Role of ASCL2 in radio-resistance and chemo-resistance of stem cells in normal esophageal epithelium

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INTRODUCTION

A rare population of basal cells of the esophageal epithelium, the stem cells, was recently identified by the marker Keratin 15 (Krt15). The transcription factor ASCL2 has been shown to be highly expressed in *Krt15*+ cells. ASCL2 is essential for the stemness of *Lgr5*+ cells in the intestine and for those in developing muscle. The biological role of ASCL2 in the esophagus is still unknown. Due to its previously described functions in other tissues, my host laboratory hypothesized that ASCL2 could be an important regulator of the gene signature of esophageal stem cells and contribute to the maintenance of esophageal stem cells. It is also known that Krt15+ cells have radio-resistance capabilities.

We hypothesize that organoids overexpressing ASCL2 (ASCL2^{OE}) will tolerate treatments better than controls as they have higher stemness properties and so that ASCL2 could be involved in the cellular response to radiotherapy and possibly chemotherapy treatments

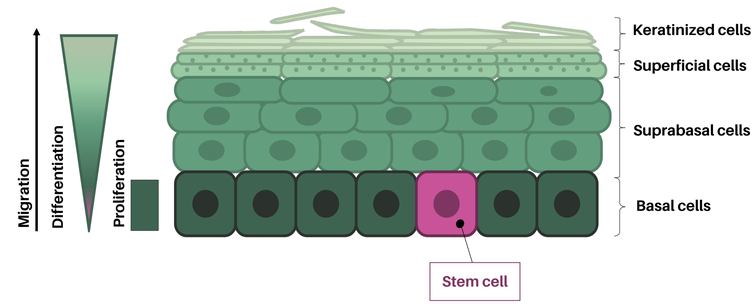

Figure 1. Representation of the murine esophageal epithelium

GOAL OF THIS RESEARCH PROJECT

Evaluate the ability of ASCL2^{OE} organoids to survive irradiation, chemotherapeutic agents, and DNA damage, and consequently investigate the role of ASCL2 in this response in esophageal epithelial biology.

METHOLOGIES

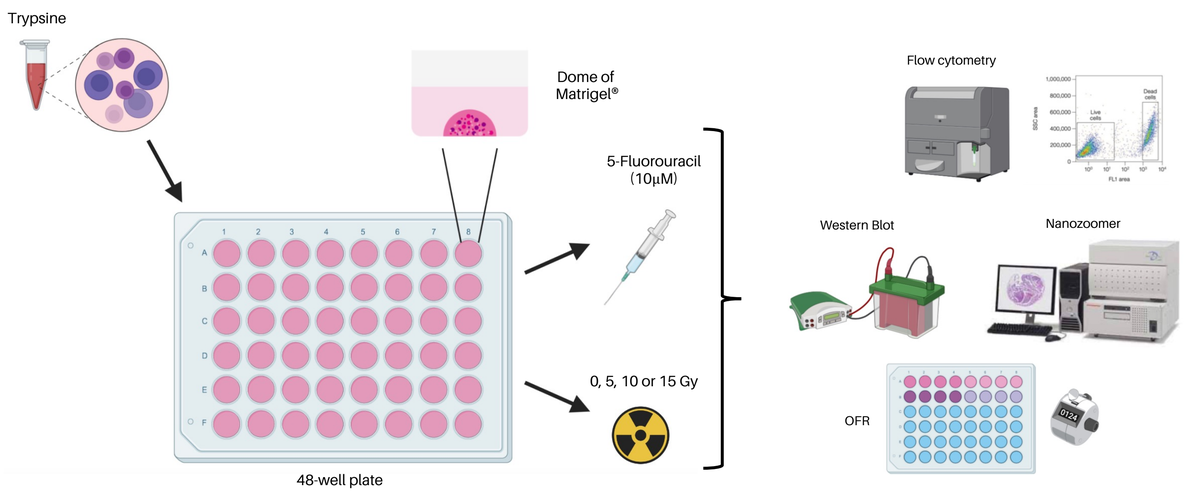

Figure 2. Methods used in the project

Cell culture, western immunoblot, flow cytometry, and organoid formation rate assay were used to study the role of ASCL2 in the response to physical and chemical treatments in the esophageal epithelium.

