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Data Article

Data describing the effects of dietary bioactive agents on colonic stem cell microRNA and mRNA expression

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A B S T R A C T

With the identification of Lgr5 as a definitive marker for intestinal stem cells, we used the highly novel, recently described, Lgr5-EGFP-IRES-cre ERT\textsuperscript{2} knock in mouse model. Mice were injected with azoxymethane (AOM, a colon carcinogen) or saline (control) and fed a chemo-protective diet containing n-3 fatty acids and fermentable fiber (n-3 PUFAs+pectin) or a control diet (n-6 PUFAs + cellulose). Single cells were isolated from colonic mucosa crypts and three discrete populations of cells were collected via fluorescence-activated cell sorting (FACS): Lgr5\textsuperscript{high} (stem cells), Lgr5\textsuperscript{low} (daughter cells) and Lgr5\textsuperscript{negative} (differentiated cells). microRNA profiling and RNA sequencing were performed from the same sample and analyzed. These data refer to ‘Comparative effects of diet and..."
carcinogen on microRNA expression in the stem cell niche of the mouse colonic crypt' (Shah et al., 2016) [5].
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### Specifications table

| Subject area        | Biology                      |
|---------------------|------------------------------|
| More specific subject area | Intestinal stem cells |
| Type of data        | Tables                       |
| How data was acquired | The raw data was generated using an Illumina sequencer and was statistically analyzed and displayed in tabular format. |
| Data format         | Analyzed                     |
| Experimental factors | Colonic crypts were isolated from the stem cell reporter mice and were sorted based on GFP into high, low and negative cell populations using a BD FACS Aria II cytometer/sorter (BD Biosciences). Total RNA was isolated from the sorted cells using a miRVana miRNA isolation kit. After checking the quality of the RNA, libraries were subjected to RNA sequencing and microRNA profiling. |
| Experimental features |                              |
| Data source location | College Station, Texas, USA (30.6014°N, 96.3144°W) |
| Data accessibility  | All the datasets mentioned in this manuscript will be uploaded to the Gene Expression Omnibus (GEO) (accession no.- SRP061188) in NCBI. |

### Value of the data

- These data will serve as a basis to compare Lgr5\textsuperscript{high} stem cells that have been perturbed by inflammation, radiation or knockout of tumor suppressors/oncogenes.
- These data describe expression values for miRNAs in Lgr5\textsuperscript{high}, Lgr5\textsuperscript{low} and Lgr5\textsuperscript{neg} cells. For example, if a researcher wants to know if a certain miRNA is expressed in the colonic crypt, this database will provide that information. The data set also details the location of the miRNA throughout the entire crypt.
- RNA sequencing data from Lgr5\textsuperscript{high}, Lgr5\textsuperscript{low} and Lgr5\textsuperscript{neg} cells also provides the basis for determining the expression of different miRNAs throughout the crypt. This database will serve as a comparative platform for future studies to determine correlations between current and future datasets.

### 1. Data

The data presented in this article represent: (a) Ct values of miRNAs expressed in mouse colonic epithelial cells (Table 1), (b) differentially expressed miRNAs in Lgr5\textsuperscript{high} versus Lgr5\textsuperscript{negative} cells (i.e., stem cells vs. differentiated cells) (Table 2), (c) the effect of diet on miRNA expression in Lgr5\textsuperscript{high} sorted cells (Table 3), (d) the effect of carcinogen on miRNA expression in Lgr5 high sorted cells (Table 4) and (e) the effect of diet and carcinogen combination on miRNA expression in GFP\textsuperscript{negative} sorted cells (Table 5).
These data refer to our recently published paper 'Comparative effects of diet and carcinogen on microRNA expression in the stem cell niche of the mouse colonic crypt' (Shah et al., 2016) [5].

2. Experimental design, materials and methods

2.1. Experimental diets

Lgr5-EGFP-IRESCreER<sup>T2</sup> mice were assigned to one of the two diet groups (fish oil / pectin or corn oil / cellulose), which differed only in the type of fat and fiber. Diets contained (g/100 g diet): dextrose, 51.00; casein, 22.40; D,L-methionine, 0.34; American Institute of Nutrition (AIN)-76 salt mix, 3.91; AIN-76 vitamin mix, 1.12; choline chloride, 0.13; pectin or cellulose, 6.00. The total fat content of each diet was 15% by weight with the n-6 PUFA diet containing 15.0 g corn oil/100 g diet (Dyets, Bethlehem, PA) and the n-3 PUFA diet containing 11.5 g fish oil/100 g diet (Omega Protein, Houston, TX).

