Expression Analyses Revealed Thymic Stromal Co-Transporter/Slc46A2 Is in Stem Cell Populations and Is a Putative Tumor Suppressor

Ki Yeon Kim¹, Gwanghee Lee³, Minsang Yoon¹, Eun Hye Cho¹, Chan-Sik Park³, and Moon Gyo Kim¹*

INTRODUCTION

Thymus produces educated T cells that can react with peptide antigen loaded on a self major histocompatibility complex (MHC) but not with self antigens. Developing thymocytes are guided and selected in the microenvironment of thymic stromal cells. Among the stromal cells, thymic epithelial cells (TECs) are major components that play important roles of thymocyte differentiation in the separate compartments, the cortex and the medulla.

TECs also play critical roles during thymic organogenesis as shown in Foxn1 mutant mice, in which early TEC differentiation is abrogated, functional thymus lacks and, therefore, no T cell is present (Nehls et al., 1996). In mice, initial thymic structure begins to form with the TEC precursor cells originated from the third pharyngeal pouch around fetal day 10.5 (Blackburn and Manley, 2004; Gill et al., 2003; Rodewald, 2008; Su et al., 2001). At this stage, fetal thymus does not show clear medullary compartmentalization yet although the cells of medullary thymic epithelial cells (mTECs) in nature are found (Roberts et al., 2012). Fetal thymus begins to express the cortex-specific markers such as CDR1 in addition to general epithelial markers, EpCAM and MHCII (Ahn et al., 2008; Boehm, 2008; Lee et al., 2012; Yang et al., 2005). Later, thymus undergoes atrophy by aging after puberty and/or by damaging insults such as radiation or stress hormones (Blackburn et al., 2002; Cheng et al., 2010; Gill et al., 2003). However, thymus can also be rejuvenated by removing steroid sex hormones or removing organs that produce sex hormones (Berzina et al., 2002; Lynch et al., 2009; Sutherland et al., 2005). The functional thymic epithelial stem cell (sTEC) in the adult or aged animals were identified (Blackburn et al., 2002; Rodewald et al., 2001; Swann and Boehm, 2007; Ucar et al., 2014; Wong et al., 2014). It is important to identify the molecular marker present in the sTEC to understand the mechanism of thymic regeneration and to translate into the clinic for the recovery of important cellular immunity.

There has been much evidence that cortical TEC (cTEC) and mTEC are derived from the single precursor TECs (pTEC) or sTEC (Bleuel et al., 2006; Rossi et al., 2006). While pTEC can be bipotent or specific lineage-committed (Park et al., 2013; Ucar et al., 2014), TEC development may be more progressive without instant commitment to a specific lineage (Alves et al., 2014). The original specific antibodies used for the identification of sTECs are MTS24 and MTS20 (Bennett et al., 2002; Gill et al., 2002). These TEC stem cells were located in the small...
medullary islets of very young thymus or cortico-medullary junction of the adult thymus (Rodewald et al., 2001). The cytokeratin K5 and K8 are also important molecules for the identification of stECs (Klug et al., 1998; 2002). It was also proposed that TEC stem cells reside in the MTS10+ cells in the medullary area.

It has been considered that Foxn1 might be the master key transcription factor controlling TEC differentiation (Chen et al., 2009; Cheng et al., 2010; Corbeaux et al., 2010; Manley and Condie, 2011; Nowell et al., 2011; Ucar et al., 2014). Some other transcription factors such as Pax1/9 and Hoxa3, and signaling molecules such as Shh, Wnt, Bmp and Fgf were also identified from the studies using the mouse lines with gene ablation (Hollander et al., 2006; Manley and Condie, 2010). Those molecules function from the stage of third pharyngeal pouch to the initial state of thymus formation. Foxn1 appears to be important for the survival and proliferation of committed TECs at the stage of thymic organ maintenance. Wnt4 is responsible for the expression of Foxn1 (Balcinuait et al., 2002).

However, the presence of sTEC without Foxn1 expression was recently shown by Bruno Keywisky’s group by using a new feature for stem cells to be able to form spheres in 3D culture (Ucar et al., 2014). Methods which can isolate stem cells based on their functionality will provide thrust for the studies on the initiation of thymic organogenesis at the molecular level and on the detailed processes on how it behaves. Our understanding of sTEC is still in a primitive state. One of the distinguished common features of stem cells, either from the specific organs or even from cancer cells, is called “side population” (SP) in flow cytometry (Golebiewska et al., 2011; Zhou et al., 2001). The cells in the side population emit both blue and red fluorescence from the DNA staining dye, Hoechst33342. This phenomenon is mediated by the ABC transporters and can be blocked by the inhibitor (Golebiewska et al., 2011; Zhou et al., 2001). Therefore, it is very useful to identify stem cells when no well-characterized stem cell marker is available.

TSCOT (Slc46a2/Ly110) is a gene encoding cTEC-specific membrane protein (Ahn et al., 2008; Chen et al., 2000; Kim et al., 2000; Yang et al., 2005), isolated from the cDNA library of SCID thymus and of fetal thymic stroma (Kim et al., 1998; Park, 1997). Its expression peaks at the early stage of thymic development and reduces when the thymus is more mature (Ahn et al., 2008; Kim et al., 2000; Lee et al., 2012; Yang et al., 2005). When LacZ reporter is inserted in the TSCOT locus, β-galactosidase expression was found in the whole thymus of new born but only in the cortex and corticomedullary junction of adults (Ahn et al., 2008). When hooked to the promoter fragments (9.1 kb), evolutionarily conserved sequences located in the upstream of the coding sequence, reporter EGFP expression copied the expression pattern of endogenous gene while a shorter promoter fragment (3.1 Kb) revealed unexpected expression in the medulla at the adult stage of transgenic mice (Chen et al., 2000; Lee et al., 2012). The Cre recombinase under the control of TSCOT promoters resulted in expression of EGFP and β-galactosidase in the bipotent pTEC by the deletion of loxP sequences harbored in the ROSA locus of the transgenic mouse lines (Park et al., 2013). Therefore, the unique restricted pattern of the TSCOT expression is of high value to study TEC differentiation and thymic organogenesis.

Expression of TSCOT has also been noticed in the male epididymal duct in conventional Northern blotting and immunohistochemistry (Obermann et al., 2003), and the TSCOT locus has been assigned in a susceptibility of cervical carcinoma by human genetic analyses (Engelmark et al., 2006; 2008). In the current era of bioinformatics, there has been many systemic data accumulating in the public database and available for analysis.

In this study, we took advantage of public database and bioinformatics tools and performed genetic profiling in addition to classical methodologies. We show TSCOT is expressed prior to bipotent pTEC, at the side population stage of thymic epithelial cells, and also even in differentiating ES cells. Its expression does not depend of Foxn1. TSCOT expression and its roles in other epithelial tissues like skin and lung are discussed.

