large tumors and ascites formation. \* indicates statistical difference ( $p < 0.01$ ) between ID8 and 28-2 groups.

## Hypothesis:

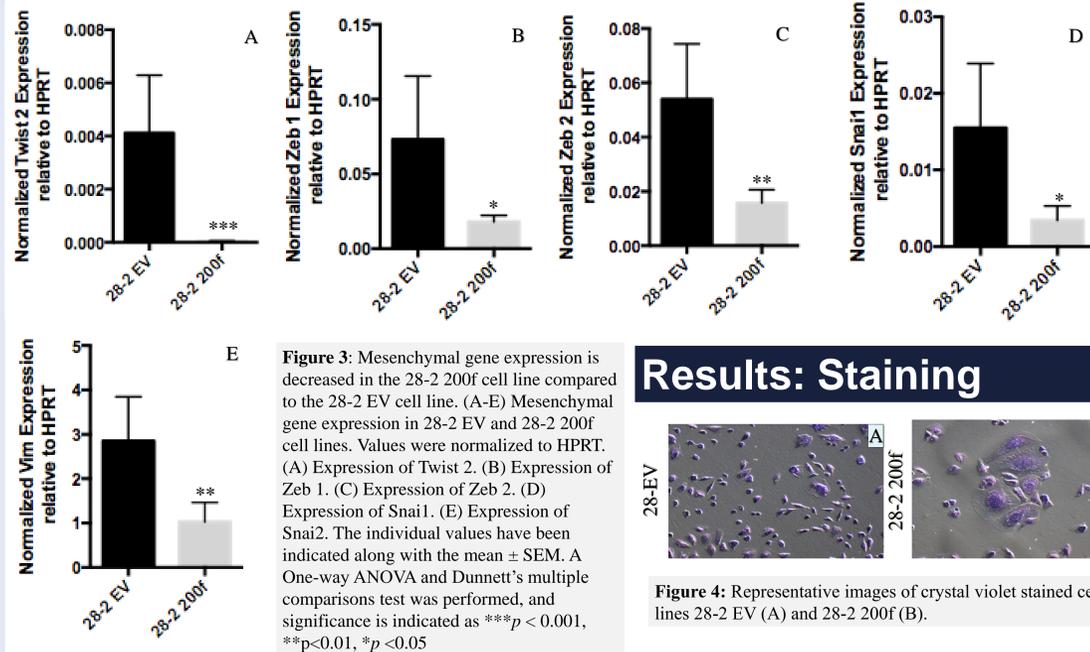
The overexpression of the miR-200 family in mouse ovarian surface epithelial cell lines ID8 and 28-2 will result in increased EMT gene expression, increased apoptosis, and decreased proliferation *in vitro*, and delayed tumour initiation, reduced growth, and inhibited metastasis *in vivo*.

## Results: miR-200 lentiviral infection in 28-2 Cells



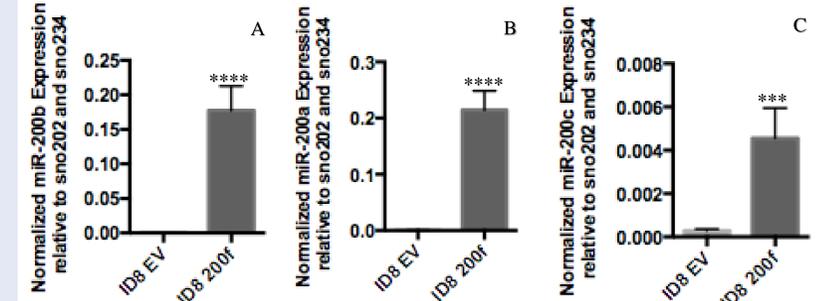
**Figure 2:** Successful lentiviral infection occurred in the 28-2 200f cell line expressing all members of the miR-200 family. (A-E) miR-200 family member expression in 28-2 EV and 28-2 200f cell lines. Values were normalized to sno202 and sno234. (A) Expression of miR-200b. (B) Expression of miR-200a. (C) Expression of miR-429. (D) Expression of miR-200c. (E) Expression of miR-141. The individual values have been indicated along with the mean  $\pm$  SEM. A One-way ANOVA and Dunnett's multiple comparisons test was performed and significance is indicated as \*\*\*\* $p < 0.0001$ , \* $p < 0.05$ .