RESULTS

Morphology

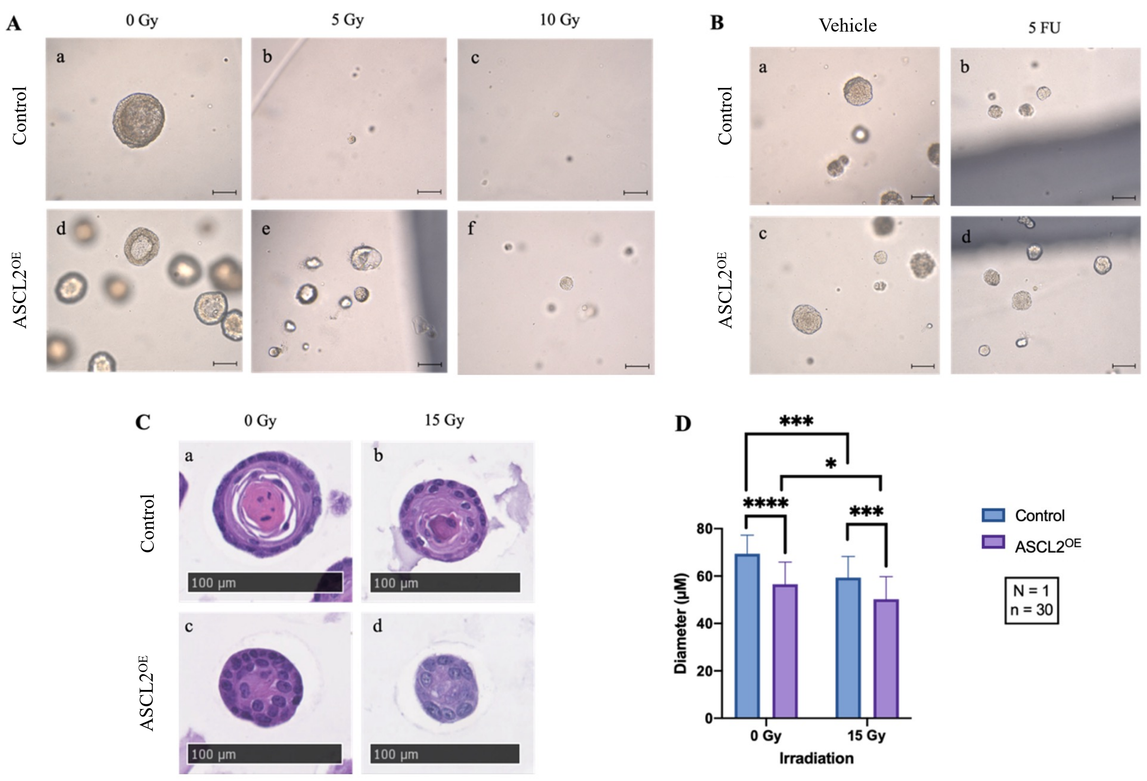

Figure 3. The morphology of control organoids seems to be more affected than that of ASCL2^{OE} after treatments with radiation or chemotherapeutic drugs.

(A, B) ASCL2-overexpressing and control organoids were seeded at 300 cells, irradiated (5Gy, 10Gy) or treated with 10µM 5-FU at Day 1 (D1) post-seeding and observed between D5 and D7. The vehicle is DMF. Scale bar represents 100 µm (C) Hematoxylin and eosin staining (H&E) of organoids with or without irradiation. Scale bar represents 100 µm. (D) Organoid diameter was assessed on H&E stains for samples that received a radiation dose of 0 or 15Gy at D5 (N=1, 30 organoids per group). The graph represents the mean diameter in µm of organoids ± standard deviation; p≤0.0001 **** using a one-way ANOVA.