MATERIALS AND METHODS

Expression profiling using public database

The data sets were obtained from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), and the extension changed as txt files to analyze in the GENESIS program (version 1.7.6) released by Graz University of Technology Institute for Genomics and Bioinformatics. Genes and the probes used are shown in Table 1. Multiple sample data are averaged before the final analysis. The normalized data of the genes were sorted by similarity to TSCOT genes or calculated by using Hierarchical Clustering to generate heatmaps. Some of the GEO data sets are drawn as graphs and calculated P values with a two-tailed T test in GraphPad Prism (version 6.0c).

Mice

The mouse lines TDlacZ (Ahn et al., 2008), 3.1T-EGFP (Chen et al., 2000), and 9.1T-NE (Lee et al., 2012) were maintained in the Laboratory of Molecular and Cellular Immunology Animal Facility of Inha University, Korea. All animal studies are in compliance with the Use of Laboratory Animals under the proper protocols. The protocols were approved by the Committees on the Ethics of Animal Experiments of NIH (LCMI Protocol 8) and Inha University (Protocol LMC1-2). Fetal mice were obtained from timed mating. The presence of a vaginal plug was considered at E0.5.

For genotyping, tail samples were extracted and used for a polymerase chain reaction with primers for the TDlacZ locus: Neo primer (ACCGCTATCAGGACATGGCGTCG), 1C12 F1 (TTACTCAAGTGAGCTGAAGCTG), 1C12 B2 (CGGAGGATTCCCTGTTGACATT), and the EGFP locus: EGFP-F (GCCACAAATGCAGGGTTCG), EGFP-R (GCTGCTGTTCGGTCTTGTG), using the red Extract-N-Amp Tissue PCR kit (Sigma).

Automatic cell counting

A fluorescence-based, automatic cell counter (Luna-FL, Logos Biosystems) was used to measure accurately the numbers of cells including thymic epithelial cells. The contamination from red blood cells could be automatically excluded because this system enumerates only nucleated cells.

Thymic stromal cell preparation

A single cell suspension was prepared as described (Lee et al., 2012). Briefly, thymic tissues or deoxyguanosine treated fetal thymic organ culture were treated with 0.25% trypsin (Invitrogen) for about 20 min, in the presence of DNase I (Sigma), and washed with phosphate buffered saline (PBS) containing 10% fetal bovine serum (FBS). For further purification of TEC, the single cell suspension was isolated using magnetic bead cell sorting after incubating with anti-FC mAb 2.4G2 and anti-mouse CD45 microbeads (Milteny Biotec) for 20 min at 4°C.
Table 1. List of Selected Gene Probe IDs used in the bioinformatics analyses

| GPL* | Gene symbol | Probe ID | GenBank access number |
|------|-------------|---------|----------------------|
| GPL570 | AIRE | 208090_s_at | NM_000658 |
| | BMP4 | 211518_s_at | D30751 |
| | CD248 | 219002_at | NM_020404 |
| | CLDN18 | 214135_at | BE551219 |
| | CLIC5 | 219866_at | NM_016929 |
| | CRIPT3 | 235720_at | A042209 |
| | CRTAC1 | 221204_s_at | NM_018058 |
| | CYP4B1 | 1555497_a_at | AY151049 |
| | EYA1 | 214608_s_at | AJ000098 |
| | FGFL7 | 205782_at | NM_002009 |
| | FGFL8 | 208449_s_at | NM_006119 |
| | FOXG1 | 206018_at | NM_005249 |
| | FOXN1 | 207683_at | NM_003593 |
| | GKN2 | 238222_at | AI821357 |
| | GRHL3 | 232116_at | AL317763 |
| | HOXA3 | 208604_s_at | NM_030661 |
| | HOXC13 | 219832_s_at | NM_017410 |
| | HSD17B6 | 205700_at | NM_003275 |
| | IL6 | 221165_s_at | D02052 |
| | ILF6 | 206939_at | NM_000880 |
| | IRF6 | 1552478_at | NM_153631.1 |
| | ISL1 | 206104_at | NM_02202 |
| | LIF | 205266_at | NM_02309 |
| | LRRK2 | 229584_at | AK029776 |
| | NOTCH3 | 203238_s_at | NM_00435 |
| | OSM | 230170_at | A075327 |
| | PAX1 | 1553492_s_at | NM_006192 |
| | PAX9 | 207059_at | NM_006194 |
| | PEBP4 | 227848_at | A218954 |
| | PLA2G1B | 206311_s_at | NM_000928 |
| | SFTPC | 215454_s_at | AI831055 |
| | SHH | 207587_at | NM_000193 |
| | SIX1 | 205813_at | NM_005982 |
| | SLC46A2 | 223816_at | AF242557 |
| | SOX2 | 220838_at | A069815 |
| | SUSD2 | 234310_s_at | AK024361 |
| | TBX2 | 207662_at | NM_005992 |
| | VEPH1 | 232122_s_at | AK022666 |
| | WNT4 | 208606_s_at | NM_030761 |
| | WNT5A | 205990_at | NM_003392 |
| | WNT5B | 221029_s_at | NM_030775 |

| GPL1261 | Gene symbol | Probe ID | GenBank access number |
|---------|-------------|---------|----------------------|
| | HOXA3 | 1452421_at | BB496114 |
| | HoxC13 | 1425874_at | AF193796 |
| | IL6 | 1450297_at | NM_031168 |
| | IL7 | 1422080_at | NM_008371 |
| | Irf6 | 1418301_at | NM_016851 |
| | Isl1 | 1422720_at | BQ176915 |
| | LIF | 1450160_at | AFO65917 |
| | Ly75 | 1449328_at | NM_013825 |
| | Meis1 | 1443260_at | BB055155 |
| | Notch1 | 1421864_at | NM_008716 |
| | Osm | 1438767_at | BB237825 |
| | Pax1 | 1449539_at | NM_008790 |
| | Pax9 | 1421246_at | BC005794 |
| | Pbx1 | 1449542_at | NM_00873 |
| | Par3 | 1453150_at | BG063941 |
| | Shh | 1436869_at | AV304616 |
| | Six1 | 1427277_at | BB137929 |
| | Six4 | 1456862_at | AI983638 |
| | SLC46A2 | 1423476_at | BB329435 |
| | Sox2 | 1416967_at | U31967 |
| | Tbx1 | 1425779_at | AF326960 |
| | Tert | 1450254_at | NM_009354 |
| | Wnt4 | 1450782_at | NM_009523 |
| | Wnt5a | 1436781_at | BB067079 |
| | Wnt5b | 1426002_at | NM_009523 |

| GPL2987 | Gene symbol | Probe ID | GenBank access number |
|---------|-------------|---------|----------------------|
| | FOXN1 | hCG31797.3 | NM_003593.2 |
| | HOXA3 | hCG1640627.4 | NM_153821.1 |
| | MEIS1 | hCG20991.2 | NM_009361.1 |
| | PAX9 | hCG20991.2 | NM_006194.1 |
| | SLC46A2 | hCG29190.4 | NM_033051.2 |

| GPL8217 | Gene symbol | Probe ID | GenBank access number |
|---------|-------------|---------|----------------------|
| | HOXA3 | HSG002011177 | (ROSETTAGENE MODEL_ID) |
| | MEIS1 | HSG00314123 | NM_002309 |
| | PAX9 | HSG00262240 | NM_030775 |
| | SLC46A2 | HSG00262263 | NM_033051 |

