

Gene Expression Profiling in Porcine Fetal Thymus

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To obtain an initial overview of gene diversity and expression pattern in porcine thymus, 11,712 ESTs (Expressed Sequence Tags) from 100-day-old porcine thymus (FTY) were sequenced and 7,071 cleaned ESTs were used for gene expression analysis. Clustered by the PHRAP program, 959 contigs and 3,074 singlets were obtained. Blast search showed that 806 contigs and 1,669 singlets (totally 5,442 ESTs) had homologues in GenBank and 1,629 ESTs were novel. According to the Gene Ontology classification, 36.99% ESTs were cataloged into the gene expression group, indicating that although the functional gene (18.78% in defense group) of thymus is expressed in a certain degree, the 100-day-old porcine thymus still exists in a developmental stage. Comparative analysis showed that the gene expression pattern of the 100-day-old porcine thymus is similar to that of the human infant thymus.

Key words: porcine, fetal thymus, EST

Introduction

The ordered and timely expression of genes is equally important to the definition and to the biology of organs. Single-pass, partial sequencing of complementary DNA (cDNA) clones from one or both ends to generate expressed sequence tags (ESTs) provides a rapid method of gene discovery that has been widely applied to human and other species.

The thymus is a bilobed gland located in the anterior part of the superior mediastinum, posterior to the sternum and anterior to the great vessels and upper part of the heart. The gland reaches its greatest size just prior to birth. The thymus is the central lymphoid organ that provides a specialized microenvironment for the maturation and selection of the majority of T lymphocytes. Only 1%-5% of thymocytes in the thymus reach maturity, and most undergo programmed cell death (apoptosis).

Interdigitated cells and macrophages appear in the fetal thymus at Week 14 of gestation. At Week 17, the thymus is fully differentiated, producing viable lymphocytes (1). The unique characteristic of the 100-day-old fetal thymus is that it is at a developmental turning point from pre-T to pro-T cell. There-

fore, locating genes related with thymus development and T cell differentiation is worthy of investigation. To obtain an initial overview of gene diversity and expression pattern in porcine thymus, 11,712 ESTs from a 100-day-old porcine fetal thymus cDNA library, named FTY, were sequenced and analyzed.

Results

The insert size of the FTY library was 0.6-6 Kb. 11,712 clones were randomly picked and sequenced from the 3' end. After removing all sequences that were either contaminated or of low quality (Q20<100 bp), 7,071 cleaned EST sequences were available for further analysis. The average read-length of cleaned ESTs was 427 bp. The cleaned ESTs were assembled into 959 contigs and 3,074 singlets (clusters) by the PHRAP program. Blast search showed that 2,345 clusters (5,442 ESTs) had homologues in GenBank. Among them, 5,112 (72.30%) ESTs had functional homologues in NR, SwissProt or human, cattle and mouse Unigene databases, representing 2,179 unique genes. The other 1,629 (23.04%) ESTs did not show any significant homology to known sequences (Table S1).

All the clusters annotated in GenBank were classified into seven groups according to their func-

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tions: signaling and communication, gene expression, cell division, structure and motility, defense and homeostasis, metabolism, and unclassified (2). Regarding the frequency of ESTs, the gene expression group represented a significant proportion (36.99%) and showed high redundancy: 1,802 ESTs represented 393 unigenes. The protein degradation genes (184 EST, 28.66% in the metabolism group) and energy metabolism genes (162 ESTs, 25.23% in metabolism group) comprised a large percentage of the metabolism group (Table S2).

Comparison of the FTY gene expression pattern with that of the human infant thymus (3) showed that the general expression profile of these two tissues is very similar. The higher representation of the cell division and gene expression groups in FTY than in the human infant might be related to the earlier developmental stage of FTY.

Discussion

Thymus-related gene expression showed that there are 73 ESTs, of which 61 represent TCR (T cell receptor), 10 CD3, and 2 CD8, together accounting for 19.3% of the cell defense and homeostasis group. The cell membrane marker of mature T cell TCR, CD4 and CD8 is expressed to a lesser degree, indicating that the pro-T cell in the 100-day-old fetal thymus developed faster than matured T cell.

Cell elimination through apoptosis is an evolutionarily conserved central tenet of biology from embryological development to immune homeostasis. The proteasome and ubiquitin are the principal components of an energy (ATP)-dependent proteolytic system that plays a central role in the regulation of proteins controlling cell-cycle progression and apoptosis (4). In the past decade, many reports of polyubiquitin conjugation of key pro- and anti-apoptotic molecules have characterized ubiquitin as an essential regulatory modification targeting proteins for proteasomal degradation (5). In FTY, there are 50 ESTs (11.84% in the signal group) contained in the apoptosis signal pathway. The energy (ATP)-dependent proteolytic related gene (184 ESTs) expression represents a significant part (26.64%) of the metabolism group. This is significantly higher than the newborn porcine thymus. Twelve ESTs represent protease serine 16 (PRSS16) that is expressed by cortical epithelial cells of the thymus (6) and is noticeably more active than in the newborn porcine thymus. This indicates that

the apoptosis process in FTY is important. Thymosin is represented by 44 ESTs, accounting for 9.0% of the signal group and indicating that at 100 days the thymus has gained its differential function.

In order to investigate the fetal thymus gene expression pattern similarities between porcine and human, the expression profiles of porcine fetal thymus and human infant thymus were compared (Figure S1). The figure shows that the general expression patterns are very similar to each other. Most expressed genes are those involving in gene expression, cell division, and DNA synthesis. The expressions of defense-related genes are very similar to other organs (2). These findings suggest that although the thymus is an immune-related organ, the 100-day-old fetal thymus is still in the stage of rapid growth rather than immune function development.

Materials and Methods (abbreviated)

References

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Supporting Online Material

[http://www.gpbjournal.org/journal/pdf/GPB1\(2\)-10.htm](http://www.gpbjournal.org/journal/pdf/GPB1(2)-10.htm)

Table S1

Table S2

Figure S1