Table 1

| miRNA     | Ct values | miRNA     | Ct values | miRNA     | Ct values |
|-----------|-----------|-----------|-----------|-----------|-----------|
| mmu-miR-31| 11.44     | mmu-miR-671-3p | 26.81     | mmu-let-7g-| 28.94     |
| rno-miR-190b| 14.92 | mmu-miR-215  | 26.81     | mmu-miR-103 | 28.95     |
| mmu-miR-872| 17.72     | mmu-miR-151-3p | 26.9     | mmu-miR-320 | 29        |
| mmu-miR-124| 20.88     | mmu-let-7b   | 26.96     | mmu-miR-218 | 29.02     |
| mmu-miR-128a| 20.99   | mmu-miR-203  | 26.97     | mmu-miR-30d | 29.2       |
| mmu-miR-429| 21.89     | mmu-miR-484  | 27.02     | mmu-miR-125b-5p | 29.2     |
| mmu-miR-148b| 22.68    | mmu-miR-29a  | 27.11     | mmu-miR-205 | 29.63     |
| mmu-miR-324-5p| 22.88 | mmu-miR-29b  | 27.24     | mmu-miR-18a | 29.63     |
| mmu-miR-322| 23.04     | mmu-miR-340-5p| 27.41     | mmu-miR-195 | 29.68     |
| mmu-miR-142-3p| 23.43 | mmu-miR-139-5p| 27.43     | mmu-miR-28  | 29.7       |
| mmu-miR-192| 23.51     | mmu-miR-15b  | 27.43     | mmu-miR-574-3p | 29.83     |
| mmu-miR-200a| 23.75    | mmu-miR-93   | 27.49     | mmu-miR-101a| 29.83     |
| mmu-miR-423-5p| 23.88 | mmu-let-7e   | 27.55     | mmu-miR-29c | 30.01     |
| mmu-miR-375| 23.94     | mmu-miR-20b  | 27.71     | rno-miR-345-3p | 30.02     |
| mmu-miR-10b| 24.03     | mmu-miR-27b  | 28.01     | mmu-miR-301b| 30.12     |
| mmu-miR-19b| 24.16     | mmu-miR-30a  | 28.04     | mmu-miR-146b| 30.29     |
| mmu-miR-30c| 24.31     | mmu-miR-27a  | 28.04     | mmu-miR-744 | 30.34     |
| mmu-miR-92a| 24.36     | mmu-miR-148a | 28.06     | mmu-miR-331-3p| 30.37     |
| mmu-miR-191| 24.43     | mmu-miR-222  | 28.13     | mmu-miR-186 | 30.38     |
| mmu-miR-30b| 24.75     | mmu-miR-141  | 28.18     | mmu-miR-196b| 30.39     |
| mmu-miR-24 | 24.78     | mmu-miR-188-5p| 28.19     | mmu-miR-340-3p| 30.44     |
| mmu-miR-126-3p| 24.79 | mmu-miR-106b | 28.22     | mmu-miR-301a| 30.45     |
| mmu-miR-17 | 24.91     | mmu-miR-19a  | 28.23     | mmu-miR-130b| 30.49     |
| mmu-miR-194| 24.92     | mmu-let-7d   | 28.23     | mmu-miR-193b| 30.65     |
| mmu-miR-200b | 24.99 | mmu-miR-25  | 28.24     | mmu-miR-155 | 30.77     |
| mmu-miR-34b-3p| 25.05 | rno-miR-196c | 28.46     | mmu-miR-152 | 30.8      |
| mmu-miR-20a | 25.16     | mmu-miR-130a | 28.46     | mmu-miR-23b | 30.98     |
| mmu-miR-106a| 25.16     | mmu-miR-100  | 28.5      | mmu-miR-183 | 31.39     |
| mmu-miR-200c| 25.38     | mmu-miR-30e  | 28.57     | mmu-miR-125a-5p| 31.84     |
| mmu-let-7c | 25.74     | mmu-miR-182  | 28.57     | mmu-miR-181a| 31.94     |
| mmu-miR-16 | 25.98     | mmu-miR-328  | 28.59     | mmu-miR-181c| 34.76     |
| mmu-miR-10a | 26.11     | mmu-let-7i   | 28.68     | mmu-miR-145 | 28.84     |
| mmu-miR-145 | 26.32     | mmu-miR-26b  | 28.84     | mmu-miR-26a | 28.86     |
| mmu-miR-26a | 26.33     | mmu-miR-140  | 28.86     | mmu-miR-21 | 28.87     |
| mmu-miR-21 | 26.48     | mmu-miR-146a | 28.87     | mmu-miR-99a| 28.92     |

