Data in Brief

Microarray profiling to analyze the effect of Snai1 loss in mouse intestinal epithelium

Gary R. Hime a,⁎, Katja Horvay c, Thierry Jardé c, Franca Casagrand a, Victoria M. Perreau b, Helen E. Abud c

a Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Vic 3010, Australia
b The Florey Institute of Neuroscience and Mental Health, Parkville, Vic 3010, Australia
c Department of Anatomy and Developmental Biology, Monash University, Clayton, Vic 3800, Australia

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Epithelial stem cells from a variety of tissues have been shown to express genes linked to mesenchymal cell states. The Snail family of transcriptional factors has long been regarded as a marker of mesenchymal cells, however recent studies have indicated an involvement in regulation of epithelial stem cell populations. Snai1 is expressed in the stem cell population found at the base of the mouse small intestinal crypt that is responsible for generating all differentiated cell types of the intestinal epithelium. We utilized an inducible Cre recombinase approach in the intestinal epithelium combined with a conditional floxed Snai1 allele to induce knockout of gene function in the stem cell population. Loss of Snai1 resulted in loss of crypt base columnar cells and a failure to induce a proliferative response following radiation damage. We induced Snai1 loss in cultured organoids that had been derived from epithelial cells and compared gene expression to organoids with functional Snai1. Here we describe in detail the methods for generation of knockout organoids and analysis of microarray data that has been deposited in Gene Expression Omnibus (GEO):GSE65005.

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1. Direct link to deposited data

The deposited data can be found at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65005.

2. Experimental design, materials and methods

2.1. Mice

Snai1 is strongly localized to nuclei of crypt base columnar stem cells [1] and was likely to regulate stem cell function so experiments were undertaken to knockout Snai1 function in the mouse intestinal epithelium. Germline loss of mouse Snai1 results in early embryonic lethality [2] thus in order to analyze the function of Snai1 in adult intestinal epithelium, a floxed Snai1 allele, Snai1fl/fl [3], was combined with an inducible Cre allele, VillinCreERT2 [4], that expressed CreERT2 in all cells of the small intestinal epithelium. Cre activity was induced via four daily intraperitoneal injections of 100 mg/kg tamoxifen dissolved in corn oil and on day 5 mice were killed and tissue harvested. At least 3 biological replicates of experimental, VillinCreERT2 Snai1fl/fl, and control, VillinCreERT2 Snai1+/+ mice were used to study the role of Snai1 in experiments as reported in Horvay et al. (2015) [5].
2.2. Generation and culture of organoids

Organoid cultures can be established from isolated crypts or individual intestinal stem cells and provide an opportunity to assay gene function in relation to stem cell function or intestinal regeneration in a defined culture system [6,7]. This system allowed us to induce loss of Snai1 with precise temporal control, assay organoid growth and apoptosis, and collect RNA from a pure population of epithelial cells [5]. The small intestinal tract from female mice was isolated by dissection just posterior to the stomach and just anterior to the caecum following removal of associated fat and mesentery. The intestinal tract was placed in cold phosphate buffered saline (PBS), cut longitudinally and gently flushed to remove luminal contents. Villi were removed by scraping the luminal surface with a glass coverslip. The intestinal tract was then cut into small pieces in cold PBS and washed 3 times before incubation in 30 ml 2 mM EDTA in PBS with gentle rotation at 4 °C for 30 min. The supernatant was discarded and replaced with 15 ml cold PBS and mechanically disso-

ected cells were pelleted. The supernatant was again discarded and replaced with another 15 ml of cold PBS. This process was repeated with crypt containing fractions 2 to 4 collected. Crypts were pelleted and the pellet containing crypts resuspended in phenol red free Matrigel (BD 356231). 50 μl of Matrigel was seeded in each well of a pre-warmed 24 well plate and incubated for 5 min until well solidified. 500 μl of cold culture medium (DMEM/F12 supplemented with B27, glutamax, N2, 10 mM HEPES, fungizone, 50 μg/ml EGF (Peprotech), 100 ng/ml Noggin, (Peprotech), penicillin/streptomycin and 600 ng/ml R-spondin 1 (R&D systems)) was added to 100 mg of isolated crypts and mechanically lysed by pipet-

ting up and down several times. Following addition of 0.2 ml of chlo-

er free Matrigel (BD 356231), 50 μl of Matrigel was seeded in each well of a pre-warmed 24 well plate and incubated for 5 min until well solidified. 500 μl of cold culture medium (DMEM/F12 supplemented with B27, glutamax, N2, 10 mM HEPES, fungizone, 50 μg/ml EGF (Peprotech), 100 ng/ml Noggin, (Peprotech), penicillin/streptomycin and 600 ng/ml R-spondin 1 (R&D systems)) was added to each well. After several days in culture, seeded organoids would start to form buds or crypt like domains. Medium on organoid cultures was changed 3 times per week. Following 7 to 10 days in culture, organoids were passed by mechanically disrupting the organoids in Matrigel by pi-

petting. Organoids were gently mechanically disrupted by pipetting, washed in 70% ethanol before being dissolved in 50 μl RNase free water. Further RNA cleanup and DNase digestion was achieved using the RNeasy mini kit from Qiagen.