*GPL, GEO platform accession number

Flow cytometry

Monoclonal antibodies used in the staining of cells include anti-MHCII (I-Ab), anti-CD45 (Ly-5), and anti-Sca-1. The antibodies were purchased from Caltag or from BD PharMingen. Anti-aminopeptidase A (CDR-1) and anti-EpCAM (G8.8) were prepared in the Custom Antibody Services Facility, NIAID, NIH. Biotinylated UEA-1 was purchased from Vector Laboratories.

Cells were washed in cold FACS buffer (PBS + 1% BSA), subsequently stained on ice with the primary and the secondary antibodies, then analyzed on FACScalibur or FACSAnality with two lasers in the presence of 1-2 μg/ml of propidium iodide.

(continued)
Fig. 1. Tissue and cell type specific TSCOT expression profiling. (A) Clustering of TSCOT with three other genes, FOXN1, PAX9, HOXA3, expression in human fetal tissues (GSE7905). (B) Expression in human adult tissues (GSE14938). (C) Gene expression during TEC development. cTEC and mTEC from adult mice are analyzed (GSE 56928). mTEC (lo): CD80lo and MHC-IIlo, mTEC (hi): CD80Hi and MHC-IIHi. Gene expression profiles are from GEO microarray data. The gene expression values are normalized as 2.0 to -2.0 in the Genesis program. High, Red; Middle, Black; Low, Green. (D) Flow cytometric analysis of 4 weeks old thymic stromal cells for the lineage TEC makers. CDR1 is for cTEC, UEA-1 is for mTEC. The left panel shows CD45- gate of whole stromal cells and the right panel shows the TSCOT+ gate.

(PI). Anti-Fc, 2.4G2 antibody was included in all flow cytometry staining to block Fc receptor. For side population analysis, a 1 x 10^6 dissociated single cell suspension of fetal thymic organ culture (FD14.5) were incubated for an hour at 4°C in the presence of 5 μg/ml Hoechst 33342 dissolved in Hanks balanced salt solution. For verification of the side population, verapamil 0.25 mM was included. After washing at 4°C, cells were resuspended and examined by a flow cytometer equipped with a UV laser (FACSariaII). For multicolor staining with SP analysis, cells were prestained with selected antibodies including home-made mAb CLVE (Yang et al., 2005). Negative control of TSCOT staining was carried out with all the same combination of antibodies except mAb CLVE. Analyses were done using the FlowJo program (http://flowjo.com).

RT-PCR
Sorted 1000 cells were used for RNA preparation. cDNA was generated with Superscript III and RT-PCR was carried out with the primers for TSCOT: F84 (5-CAGTCTTCCAATAACCTGCTTTGGCCT-3) and B83 (5-CGATTCCATGTGCCCCATTG-3) to amplify a 310 bp fragment and for GAPDH (Ahn et al., 2008; Kim et al., 2000). The primers for TSCOT are located in the separate exons with one intron and RT control sampled did not show any band in the gel.

Histostaining and microscopy
The immunofluorescence and X-gal staining the sections is described (Lee et al., 2012). An isolated thymus was washed in PBS and fixed in 1% para-formaldehyde, 0.2% glutaraldehyde, 0.02% NP-40, 1 mM MgCl2 in PBS for 1 or 2 h on ice and was embedded in Tissue Freezing Medium (Triangle Biomedical Sciences, USA). The 4 μm sections were fixed for 2 min in 1% formaldehyde, 0.2% glutaraldehyde, 0.02% NP-40 1 mM NaCl, then incubated with X-gal solution (1 part X-gal 40 μg/ml in dimethyl formamide, in 40 parts 2 mM MgCl2, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide in PBS) at 37°C for 48 h. For the detection of EGFP for fetal thymus sections, confocal microscopy was performed on the frozen sections in NIAID confocal facility (Leica SP2).

RESULTS
Gene profiling analysis verifies the tissue-specific TSCOT expression
In order to study the expression pattern of TSCOT at the genome level, we first used Google to identify any data and downloaded from the public database (http://www.ncbi.nlm.nih.gov/geo/) that shows the differential expression pattern (GSE7905). From the GEO database, the fetal tissue-specific expression was first examined (Fig. 1A). TSCOT expression was found only in the human fetal thymus, not in the fetal brain, fetal liver, nor fetal kidney. FOXN1 expression showed a similar but not an identical pattern. PAX9 and HOXA3 that are previously associated with the third pharyngeal pouch formation are even more different from the TSCOT pattern. In human adult tissues (GSE14938), TSCOT was found in the thymus and skin. In addition, it was present in lung and epididymis at lower levels (Fig. 1B). FOXN1 expression is also strongly expressed in the skin and thymus. However, it is not strongly expressed in any of the tissues that TSCOT is expressed at in the lower levels. Instead, FOXN1 is expressed in the liver, stomach and placenta. These suggest that TSCOT and FOXN1 may not be strongly associated in differentiated adult tissues.

Expression profiles of TSCOT and selected genes are investigated for the expression in the isolated mouse thymic epithelial cells (GSE56928). The genes for profiling (Table 2) are selected based on the literature which contains the information on the expression of the genes in the thymic epithelium or third pharyngeal pouch (references in Table 2). As shown in Fig. 1C, expression patterns are clustered as six different groups. A
Table 2. List of genes used in expression profiling during organogenesis