Expression of miRNAs was quantified by reverse transcription using miRNA-specific primers followed by real-time PCR TaqMan low-density array analysis. Ct values represent means of 60 samples, mmu, mouse; rno, rat; Ct, cross threshold.
TX) plus 3.5 g corn oil/100 g diet to prevent essential fatty acid deficiency. All diet ingredients except oils were obtained from Bio-serv (Frenchtown, NJ). To prevent the formation of oxidized lipids, diets were stored at –20 °C and provided fresh to animals every day.

Table 2
Differentially expressed miRNAs in Lgr5<sup>high</sup> versus Lgr5<sup>negative</sup> cells.

| miRNA              | Expression ratio Lgr5<sup>high</sup>/Lgr5<sup>negative</sup> | P-value | miRNA              | Expression ratio Lgr5<sup>high</sup>/Lgr5<sup>negative</sup> | P-value |
|--------------------|-------------------------------------------------------------|---------|--------------------|-------------------------------------------------------------|---------|
| mmu-miR-342-3p     | 2.64                                                        | 0.012   | mmu-miR-652        | 0.32                                                        | 0.000   |
| mmu-miR-671-3p     | 2.29                                                        | 0.008   | mmu-miR-145        | 0.40                                                        | 0.009   |
| rno-miR-345-3p     | 2.14                                                        | 0.022   | mmu-miR-27a        | 0.42                                                        | 0.000   |
| rno-miR-190b       | 1.95                                                        | 0.018   | mmu-miR-215        | 0.42                                                        | 0.000   |
| mmu-miR-155        | 1.90                                                        | 0.033   | mmu-miR-532-5p     | 0.50                                                        | 0.040   |
| mmu-miR-191        | 1.81                                                        | 0.001   | mmu-miR-7b         | 0.60                                                        | 0.027   |
| mmu-miR-20b        | 1.68                                                        | 0.011   | mmu-miR-21         | 0.62                                                        | 0.024   |
| mmu-miR-17         | 1.68                                                        | 0.000   | mmu-miR-30d        | 0.62                                                        | 0.017   |
| mmu-miR-125a-5p    | 1.58                                                        | 0.036   | rno-miR-224        | 0.62                                                        | 0.018   |
| mmu-miR-186        | 1.58                                                        | 0.006   | mmu-miR-30a        | 0.66                                                        | 0.000   |
| mmu-miR-218        | 1.44                                                        | 0.003   | mmu-miR-200b       | 0.69                                                        | 0.013   |
| mmu-miR-10a        | 1.30                                                        | 0.047   | mmu-miR-203        | 0.76                                                        | 0.003   |
| mmu-miR-92a        | 1.29                                                        | 0.018   |                  |                                                             |         |
| mmu-miR-200a       | 1.27                                                        | 0.031   |                  |                                                             |         |

Expression of miRNAs were quantified as described in Table 1. GFP<sup>high</sup> stem cells (n=20, pooled samples); Lgr5<sup>negative</sup> cells (n=20, pooled samples). Only miRNAs with P < 0.05 are shown. mmu, mouse; rno, rat.

Table 3
Effect of carcinogen on miRNA expression in Lgr5<sup>high</sup> sorted cells.