After 24 h of tamoxifen treatment, the matrigel-embedded organoids were gently mechanically disrupted by pipetting, washed with PBS and centrifugated at 1500 rpm for 3 min. The supernatant was discarded and the pellet containing organoids was resuspended in RLT lysis buffer (Qiagen RNeasy Micro kit). The solution was then homogenized using QiAShredder columns (Qiagen) and extraction of RNA was subsequently achieved following manufacturer’s recommend-

ations (Qiagen RNeasy Micro kit).

cDNA was prepared from epithelial crypt preps (1 μg RNA) or organoid cultures (0.5 μg) using Superscript III Reverse Transcriptase (Invitrogen) with random hexamers or QuantiTect Reverse Transcrip-

tion Kit (Qiagen).

2.4. Microarray analysis

Labelling and hybridization of RNA was conducted by the Australian Genome Research Facility. Total RNA quality and quantity was assayed with an Agilent Bioanalyzer 2100 using the NanoChip protocol. 500 ng of total RNA from each sample was used to prepare a probe cocktail (cRNA @ 0.05 μg/μl). 30 μl of total hybridization volume for each sample was loaded into a single array on the Illumina MouseWG-6_V2 Expression BeadChip. The chip was hybridized at 58 °C for 16 h. Chips were then washed as per the Illumina manual and coupled with Cy3 and scanned in an Illumina iScan Reader. The LumiR package of R Bioconductor was applied to raw signal intensity values to conduct background correction, log2 transformation and variance stabilization. Partek Genomics Suite™ software was used to perform ANOVA of normalized probe intensities and calculate significance of variation between groups. The SerinC3 gene was found to be downregulated at 24 h after induction of Snai1 loss [5]. Little is known of SerinC3 (also known as TDE1) function except that it has been associated with protection from apoptosis [8]. Loss of Snai1 resulted in apoptosis of the crypt base columnar stem cell population. Down regulation of SerinC3 was confirmed by quantitative PCR of RNA isolated from organoids 24 and 72 h post treatment with tamoxifen [5].

2.5. Droplet digital PCR

ddPCR is a form of quantitative PCR that is based on partition of the PCR reaction mixture into many thousands of droplets prior to initi-
ation of the PCR reaction. Some droplets will contain a target mole-
cule and some will not, hence PCR products will only be generated in a percentage of the droplets and counting these “positive” drops-

lets allows a calculation of the number of target cDNA molecules that were present in the reaction mix. We used this sensitive method [9] to measure the expression of SerinC3 in total RNA isolated from Snai1 mutant and control mouse small intestinal crypt preps, 5 days post treatment with tamoxifen, cDNA was generated as above. Reaction mix [2×ddPCR Supermix (BioRad # 186–3010)], 20 × stock concentration of primers and probe mix (IDT PrimeTime qPCR assay 500 nM primers and 250 nM probe) and cDNA (variable volume) in a 25 μl total volume. Droplets were generated and

Fig. 1. Droplet digital PCR of SerinC3 (normalized to HPRT) expression in VillinCreERT2 Snai1fl/fl intestinal crypts, 5 days after tamoxifen treatment, compared to control crypts, VillinCreERT2 Snai1+/+. p = 0.004, Student’s T-test.
subjected to a 2-step thermocycling protocol [95 °C × 10 min; 40 cycles x [(94 °C × 30 s, 60 °C × 60 s); 98 °C × 10 min, ramp rate set at 2.5 °C/s]. Droplet fluorescence was counted in a QX100 Droplet Reader (BioRad) and analyzed with QuantaSoft software (BioRad). The SerinC3 assay Mm.Pt.58.28709379 (IDT) was normalized with a HPRT (hypoxathine phosphoribosyltransferase) housekeeper assay Mm.PT.39a.22214828 (IDT). Expression of SerinC3 was reduced approximately 4-fold in the Snai1 knockout crypts (Fig. 1) in a similar manner to the reduction of expression exhibited by the Snai1 knockout organoids. The reduction in SerinC3 is thus a direct consequence of the loss of Snai1 in vivo within the small intestinal epithelium.

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Author/s:
Hime, GR; Horvay, K; Jarde, T; Casagranda, F; Perreau, VM; Abud, HE

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