| Gene* name | Full name | Function | Reference | TSCOT expression from GEO data |
|------------|-----------|----------|-----------|-------------------------------|
| AIRE       | Autoimmune regulator | Regulate mTEC development and differentiation, Transcription factor | Gordon and Manley, 2011; Sun et al., 2013 | |
| BCL2       | Growth Arrest-Specific 1 | Antiapoptotic gene | Wong et al., 2014 | |
| BMP4       | Bone morphogenetic protein 4 | Essential for thymus and parathyroid morphogenesis prior to Foxn1 | Gordon and Manley, 2011 | |
| CD248      | CD248 Molecule, Endosialin | Required for postnatal thymic growth and regeneration following infection-dependent thymic atrophy | Liu et al., 2014 | |
| CRIP3 (TLP) | Cystein-Rich Protein 3 (Thymus Lim Protein) | Appears to have a role in normal thymus development | Kirchner et al., 2001 | |
| DAB2       | Mitogen-Responsive Phosphoprotein, Homolog | Wnt-inhibitors, Control proliferation and differentiation of stem cells into lineage-restricted cells | Wong et al., 2014 | |
| DKK3       | Dickkopf WNT Signaling Pathway Inhibitor 3 | Wnt-inhibitors, Control proliferation and differentiation of stem cells into lineage-restricted cells | Wong et al., 2014 | |
| EYA1       | Eyes absent 1 homolog | Necessary for 3rd pouch development | Wei and Condie, 2011; Gordon and Manley, 2011 | |
| FGF7 (KGF) | Keratinocyte growth factor | Induces mature and immature TECs and promotes differentiation of immature TECs | Rossi et al., 2006 | |
| FGF8       | Fibroblast growth factor 8 | Indirectly influence TECs by regulating neural crest cells survival and differentiation, relate to early pouch formation | Gordon and Manley, 2011; Sun et al., 2013 | |
| FOXG1      | Forkhead Box G1 | May play a role in the regulation of TEC differentiation during fetal and postnatal stages, Transcription factor | Wei and Condie, 2011 | |
| FOXN1      | Forkhead Box N1 | Necessary for the development of immature TEC progenitor cells into cTECs and mTECs, Transcription factor | Blackburn et al., 1996; Bennett et al., 2002; Gordon and Manley, 2011; Bredenkamp et al., 2014 | |
| GAS1       | Growth Arrest-Specific 1 | Cell-cycle suppressor gene | Wong et al., 2014 | Reduced TSCOT level in Get-1 KO skin (GDS2629/GSE7381) (P: 0.0042**) |
| GRHL3 (Get-1) | Grainyhead-Like 3 | Ancient mediator of epithelial integrity, Transcription factor | Yu et al., 2008; de la Garza et al., 2012 | |
| HOXA3      | Homeobox A3 | Early pouch patterning and initial organ formation, Transcription factor | Manley and Capocchi, 1995; Su et al., 2001; Gordon and Manley, 2011 | |
| HOXC13     | Homeobox C13 | Mediates transcriptional regulation of Foxn1, Transcription factor | Potter et al., 2010 | |
| IL22       | Interleukin 22 | Leads to regeneration of supporting epithelial microenvironment for enhanced thymopoiesis after thymic injury | Dudakov et al., 2012 | Reduced TSCOT level of IL22 treated epidermal keratinocytes (GDS2611/ GSE7216) (p < 0.0001****) |
| IL6        | Interleukin 6 | Associated with thymic involution | Chinn et al., 2012 | |
| IL7        | Interleukin 7 | Cofactor for V(D)J rearrangement of the T cell receptor beta during early T cell development | Huang and Muegge, 2001; Zamisch et al., 2005 | |

(continued)
| Gene name | Full name | Function | Reference | TSCOT expression from GEO data |
|-----------|-----------|----------|-----------|-------------------------------|
| IRF6      | Interferon regulatory factor 6 | Key determinant of keratinocyte proliferation-differentiation switch, Transcription factor | Richardson et al., 2006 | Reduced TSCOT level in IRF6 KO skin (GDS2359/GSE5800) (P< 0.0001****) |
| ISL1      | ISL LIM Homeobox 1 | May play a role in the regulation of TEC differentiation during fetal and postnatal stages, Transcription factor | Wei and Condie, 2011 |  |
| LIF       | Leukemia inhibitory factor | Maintenance mouse ES cell pluripotency, Associated with thymic involution | Shen and Leder, 1992; Graf et al., 2011; Chinn et al., 2012 | Increased TSCOT level in murine CGR8 ES cells treated LIF (GDS3729/ GSE6689) (P: 0.1181) |
| LY75      | Lymphocyte antigen 75 | Contribute to antigen presentation, Marker of cTEC in adult thymus | Jiang et al., 1995; Shakib et al., 2009 |  |
| MEIS1     | Myeloid ecotropic viral integration site 1 | Functional and physical partners of Pbx1 and Hoxa3, Required for maintenance of the postnatal thymic microenvironment, Transcription factor | Hirayama et al., 2014 |  |
| NOTCH3    | Notch homolog protein 3 | Regulate murine T cell differentiation and leukemogenesis | Bellavia et al., 2008 |  |
| OSM       | Oncostatin M | Plays an inhibitory role in normal and malignant mammary epithelial cell growth in vitro, Associated with thymic involution | Liu et al., 1998; Chinn et al., 2012 |  |
| PAX1      | Paired Box 1 | Early pouch formation and parathyroid development, minor role in thymus size, Transcription factor | Wallin et al., 1996; Gordon and Manley, 2011 |  |
| PAX9      | Paired Box 9 | Pouch and initial organ formation, TEC differentiation, Transcription factor | Hetzer-Egger et al., 2002; Gordon and Manley, 2011 |  |
| PBX1      | Pre-B-cell leukemia homeobox | Required for embryonic thymic organogenesis, Transcription factor | Hirayama et al., 2014 |  |
| PSMB11    | Proteasome subunit, beta type, 11 | Positive selection of CD8+ T cells, cTEC specific proteasome subunit | Murata et al., 2007; Shakib et al., 2009 |  |
| SHH       | Sonic hedgehog | Regulate pharyngeal region development | Moore-Scott and Manley, 2005; Gordon and Manley, 2011 | Increased TSCOT level in SHH treated human fibroblasts (GDS4512/ GSE29316) (P: 0.1122) |
| SIX1/4    | Sine oculis-related homeobox 1/4 | Necessary for 3rd pouch development, Transcription factor | Wei and Condie, 2011; Gordon and Manley, 2011 |  |
| SOX2      | SRY (sex determining region Y)-box 2 | Regulate self-renewal of the mouse and human ESCs, important for the maintenance of stem cells in multiple adult tissue, establish induced pluripotent stem cells, Transcription factor | Cimpean et al., 2011; Liu et al., 2013 | Higher TSCOT level in SOX2+ follicle dermal cells (GDS3753/ GSE18690) (P: 0.0015**) |
| TBX1      | T-box transcription factor | Pouch formation and patterning, might establish parathyroid fate, Transcription factor | Jerome and Papaioannou, 2001; Hollander et al., 2006; Gordon and Manley, 2011 |  |

