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Technical note

Identification of novel genetic markers for mouse yolk sac cells by using microarray analyses

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ABSTRACT

The mouse embryonic yolk sac consists of a visceral yolk sac (VYS) and parietal yolk sac (PYS), and may function as a materno-fetal exchange system for nutrients and wastes, and physical protector for the embryo/fetus. The present study was undertaken to characterize gene expression of the VYS and PYS endodermal cells, and to identify their novel genetic markers from microarray data. *Apoa4*, *Lrp2*, *Fxyd2*, *Slc34a3* and *Entpd2* were predominantly expressed in VYS epithelial cells. *Gkn2* and *Pga5* were selected as markers for PYS cells. These genetic markers may be useful for characterization of murine yolk sacs during development.

1. Introduction

The mouse yolk sac is composed of a visceral yolk sac (VYS) and parietal yolk sac (PYS), and their endodermal cells both originate from the primitive endoderm [1,2]. The VYS epithelial cells have important functions as a materno-fetal exchange system for nutrients, waste and gas, which is later assisted by the chorioallantoic placenta [3,4]. The PYS may be involved in not only nutrient-waste exchange but also protection of the embryo/fetus through Reichert's membrane [5]. However, it remains to be demonstrated in detail what functions VYS and PYS cells have and what genes they specifically express.

The present study was undertaken to compare gene expression of the VYS and PYS with that of the placenta, liver and small intestine, and to identify their novel genetic markers via microarray analyses. Because gene expression of the liver and small intestine, and the placenta may resemble that of the VYS and PYS, respectively [6–11], they were used as a selective reference to obtain novel markers of the yolk sacs in the microarray analyses.

2. Methods

2.1. Animals

C3H/HeSlc strain mice (Japan SLC) were mated overnight, and noon of the day of vaginal plug appearance was considered day 0.5 of gestation (E0.5). VYSs, PYSs, placentas, livers and small intestines at E11.5, E12.5 and E15.5 were used for microarray and gene expression analyses. All animal experiments were carried out in compliance with the “Guide for Care and Use of Laboratory Animals” of Shizuoka University.

2.2. Microarray analyses

Total RNA was extracted from the VYS, PYS (endodermal cells and Reichert's membrane), placenta, liver and small intestine at E12.5 with RNeasy Mini kit (Qiagen) in accordance with the manufacturer's instructions. Quality assessment of the total RNA was performed using an Agilent 2100 Bioanalyzer. Cy3-labeled cRNA was prepared from total RNA using a Low Input Quick Amp Labeling Kit (Agilent Technologies) according to the manufacturer's instructions. Labeled cRNA was hybridized to a SurePrint G3 Mouse GE 8 × 60K Microarray (Agilent Microarray Design ID 028005) according to the manufacturer's protocol. The array slide was scanned using an Agilent G2565CA Microarray Scanner System, and probe signals were quantified using Agilent's Feature Extraction Software version 9.5. Quantified probe signals were normalized

Abbreviations: PYS, parietal yolk sac; VYS, visceral yolk sac.

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and processed for differences among organ tissues by Subio platform version 1.16 with Basic Plug-in. The data are available in NCBI's GEO database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86820) under accession number GSE86820.

2.3. RT-PCR

Total RNA was extracted from VYS, PYS, placenta, liver and small intestine tissues at E11.5 and E15.5 using ISOGEN II (Nippon Gene Co.). cDNA was synthesized from the total RNA, and the PCR reaction was conducted according to Akai et al. [12]. The primers used were shown in Supplementary Table 1.

2.4. In situ hybridization

Sense and antisense digoxigenin-labeled riboprobes for mouse *Apoa4*, *Entpd2*, *Fxyd2*, *Gkn2*, *Lrp2*, *Pga5*, *Pi16*, *Scube2* and *Slc34a3* mRNAs (Supplementary Table 2) were directly prepared using a DIG RNA labeling kit (Roche Diagnostics) with T7 and Sp6 RNA polymerases from PCR fragments that were flanked by T7 and Sp6 promoters on each side. *In situ* hybridization of frozen sections of E11.5 embryonic and extraembryonic tissues was carried out according to Akai et al. [12].