## Results: EMT-related gene expression in 28-2 Cells



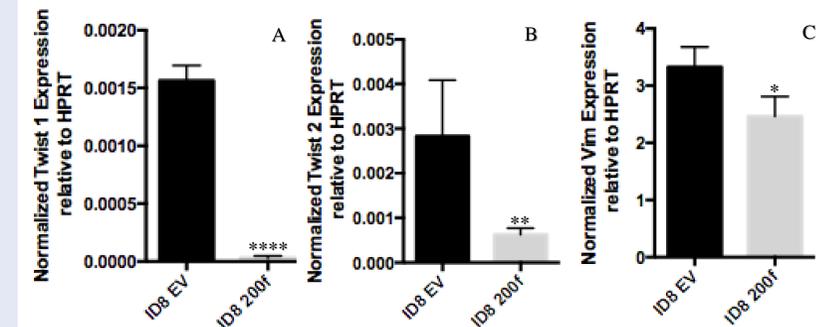
**Figure 3:** Mesenchymal gene expression is decreased in the 28-2 200f cell line compared to the 28-2 EV cell line. (A-E) Mesenchymal gene expression in 28-2 EV and 28-2 200f cell lines. Values were normalized to HPRT. (A) Expression of Twist 2. (B) Expression of Zeb 1. (C) Expression of Zeb 2. (D) Expression of Snai1. (E) Expression of Vim. The individual values have been indicated along with the mean  $\pm$  SEM. A One-way ANOVA and Dunnett's multiple comparisons test was performed, and significance is indicated as \*\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

## Results: miR-200 lentiviral infection in ID8 Cells



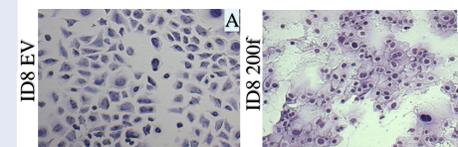
**Figure 5:** Lentiviral infection occurred in the ID8 cell line overexpressing three of the five members of the miR-200 family. (A-C) miR-200 family member expression in ID8 EV and ID8 200f cell lines. Values were normalized to sno202 and sno234. (A) Expression of miR-200b. (B) Expression of miR-200a. (C) Expression of miR-200c. The individual values have been indicated along with the mean  $\pm$  SEM. A One-way ANOVA and Dunnett's multiple comparisons test was performed and significance is indicated as \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ .

## Results: EMT-related gene expression in ID8 Cells



**Figure 6:** Mesenchymal gene expression is decreased in the ID8 200f cell line compared to the ID8 EV cell line. (A-C) Mesenchymal gene expression in ID8 EV and ID8 200f cell lines. Values were normalized to HPRT. (A) Expression of Twist 1. (B) Expression of Twist 2. (C) Expression of Vim. The individual values have been indicated along with the mean  $\pm$  SEM. A One-way ANOVA and Dunnett's multiple comparisons test was performed, and significance is indicated as \*\*\*\* $p < 0.0001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .

## Results: Staining



**Figure 7:** Representative images of crystal violet stained cell lines ID8 EV (A) and ID8 200f (B).

## Conclusions

- Overexpression of the miR-200 family via lentiviral infection was successful in the 28-2 cell line and was sufficient to significantly overexpress miR-200a, miR-200b, and miR-200c in the ID8 cell line.
- miR-200 family overexpressing cell lines had decreased expression of EMT-related genes, lending them a more epithelial phenotype compared to their empty vector counterparts.

## Next Steps

- Investigating the effect of miR-200s on ID8 and 28-2 cell migration, senescence, apoptosis, proliferation, and response to chemotherapy agents.
- Cell sequencing to identify all the genes differentially expressed between EV and 200f cells.
- Investigating the *in vivo* effects of miR-200s by injecting syngeneic mice (C57BL6) with each cell line and evaluating peritoneal tumour growth, angiogenesis, and cancer cell proliferation and apoptosis.

## Funding