(continued)
group contains TSCOT, Wnt4, Meis1, Gas1, Pax1, Isl1, Pax9, Six1, Pbx1, IL7, Bcl2, Bmp4, Dab2, Foxg1, Sox4, and Hoxa3. These genes are expressed in both cTEC and mTEC. Among them, TSCOT, Wnt4, Meis1, and Pax1 showed the strongest expression in the cTEC of the youngest mouse. Our earlier study on the expression kinetics (Kim et al., 2000) is consistent with these results. In this group, Pax1, Pax 9, Six1, Meis1 and Hoxa3 are the genes involved in the pouch stages (Manley et al., 2004). Wnt4 and Bmp4 were shown to be involved in the thymic organogenesis at the upstream of Foxn1 (Bleul and Boehm, 2005). It is interesting to note that Dab2 is a Wnt inhibitor. Gas1, Bcl2 and IL7 are the genes involved in the general cell cycle and survival. Group B contains Psmb11 (iµST) and Ly75 (NLDC202/DEC205) that are genuine cTEC-specific genes. Group C (Wnt5a, Wnt5b, Eya1, Fgf8, Notch3 and Dkk3) includes genes that are expressed higher in the later stages of cTEC and mTEC. Eya1 is known for roles in the third pharyngeal pouch (Gordon and Manley, 2011; Wei and Condle, 2011). However, its expression profile is somewhat different from the other genes involved in the same stage. Next, group D contains Foxn1 and Crip3 (TLP) that show the expression in cTEC and mTEC. Here again, it clearly shows a deviation of expression pattern between TSCOT and Foxn1. Group E genes (Lif, Tert, and Fgf7) show the highest expression in the mTEC or mTEC. Given the known functions of Tert high expression in less divided cells, this result suggests that mTEC may be found in more immature cells. The last group, group F contains Hoxc13, Osm, Il6, Cd248 (Endosialin), Tbx1, Aire, Shh, Sox2, and Ghrl3. Those genes show the highest expression in the mTEC population. Hoxc13 regulates Foxn1 expression, and three genes, Osm, Il6, and Cd248 are involved in thymic atrophy and involution. The roles of Tbx1 and Shh in mTEC are not completely understood yet even if they are known for involvement in pouch formation.

The expression of TSCOT in mTEC is not so surprising since it was found in the corticomedullary junction of young adult thymus where precursor or stem cells for thymic epithelial cells resides. When 4 week old thymic stromal cells were investigated by flow cytometry, the CD45 population contains transitory cells with both cortical and medullary markers (CDR1+/ UEA-1). Those cells are included in the TSCOT+ gated cells beside CDR1+/UEA-1 cTECs (Fig. 1D).

From these analyses, it was concluded that TSCOT is expressed in cTEC and undifferentiated and/or precursor mTEC. These expression profiles are common among the genes involved in early thymic organogenesis.

TSCOT is expressed in all TEC-committed stromal cells in fetal thymus

Next, we investigated the expression of TSCOT and reporters at the fetal stages in the different mouse models that we have previously characterized for the postnatal stages. The β-galactosidase reporter expression in TDLacZ thymus is restricted in the thymus as two dots at FD11 (Ahn et al., 2008). Figure 2A shows the β-galactosidase expression in the thymic sections at FD14.5. Expression of β-galactosidase is evenly distributed in the whole thymus, indicating most, if not all, the thymic epithelial cells at this stage express β-galactosidase. Another reporter mouse line, 3.1T-EGFP, which expresses EGFP in all TECs at the newborn stage (Park et al., 2013), showed EGFP expression earlier during fetal stages (Fig. 2B). At FD14 and 17, EGFP expression is also evenly distributed in the whole thymus. These results are consistent with the conclusion we previously described in which TSCOT is expressed in the pTEC stage (Park et al., 2013).

Fetal thymic stromal cells from normal C57BL/6 mouse (FD14) were analyzed for TSCOT expression with specific mAb CLVE (Yang et al., 2005). At this stage, EpCAM+ cells were all TSCOT+ (data not shown). When CD45 stromal cells were displayed for CD31 as an endothelial lineage marker along with MHCI, it became clear that TSCOT expression is present in all MHCI+ cells and CD31+ MHCI+ cells (Fig. 2C). Only CD31+MHCI cells of endothelial lineage were TSCOT+. From these results, it is concluded that endothelial cells either lost TSCOT expression due to lineage commitment from the common stem cell or originated from other type of precursor cells that do not express TSCOT.

TSCOT is expressed in the side population of TEC preparation

By using the TEC preparation from the deoxyguanosine treated FTOC of FD14.5, the presence of SP was tested with Hoechst 33342. In Fig. 3A, SP, which is ABC transporter sensitive, is clearly visible. When the inhibitor Verapamil was included, SP had decreased to 0.21% from 1.45%. Side population analyses were also applied with the TEC preparation using the same type culture of fetal thymus from 9.1T-NE mouse that shows

---

**Table 2. List of genes used in expression profiling during organogenesis**

| Gene* | Full name | Function | Reference | TSCOT expression from GEO data |
|-------|-----------|----------|-----------|------------------------------|
| TERT  | Telomerase Reverse Transcrptase | Telomerase reverse transcriptase | Wong et al., 2014 |
| WNT4  | Wingless-type MMTV integration site family, member 4 | Controls thymopoiesis and thymus size by regulating TEC, thymocyte and their progenitor proliferation, regulate Foxn1 expression in TECs | Sun et al., 2013 |
| WNT5A | Wingless-type MMTV integration site family, 5A | Regulate the survival of αβ lineage thymocytes, regulator of cell growth in hematopoietic tissue | Liang et al., 2007 |
| WNT5B | Wingless-type MMTV integration site family, 5B | Produced by TECs and thymocytes, regulate Foxn1 expression in TECs | Gordon and Manley, 2011; Sun et al., 2013 |

*Gene names are listed in alphabetical order.
EGFP expression patterns in the same way as endogenous TSCOT (Lee et al., 2012). As shown in Fig. 3B, a portion of the SP of 9.1T-NE TECs expresses EGFP when compared with that of normal C57BL/6 TEC preparation. In contrast, the side population of 3.1T-EGFP TEC population did not show any EGFP expression (data not shown).

When SP analysis was carried out along with antibody staining, TSCOT expression in SP and the major population (MP) were also clear. SP, either MHCII negative or positive, showed specific TSCOT expression with mAb CLVE. In addition, 84% of the SP cells and 95% of the MP cells were TSCOT+ (Fig. 3C). The next experiment was to verify TSCOT expression at the RNA level using a sorted side population of TECs prepared from normal C57BL/6. RT-PCR using the RNA prepared from the sorted cells clearly showed TSCOT expression in SP and in MP (Fig. 3D).

Many different types of stem cells express Sca-1 marker and a recent report mentioned that a TEC progenitor population is Sca-1+ (Golebiewska et al., 2011). We also tested expression of Sca-1 in fetal TEC preparation (Fig. 3E). Sca-1+ populations are present in both EpCAM+/MHCII+ and EpCAM+/MHCII- populations and most of them are TSCOT+. From these results, fetal TEC contains significant portion of sTECs that are TSCOT+.