A.

| miRNA              | Expression ratio AOM-Lgr5<sup>high</sup>/Saline-Lgr5<sup>high</sup> | P-value |
|--------------------|-----------------------------------------------------------------|---------|
| mmu-miR-532-3p     | 2.61                                                            | 0.020   |
| rno-miR-196c       | 1.66                                                            | 0.008   |
| mmu-miR-331-3p     | 1.45                                                            | 0.032   |
| mmu-miR-92a        | 1.41                                                            | 0.042   |
| mmu-miR-100        | 0.19                                                            | 0.050   |
| mmu-miR-124        | 0.25                                                            | 0.021   |

B.

| miRNA              | Expression ratio AOM-Lgr5<sup>neg</sup>/Saline-Lgr5<sup>neg</sup> | P-value |
|--------------------|-----------------------------------------------------------------|---------|
| mmu-let-7e         | 1.97                                                            | 0.008   |
| mmu-miR-18a        | 1.84                                                            | 0.050   |
| mmu-miR-20b        | 1.59                                                            | 0.029   |
| mmu-miR-101a       | 1.52                                                            | 0.045   |
| mmu-let-7i         | 1.45                                                            | 0.039   |
| mmu-miR-375        | 1.26                                                            | 0.046   |
| mmu-miR-224        | 0.65                                                            | 0.030   |
| mmu-miR-193b       | 0.65                                                            | 0.045   |
| mmu-miR-10a        | 0.62                                                            | 0.008   |

miRNA expression was quantified as described in Table 1. AOM, azoxymethane (n=20); saline (n=20); Lgr5<sup>high</sup>, stem cells; Lgr5<sup>neg</sup>, non-stem cells. Only miRNAs with P < 0.05 are shown.
2.2. Fluorescence activated cell sorting of colonic stem cells

Colonic crypts from individual mice were isolated as previously described Sato et al. [1] with minor modification. The intact colons were everted on a disposable mouse gauge needle (Instech).

### Table 4
Effect of diet on miRNA expression in Lgr5\(^{\text{high}}\) sorted cells.

#### A. Carcinogen

| miRNA     | Expression ratio CCA-Lgr5\(^{\text{high}}\)/FPA-Lgr5\(^{\text{high}}\) | P-value |
|-----------|---------------------------------------------------------------|---------|
| miR-21    | 2.2                                                           | 0.030   |
| miR-26b   | 2.0                                                           | 0.010   |
| miR-200a  | 1.8                                                           | 0.000   |
| miR-10a   | 1.7                                                           | 0.040   |
| miR-26a   | 1.7                                                           | 0.020   |
| miR-29c   | 1.6                                                           | 0.010   |
| miR-30c   | 1.5                                                           | 0.040   |
| miR-203   | 1.5                                                           | 0.040   |
| miR-30a   | 1.4                                                           | 0.020   |
| miR-19b   | 1.3                                                           | 0.040   |
| miR-181a  | 0.6                                                           | 0.030   |
| miR-34b-3p| 0.1                                                           | 0.000   |

#### B. Saline

| miRNA     | Expression ratio CCS-Lgr5\(^{\text{high}}\)/FPS-Lgr5\(^{\text{high}}\) | P-value |
|-----------|---------------------------------------------------------------|---------|
| mmu-miR-188-5p | 5.3                                                            | 0.027   |
| mmu-miR-218   | 0.6                                                            | 0.016   |
| mmu-miR-125a-5p| 0.5                                                            | 0.047   |
| mmu-miR-574-3p| 0.5                                                            | 0.028   |
| mmu-miR-200c  | 0.5                                                            | 0.047   |
| mmu-miR-222   | 0.4                                                            | 0.016   |
| mmu-miR-429   | 0.3                                                            | 0.034   |
| mmu-miR-106a  | 0.1                                                            | 0.047   |

Expression of miRNAs was quantified as described in Table 1. CCA, Corn oil + cellulose + azoxymethane (AOM) (n = 5); FPA, Fish oil + pectin + AOM (n = 5); CCS, Corn oil + cellulose + saline (n = 5); FPS, Fish oil + pectin + saline (n = 5); Lgr5\(^{\text{high}}\), stem cells. Only miRNAs with P ≤ 0.05 are shown.