Viability

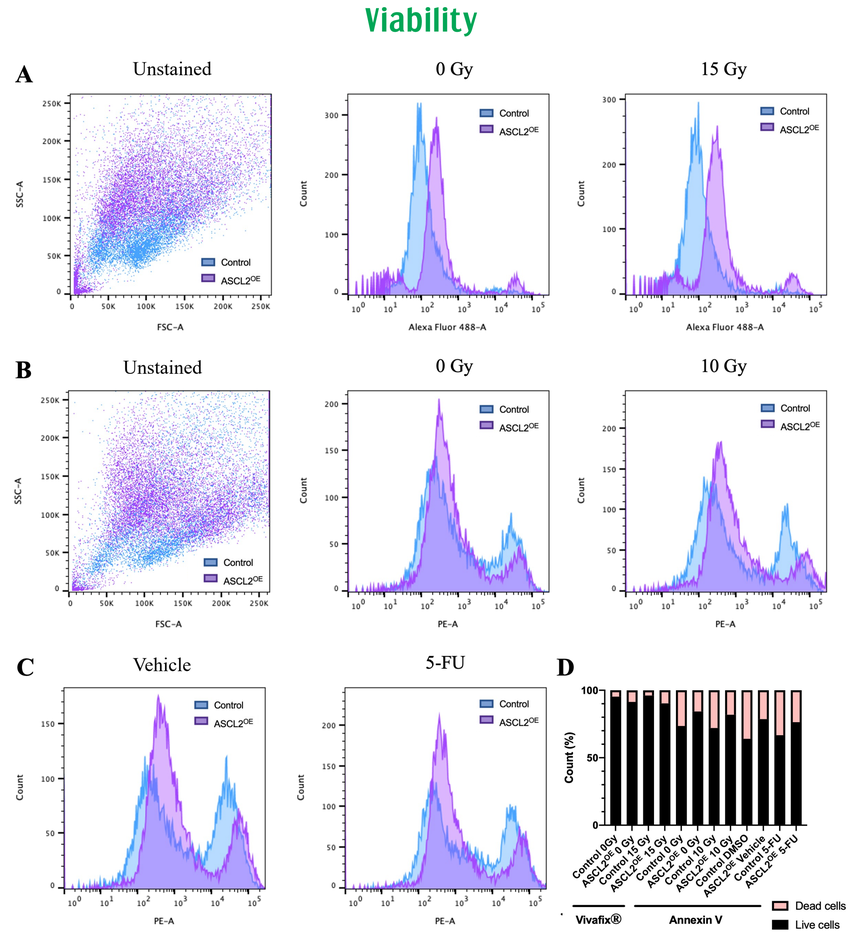

Figure 4. The viability of organoids does not seem to be affected by 5-FU and irradiation

ASCL2-overexpressing and control organoids were seeded, irradiated (10 or 15Gy) or treated with 5-FU at D5 and collected 24 hours after treatments. The vehicle is DMSO. (A) Vivafix assay for irradiated organoids (N = 3). (B) Annexin V assay for irradiated organoids (N = 1). (C) Annexin V assay for organoids treated with chemotherapeutic drug (N = 1). (D) Percentage of live or dead cells in the different tests.

Resistance

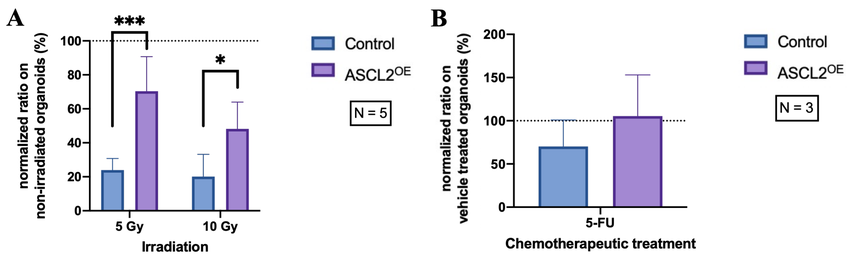

Figure 5. ASCL2^{OE} organoids are more resistant to treatments

(A, B) ASCL2-overexpressing and control organoids were seeded at 300 cells, irradiated (5Gy, 10Gy) or treated with chemotherapeutic drugs (5-FU) at D1 and observed between D5 and D7. The vehicle is DMF. Graphs represent the average count of organoids ± standard deviation; p≤0.0002 *** using a one-way ANOVA.

DNA damages

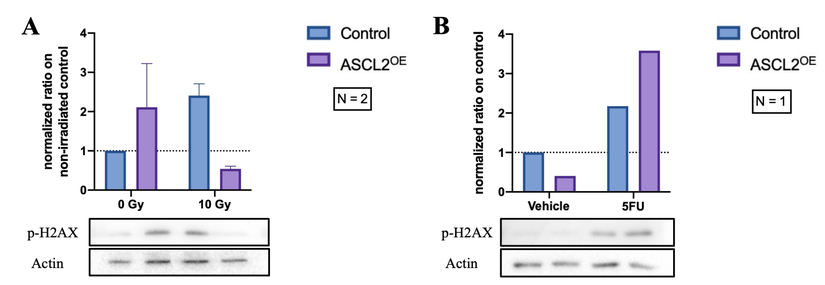

Figure 6. The overexpression of ASCL2 results in radio-resistant organoids, but not chemo-resistant ones

(A, B) ASCL2-overexpressing and control organoids were seeded and irradiated (10Gy) or treated with 10µM of 5-FU at D5 and harvested 48h later. The vehicle is DMF. Western blot experiments were performed on whole organoids extracts. Data show protein expression patterns of pH2AX (lower panel) and quantitative analysis of the expression in comparison to actin (upper panel).

CONCLUSION & PERSPECTIVES

We have identified that organoids overexpressing ASCL2 appear to be radioresistant. Further analysis regarding treatment with chemotherapeutic drugs is needed to confirm the chemoresistance observed in OFR assays. These findings could help to understand stem cell radioresistance in esophageal cancer as recently ASCL2 has been described as a new prognostic predictor in esophageal adenocarcinoma.

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REFERENCES