**TSCOT is expressed in differentiating embryonic stem cells**

We searched the available data on TSCOT expression in the embryonic stem cell (ES) population. Two GEO sets of data (GSE14503 and GSE9440) contain an expression profile of T3 ES cell culture, embryonic body formation, and differentiating T3 ES cell into pancreatic islet-like cell clusters or fibroblasts (Fig. 4A). Expression of TSCOT is found in embryonic bodies but not in the undifferentiated ES nor in differentiated pancreatic islets and fibroblasts. TSCOT expression is clustered with
Because FOXN1 was considered as a putative TEC key transcript factor, we searched for TSCOT expression in the remaining thymic rudiment of nude mouse. In RNA prepared from several tissues, TSCOT expression was found in the thymic rudiment of nude mice (Fig. 5A). RNA samples that were not treated with reverse transcriptase did not generate any bands (data not shown). This result is consistent with the conclusion derived from the gene profiling analyses.

In order to find the putative regulatory factors, differential TSCOT expressions were also examined in the skins of mouse lines with various mutations (Figs. 5B and 5C). In IRF6 KO mouse (GSE5800), TSCOT expression had reduced along with, Foxn1, Fgfl, Tbx1, and Hoxa3 while Notch3, Foxn1, Grhl3, and Wnt4 had increased. The opposite expression profiles of TSCOT and Foxn1 in IRF6 KO skin suggest that these genes are independently regulated (Fig. 5B). It is interesting to note that the binding sites of IRF6 are located in the regulatory regions of Grhl3 (Botti et al., 2011; de la Garza et al., 2012). More interestingly, TSCOT expression is also reduced in the skin of GRHL3 KO mice (GSE7381) (Fig. 5C). To investigate the actual involvement of those transcription factors will require more investigation. The effects of various cytokines for the TSCOT expression in the human epidermal keratinocytes (GSE7216) can also be visualized in Figure 5D. TSCOT expression is down regulated by IL1b, IL22 and IL24, but not by KGF, IFNγ, IL19, IL20 and IL26d (Fig. 5D) in the keratinocytes.

These results suggest a regulatory mechanism for TSCOT expression by the transcription factors, such as IRF6 and GRHL3, and by cytokines, but not by FOXN1.

**Is TSCOT a tumor suppressor?**

We also researched TSCOT expression in the lung development. Human fetal lungs at various stages between 54-154 days (GSE14334) are clustered in Fig. 6A. The genes that are expressed in a similar way to those of TSCOT are GRHL3, FOXN1, PAX9, and CRIPT. Those genes are known to be upregulated during the fetal lung developmental process (Kho et al., 2010). Therefore, it is likely that those transcription factors are involved in the positive regulator of TSCOT in the lung. The general patterns of IRF6, SHH, ISL1, and SOX2 are downregulated during lung development, the opposite pattern to that of TSCOT. This suggests that those genes are potentially involved in the negative regulation of TSCOT in lung development.

In Fig. 6B, the cluster analysis of 20 genes coexpressed in the same fashion as TSCOT is shown in Table 3. To our surprise, TSCOT expression is clearly missing in three types of lung cancers, suggesting TSCOT may function as a tumor suppressor for lung cancer. The top genes clustered for the similar expression profiles are listed in Table III. As shown, many of the genes show possible tumor suppressor phenotypes (references in Table 3).

**DISCUSSION**

Using bioinformatics approaches and conventional molecular and cellular methods, we showed that TSCOT expression is turned on in TEC at the stem cell stage, and even prior to commitment of TEC lineages. In addition, we identified putative regulatory transcription factors and cytokines during thymus, skin and lung development.

**TSCOT expression is turned on early thymic organogenesis and some other epithelial tissues**

During last several years, gene expression profiling at the gene...
Table 3. List of genes down-regulated along with TSCOT during lung cancer development

| Gene name* | Full name | Relation with cancer | Reference |
|------------|-----------|----------------------|-----------|
| CLDN18    | Claudin-18| Ectopic activation in pancreatic, esophageal, ovarian, and lung tumors | Sahin et al., 2008 |
| CRTAC1    | Cartilage acidic protein 1 | Copy number alteration in CRTAC1 gene have been observed in neurofibromatosis Type 1-associated glomus tumors | Brems et al., 2009 |
| CYP4B1    | Cytochrome P450, Family 4, Subfamily B, Polypeptide 1 | High expression of CYP4B1 increases the risk of bladder cancer by activation of carcinogenic aromatic amines | Imaoka et al., 2000 |
| GKN2      | Gastrokine-2 | Gastrointestinal tract specific gene GKN2 might inhibit gastric cancer growth in a TFF1 dependent manner | Chu et al., 2012 |
| LRRK2     | Leucine-rich repeat serine | LRRK2 G2019S mutations are associated with an increased cancer risk in Parkinson’s disease | Saunders-Pullman et al., 2010 |
| SUSD2     | Sushi domain-containing protein 2 | SUSD2 increases the invasion of breast cancer cells and contributes to a potential immune evasion | Watson et al., 2013 |

*Gene names are listed in alphabetical order

Fig. 5. Tissue specific expression of TSCOT reveals Foxn1 independency. (A) RT-PCR analysis of adult nude tissue. (B) Gene expression profiles from embryonic day 17.5 IRF6 KO and wild type mouse skin (GSE5800). Right panel: Comparison of TSCOT expression from skin between IRF6 KO mice and wild-type (P value < 0.0001****). (C) Gene expression profiles from embryonic day 18 GRHL3 KO and wild type mouse skin (GSE7381). Right panel: Comparison of TSCOT expression from skin between GRHL3 KO mice and wild-type (P value: 0.0042**). (D) TSCOT expression changes in human epidermal keratinocytes after treatment of KGF and various cytokines (GSE7216). Significant changes are compared to untreated cells (P < 0.0001****, P: 0.0025**, P: 0.0217*). Y axis are arbitrary units of process data.
profiling (Figs. 1, 4, and 5). Furthermore, we learned that more tissues such as skin and lung also express TSCOT. The transcription factors involved in the thymic organogenesis may be also involved in skin and lung development (Figs. 1A-1C, 5B, 5C, and 6A). In addition, kinetic profiling of expression of TSCOT during cTEC lineage development has also been verified (Fig. 1C). TSCOT expression is highest in the youngest cTEC as we described earlier (Kim et al., 2000). TSCOT expression in mTEClo provides an interpretation that transitional cells found in the postnatal TEC preparation with UEA-1+CDR1lo cells (Fig. 1D) are most likely the same kind. Earlier findings of sTECs in the medullary islet (Rodewald et al., 2001) and in the cortical medullary junction (Ahn et al., 2008) are consistent with the idea that TEC stem cells may overlap or share early mTEC features.