3. Results and discussion

Heat map analyses of gene expression of the E12.5 VYS, PYS,

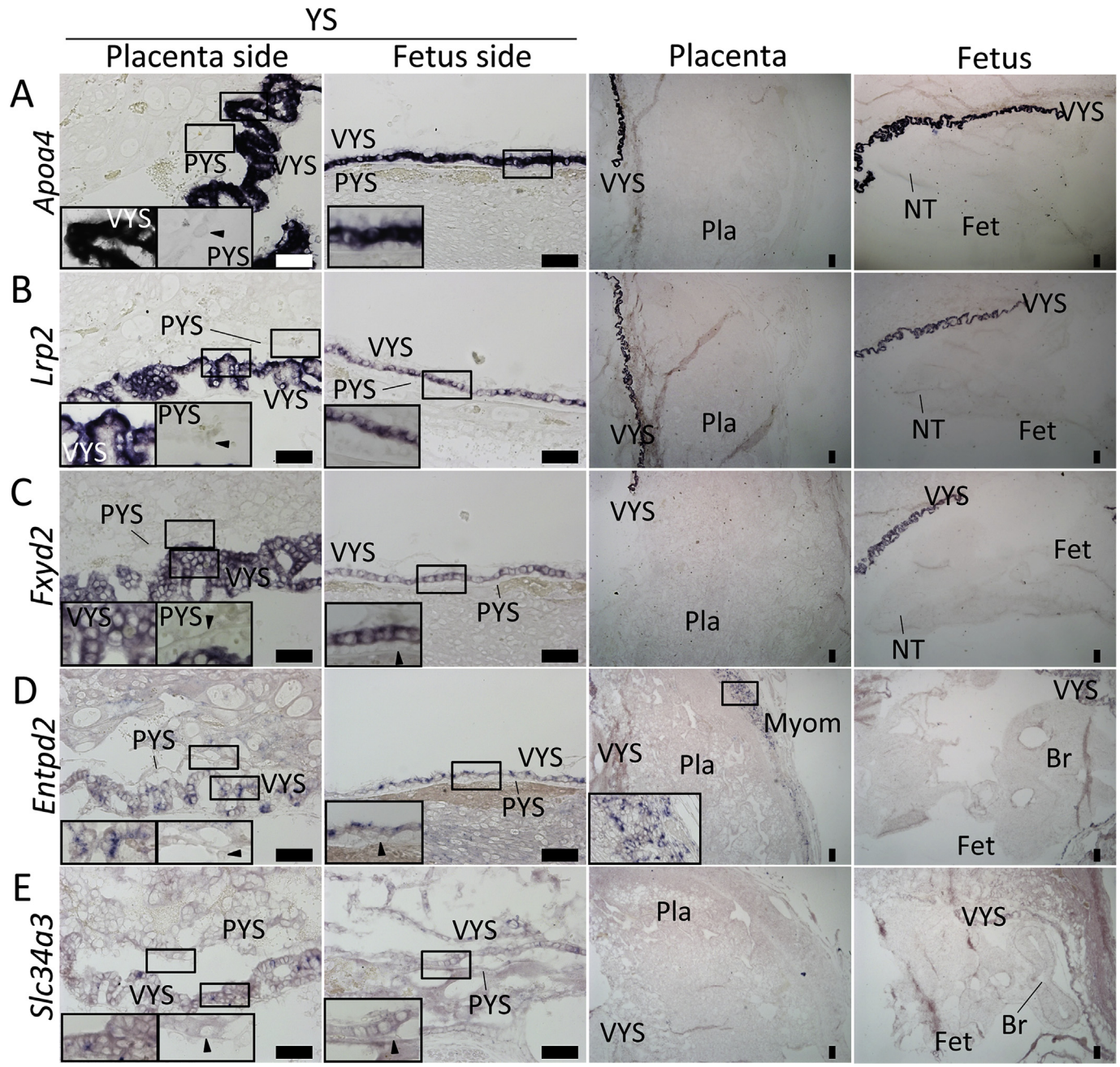

Fig. 1. *In situ* hybridization of genetic marker candidates for the VYS in E11.5 tissues. (A) *Apoa4*. (B) *Lrp2*. (C) *Fxyd2*. (D) *Entpd2*. (E) *Slc34a3*. *Apoa4*, *Lrp2*, *Fxyd2* and *Slc34a3* are expressed in VYS epithelial cells, but not in other tissues. *Entpd2* is expressed in VYS epithelial cells and myometrium cells of the uterus. Arrowheads indicate PYS cells. Br, brain; Fet, fetus; NT, neural tube; Myom, myometrium; Pla, placenta. Scale bar: 100 μm.