Data Article

Gene expression profile data of the developing small intestine of Id2-deficient mice

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A B S T R A C T

This article contains data related to the research article entitled “Id2 determines intestinal identity through repression of the foregut transcription factor, Irx5” [1]. Id2 deficient (Id2−/−) mice developed gastric tumors and heterotopic squamous epithelium in the small intestine. These tumors and heterotopic tissues were derived from ectopic gastric cells and squamous cells formed in the small intestine respectively during development. In this study, microarray data of the developing small intestine of Id2−/− mice was analyzed.

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1. Data

Microarray analysis was performed in the developing small intestine of Id2−/− mice. In total, 34 genes were differentially expressed in Id2−/− embryo compared with Id2+/+ embryo with criteria of fold change >2. Of these differentially expressed genes, 14 genes were upregulated and 20 genes were downregulated in Id2−/− embryo (Table 1) [1].

Furthermore, the expression levels of the selected 24 genes that are preferentially expressed in a specific embryonic gut segment, including foregut (eight genes), anterior-midgut (eight genes) and posterior-midgut (eight genes) were analyzed [16]. Heatmap visualization indicated that the expression of six of foregut-enriched genes were upregulated in Id2−/− embryo (Fig. 1) while the expressions of three of the midgut-enriched genes were remarkably downregulated in Id2−/− embryo. The remaining two foregut-enriched genes and 12 midgut enriched genes were not altered.

Sox21 is highly expressed in the anterior region over the period of foregut endoderm formation [3,16,17]. qRT-PCR analysis revealed that Sox21 expression increased only in the posterior part of the Id2−/− mice midgut (Fig. 2A). RT-PCR analysis clearly showed that heterotopic Sox21 expression was confined to the midgut of Id2−/− embryo, but not to the posterior part of midgut or hindgut (Fig. 2B).

2. Experimental design, materials and methods

2.1. Animals

Id2 mutant mice with 129/Sv genetic background were used for analysis [18]. Preparation of Id2+/+ and Id2−/− embryos was performed by crossing 8-week-old Id2+/+ male and Id2−/− female mice.

2.2. RNA extraction

Total RNA samples were extracted using an RNeasyMini Kit (QIAGEN, Valencia, CA, USA). Tissue lysate was purified by QIAshredder (QIAGEN) and treated with DNasel to remove genomic DNA. For
microarray analysis, total RNA from three midguts of the same genotype was taken as one sample. RNA quality was measured using the Agilent 2100 Bioanalyzer 2100 (Agilent Technology, Wilmington, DE, USA), and samples with 28S/18S ribosome ratio >2.0 were used for analysis.

2.3. Microarray

One microgram of total RNA was amplified and labeled with digoxigenin (DIG) for one round using a NanoAmp RT-IVT Labeling Kit (Applied Biosystems, Foster City, CA, USA). DIG labeled cRNA was fragmented and hybridized to Mouse Genome Survey Microarray ver.2.0 (Applied Biosystems) followed by chemiluminescence detection.

2.4. Data analysis

Raw signal values were normalized by the median. In all probe sets with false spots (flag < 5000) and signal-to-noise values < 3 (as determined by the software) were excluded. Normalized signal values were converted to log2 ratios. Fold changes between Id2⁻/⁻ and wild-type samples were calculated for each of the resulting probe sets. Heatmap visualization was constructed by Cluster 3.0 and Treeview software [19].

2.5. RT-PCR

Oligo(dT)-primed cDNA synthesis was performed using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using the Power SYBR green PCR master mix and a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Primer sequences for RT-PCR analysis are as follows: Sox21-forward, TACATGATCCCGTCACTG; and Sox21-reverse, TGCAGCCTGTCAAACAC. PCR primer sequences for qRT-PCR and Actb primers for RT-PCR analysis were described previously [1].

### Table 1
Differentially expressed genes in Id2 KO midgut.

| Gene       | Expression pattern in the developing digestive tract | Reference |
|------------|---------------------------------------------------|-----------|
| **Up-regulated genes** (KO/WT, fold change > 2) | | |
| Cym, Irx3, Irx5 | Specifically expressed in foregut endoderm | [2], [3] |
| Krt15, Foxa2, Adcy8 | Preferentially expressed in foregut endoderm | [4], [5], [6] |
| Traf6, Orfr1337, Cacng7, Wdr86, Ocr1, C030016D13Rik, Cdc96 | Oral endoderm and mesenchyme not annotated | [7] |
| **Down-regulated genes** (KO/WT, fold change < 0.5) | | |
| Sulp1, Spink3, Anxa13, Muc13, Lingo1, Bspry, Fabpl | Highly expressed in midgut endoderm | [8], [9], [10], [11], [12], [13], [14] |
| Cbhn2 | Preferentially expressed in midgut mesenchyme | [13] |
| Myl1, Slc27a2, Foxq1 | Highly expressed in the other region of midgut endoderm | [13], [15] |
| Them7, Kynu, Ppp1r1b, Mkrn2os, Hapln2, BC030870, 2610044015Rik, Ifi203, Faim3 | not annotated | |
Fig. 1. Heatmap of specific gene expressions in the midgut of Id2 wild-type (WT) and Id2 deficient (KO) mice embryos. The colored scale at the top of heatmap is log based. Genes are preferentially expressed in the specific gut segment. Foregut enriched genes, Anterior-Midgut enriched genes and Posterior-Midgut enriched genes were represented with different colors; cyan, orange and magenta respectively. Hierarchical clustering was performed with the complete-linkage method.
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Fig. 2. Sox21 expression in the developing gastrointestinal tract of Id2+/− embryo. (A) qRT-PCR analysis of Sox21 expression in E13.5 midguts. Midgut tissues were subdivided into anterior and posterior parts (n=7 per genotype). (B) RT-PCR analysis of Sox21 expression in E15.5 gastrointestinal tract. Midgut tissues were subdivided into three segments along the anterior-posterior axis. Ant - anterior segment of midgut; Mid - middle segment of midgut; Pos - posterior segment of midgut.
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