To further investigate cells at earlier stages than pTEC expression, we first utilized a functional SP analysis and showed that SP of fetal TEC preparation expresses TSCOT (Fig. 3). We like to call those cells sTEC for SP and stem TECs. In the SP of other type of tissues, such as mouse mammary glands, SP showed a slightly higher TSCOT expression than non SP (Fig. 4C). Our search for TSCOT expression in ES cells produced interesting results that TSCOT is induced in the differentiating embryonic bodies or ES cell cultures without LIF. TSCOT expression is off when the cells are differentiated into pancreatic epithelium or fibroblasts (Fig. 4A), and in the endothelial lineage (Fig. 2C). These results support the idea that TSCOT is expressed at the stem cell stage during certain organogenesis.

Given the concept that TSCOT is expressed in sTEC, its expressions in the skin and lung are not very surprising. They all express epithelial markers such as EpCAM and Keratins. There are cases that these cell lineages are actually interconvertible under certain circumstances. The stem cell preparation from TEC can be differentiated into skin type keratinocyte (Bonfanti et al., 2010) and thymic epithelium of nude mouse has shown to have lung epithelial morphologies (Dooley et al., 2005). This phenomenon can be interpreted as that of a reprogramming of gene expression, transforming stem cells which are committed to one organ type, into another at the level of master gene expression.

A schematic model in Figure 7 summarizes the findings of the expression profile during organogenesis. TSCOT expressions are indicated at the bottom of each text box. ES, embryonic stem cells; ESdiff, differentiating ES cells.
pression is downregulated (Fig. 6A). These phenomena suggest the complex network of expression regulation and/or cross checking regulation of genes during different epithelial tissue development.

**TSCOT may be a new member of the tumor suppressors**

Besides the structural features of a transporter that appeared containing primary amino acid sequences (Kim et al., 2000), it is still unclear what biological and biochemical functions that TSCOT plays. TSCOT is a member of Slc46A and another member, Slc46A1, has been characterized as a proton coupled folate transporter (Diop-Bove et al., 2013). The heavily hydrophobic nature of the TSCOT amino acid composition and a simple twelve membrane spanning feature, with the presence of a central inner loop in the absence of ATP binding domain, suggests that TSCOT may transport small hydrophobic molecules. We proposed earlier that it may function in the survival of TECs based on the expression (Kim et al., 2000).

It is exciting to find that TSCOT expression in the lung disappears in three types of lung cancers, large lung cell carcinoma, lung adenocarcinoma, and squamous lung carcinoma (Fig. 6B). This expression strongly implies TSCOT may function as a type of tumor suppressor. This supports the fact that TSCOT also function in the same way for the genetic type of cervical cancer susceptibility proposed (Engelmark et al., 2006; 2008). In fact, the Human TSCOT locus (9q32) was mapped to the susceptibility of cervical cancer through a SNP polymorphism study (Engel mark et al., 2006; 2008). It may function as a necessary component to maintain normal epithelium. When it is missing in lung epithelium, carcinogenesis progresses without hindrance. Other genes expressed in a similar fashion (Fig. 6) also show the functionality in tumor suppressors as described in Table III.

**ACKNOWLEDGMENTS**

We would like to thank Dr. Polly Matzinger for the help on finding nude thymic rudiments and also to Minchull Kim and Soo Jung Yang for technical help. This work was supported by the National Research Foundation of Korea (NRF) (a grant funded by the Korean government (MSIP) (NRF-2013R1A2A2A01069080), National Research Foundation of Korea (NRF), a grant funded by the Korean government (MSIP) (NRF-2013R1A2A2A01069080), and Inha University Research Grant.

**REFERENCES**

Ahn, S., Lee, G., Yang, S.J., Lee, D., Lee, S., Shin, H.S., Kim, M.C., Lee, K.N., Palmer, D.C., Theoret, M.R., et al. (2008). TSCOT-thymic epithelial cell-mediated sensitive CD4 tolerance by direct presentation. PLoS Biol. 6, e191.

Alves, N.L., Takahama, Y., Ohigashi, I., Ribeiro, A.R., Baik, S., Anderson, G., and Jenkinson, W.E. (2014). Serial progression of cortical and medullary thymic epithelial microenvironments. Eur. J. Immunol. 44, 16-22.

Balcunaita, G., Keller, M.P., Balcunaita, E., Piali, L., Zulys, S., Mathieu, Y.D., Gill, J., Boyd, R., Sussman, D.J., and Hollander, G.A. (2002). Wnt glycoproteins regulate the expression of FoxN1, the gene defective in nude mice. Nat. Immunol. 3, 1102-1108.

Bellavia, D., Checuolo, S., Campese, A.F., Felli, M.P., Gulino, A., and Screpanti, I. (2008). Notch3 from subtle structural differences to functional diversity. Oncogene 27, 5092-5098.

Bennett, A.R., Farley, A., Blair, N.F., Gordon, J., Sharp, L., and Blackburn, C.C. (2002). Identification and characterization of thymic epithelial progenitor cells. Immunity 16, 803-814.

Berzins, S.P., Udrich, A.P., Sutherland, J.S., and Gill, J. (2002). Thymic regeneration: teaching an old immune system new tricks. Trends Mol. Med. 8, 469-476.

Blackburn, C.C., and Manley, N.R. (2004). Developing a new paradigm for thymus organogenesis. Nat. Rev. Immunol. 4, 278-289.

Blackburn, C.C., Augustine, C.L., Li, R., Harvey, R.P., Malin, M.A., Boyd, R.L., Miller, J.F., and Morahan, G. (1996). The nu gene acts cell-autonomously and is required for differentiation of thymic epithelial progenitors. Proc. Natl. Acad. Sci. USA 93, 5740-5746.

Brems, H., Park, C., Meliones, O., Pernov, A., Messiaen, L., Upadhya, M., Claes, K., Beert, E., Peeters, K., Mautner, V. (2009). Glomus tumors in neurofibromatosis type 1: genetic, functional, and clinical evidence of a novel association. Cancer Res. 69, 7393-7401.

Chen, C., Kim, M.G., Soo Lyu, M., Kozak, C.A., Schwartz, R.H., and Flomrell, F.A. (2000). Characterization of the mouse gene, human promoter and human cDNA of TSCOT reveals strong interspecies homology. Biochim. Biophys. Acta 1493, 159-169.

Chen, L., Xiao, S., and Manley, N.R. (2009). Foxn1 is required to maintain the postnatal thymic microenvironment in a dosage-sensitive manner. Blood 113, 567-574.

Cheng, L., Guo, J., Sun, L., Fu, J., Bames, P.F., Metzger, D., Chambon, P., Oshima, R.G., Amagai, T., and Su, D.M. (2010). Postnatal tissue-specific disruption of transcription factor Foxn1 triggers acute thymic atrophy. J. Biol. Chem. 285, 5836-5847.

Chinn, I.K., Blackburn, C.C., Manley, N.R., and Sempowski, G.D. (2012). Changes in primary lymphoid organs with aging. Semin. Immunol. 24 309-320.