### Table 5
Effect of diet and carcinogen combination on miRNA expression in GFP\(^{\text{negative}}\) sorted cells.

| miRNA     | Expression ratio CCA- Lgr5\(^{\text{neg}}\)/FPA- Lgr5\(^{\text{neg}}\) | P-value |
|-----------|---------------------------------------------------------------|---------|
| miR-29b   | 3.8                                                           | 0.008   |
| let-7e    | 2.1                                                           | 0.004   |
| Let-7c    | 1.3                                                           | 0.048   |
| miR-19b   | 0.6                                                           | 0.029   |
| miR-484   | 0.5                                                           | 0.019   |
| miR-19a   | 0.4                                                           | 0.034   |
| mmu-miRNA | Expression ratio CCA- Lgr5\(^{\text{neg}}\)/FPA- Lgr5\(^{\text{neg}}\) | P-value |
| miR-29b   | 3.8                                                           | 0.008   |
| let-7e    | 2.1                                                           | 0.004   |
| Let-7c    | 1.3                                                           | 0.048   |
| miR-19b   | 0.6                                                           | 0.029   |
| miR-484   | 0.5                                                           | 0.019   |
| miR-19a   | 0.4                                                           | 0.034   |

Expression of miRNAs was quantified as described in Table 1. CCA, Corn oil + cellulose + azoxymethane (AOM) (n = 5); FPA, Fish oil + pectin + AOM (n = 5); Lgr5\(^{\text{neg}}\), differentiated cells. Only miRNAs with P ≤ 0.05 are shown.

2.2. Fluorescence activated cell sorting of colonic stem cells

Colonic crypts from individual mice were isolated as previously described Sato et al. [1] with minor modification. The intact colons were everted on a disposable mouse gauge needle (Instech...
Laboratories) and incubated with 20 mM EDTA in PBS at 37 °C for 30 min. Following transfer to chilled Ca/Mg free HBSS, colons were vigorously vortexed to release crypts. The crypts were then incubated with 50 ul of DNase (stock concentration – 20 units/ml) in 10 ml of trypsin solution and single cells were then passed through a 40 micron cell strainer. Cells were counted and resuspended to a final cell density of 2 × 10^6 cells/mL. FACS (Fluorescence activated cell sorting) was then carried out to isolate the Lgr5<sup>high</sup> expressing stem cells, Lgr5<sup>low</sup> expressing daughter cells and Lgr5<sup>negative</sup> cells isolated from the colon using a BD FACS Aria II cytometer /sorter (BD Biosciences). Cells from wild type mice were used to set the gates for sorting.

2.3. RNA analyses

Total RNA from Lgr5<sup>high</sup>, Lgr5<sup>low</sup> and Lgr5<sup>negative</sup> sorted cells was isolated. For this purpose, cells from individual mice from all 4 groups (total of 60 samples) were separately processed using the mirVana miRNA Isolation Kit according to manufacturer’s instructions (Ambion, Austin, TX). Expression of 368 mature miRNAs was determined using TaqMan Rodent MicroRNA A Array 2.0 (Life Technologies, Grand Island, NY) as we have previously described [2,3]. For mRNA profiling, samples were randomized prior to RNASeq library preparation. Sequencing libraries from RNA (10 ng) were generated using the TruSeq RNA Sample Preparation kit (Illumina, San diego, CA). ERCC (Life Technologies, Grand Island, NY) was added at the appropriate level as per manufacturer instructions. The libraries were pooled and sequenced using an Illumina HiSeq 2500 at SeqWright Genomic Services (Houston, TX). Sequencing data was provided demultiplexed and aligned using STAR with default parameters [4] and referenced against Mus musculus (UCSC version mm10).

2.4. Statistical analyses

For miRNA and mRNA profiling, two sided t-tests with Welch correction for unequal variance were performed on select miRNA across the specific treatment comparisons of interest. Mann–Whitney U nonparametric tests were also performed as a control against non-normal data and similar P-values were obtained. Standard error bars were plotted in order to document the variation in the population mean. P values < 0.05 were considered to be statistically significant, and genes were selected for analysis using prior knowledge without considering P-values. Therefore, no multiple testing correction procedure was used. Standardized differences for the miRNAs and mRNAs were computed and a two-sample t-test was utilized to compare them. Small p-values indicated strong evidence of the hypothesized trend.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.12.026.

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