Chu, G., Qi, S., Yang, G., Dou, K., Du, J., and Lu, Z. (2012). Gastrointestinal tract specific gene GDDR inhibits the progression of gastric cancer in a TFF1 dependent manner. Mol. Cell. Biochem. 359, 369-374.

Cimpan, A.M., Enicca, S., Raica, M., and Ribatti, D. (2011). SOX2 gene expression in normal human thymus and thymoma. Clin. Exp. Med. 11, 251-254.

Corbeaux, T., Hess, I., Swann, J.B., Kanzer, B., Haas-Assenbaum, A., and Boehm, T. (2010). Thymopoiesis in mice depends on a Foxn1-positive thymic epithelial cell lineage. Proc. Natl. Acad. Sci. USA 107, 16613-16618.

de la Garza, G., Schleiffarth, J.R., Dunnwald, M., Mankad, A., Weirather, J.L., Bonde, G., Butcher, S., Mansour, T.A., Kousa, Y.A., Fukazawa, C.F., et al. (2012). Interferon regulatory factor 6 promotes differentiation of the periderm by activating expression of grainyhead-Like 3. J. Invest. Dermatol. 133, 68-77.

Diop-Bove, N., Jain, M., Scaglia, F., and Goldman, I.D. (2013). A novel deletion mutation in the proton-coupled folate transporter (PCFT, SLC46A1) in a Nicaraguan child with hereditary folate malabsorption. Gene 527, 673-74.

Dooley, J., Erickson, M., Roelink, H., and Farr, A.G. (2005). Nude thymic rudiment lacking functional foxn1 resembles respiratory epithelium. Dev. Dyn. 233, 1605-1612.

Dudakov, J.A., Hanash, A.M., Jenq, R.R., Young, L.F., Ghosh, A., Singer, N.V., West, M.L., Smith, O.M., Holland, A.M., Tsai, J.J., et al. (2012). Interleukin-22 drives endogenous thymic regeneration in mice. Science 336, 91-95.

Engelmark, M.T., Ivarsson, E.L., Magnusson, J.J., Gustavsson, I.M., Beskow, A.H., Magnusson, P.K.E., and Gylensten, U.B. (2006). Identification of susceptibility loci for cervical carcinoma by genome scan of affected sib-pairs. Hum. Mol. Genet. 15.
rived from a single progenitor. Nature 414, 763-768.
Rossi, S.W., Jenkinson, W.E., Anderson, G., and Jenkinson, E.J. (2006). Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. Nature 447, 988-991.
Sahin, U., Koslowski, M., Dhaene, K., Usener, D., Brandenburg, G., Seitz, G., Huber, C., and Tureci, O. (2008). Claudin-18 splice variant 2 is a Pan-cancer target suitable for therapeutic antibody development. Clin. Cancer Res. 14, 7624-7634.
Saunders-Pullman, R., Barrett, M.J., Stanley, K.M., Luciano, M.S., Shanker, V., Severt, L., Hunt, A., Raymond, D., Ozelius, L.J., and Bressman, S.B. (2010). LRRK2G2019S mutations are associated with an increased cancer risk in Parkinson disease. Mov. Disord. 25, 2536-2541.
Shakib, S., Desanti, G.E., Jenkinson, W.E., Parnell, S.M., Jenkinson, E.J., and Anderson, G. (2009). Checkpoints in the development of thymic cortical epithelial cells. J. Immunol. 182, 130-137.
Shen, M.M., and Leder, P. (1992). Leukemia inhibitory factor is expressed by the preimplantation uterus and selectively blocks primitive ectoderm formation in vitro. Proc. Natl. Acad. Sci. USA 89, 8240-8244.
Su, D., Ellis, S., Napier, A., Lee, K., and Manley, N.R. (2001). Hoxa3 and Pax1 regulate epithelial cell death and proliferation during thymus and parathyroid organogenesis. Dev. Biol. 236, 316-329.
Sun, L., Luo, H., Li, H., and Zhao, Y. (2013). Thymic epithelial cell development and differentiation: cellular and molecular regulation. Protein Cell 4, 342-355.
Sutherland, J.S., Goldberg, G.L., Hammett, M.V., Udriich, A.P., Berzins, S.P., Heng, T.S., Blazar, B.R., Miller, J.L., Malin, M.A., Chidgey, A.P., et al. (2005). Activation of thymic regeneration in mice and humans following androgen blockade. J. Immunol. 175, 2741-2753.
Swann, J.B., and Boehm, T. (2007). Back to the beginning – the quest for thymic epithelial stem cells. Eur. J. Immunol. 37, 2364-2366.
Ucar, A., Ucar, O., Klug, P., Matt, S., Brunk, F., Hofmann, T.G., and Kyewski, B. (2014). Adult thymus Contains FoxN1– epithelial stem cells that are bipotent for medullary and cortical epithelial lineages. Immunity 41, 257-269.
Wallin, J., Eibl, H., Neubüser, A., Willing, J., Koseki, H., and Ballinger, R. (1996). Pax1 is expressed during development of the thymus epithelium and is required for normal T-cell maturation. Development 122, 23-30.
Watson, A.P., Evans, R.L., and Egland, K.A. (2013). Multiple functions of sushi domain containing 2 (SUSD2) in breast tumorigenesis. Mol. Cancer Res. 11, 74-85.
Wei, G., and Condie, B.G. (2011). A focused in situ hybridization screen identifies candidate transcriptional regulators of thymic epithelial cell development and function. PLoS One 6, e26795.
Wong, K., Lister, N.L., Barsanti, M., Lim, J.M., Hammet, M.V., Khong, D.M., Siatskas, C., Gray, D.H., Boyd, R.L., and Chidgey, A.P. (2014). Multilineage potential and self-renewal define an epithelial progenitor cell population in the adult thymus. Cell Rep. 8, 1198-1209.
Yang, S.J., Ahn, S., Park, C.S., Choi, S., and Kim, M.G. (2005). Identifying subpopulations of thymic epithelial cells by flow cytometry using a new specific thymic epithelial marker, Ly110. J. Immunol. Methods 297, 265-270.
Yu, Z., Bhandari, A., Mannik, J., Pham, T., Xu, X., and Andersen, B. (2008). Grainyhead-like factor Get1/Grhl3 regulates formation of the epidermal leading edge during eyelid closure. Dev. Biol. 319, 64-67.
Zamisch, M., Moore-Scott, B., Su, D.M., Lucas, P.J., Manley, N., and Richie, E.R. (2005). Ontogeny and regulation of IL-7-expressing thymic epithelial cells. J. Immunol. 174, 60-67.
Zhou, S., Schuetz, J.D., Bunting, K.D., Colapietro, A.M., Sampath, J., Morris, J.J., Lagutina, I., Grosveld, G.C., Osawa, M., Nakauchi, H., et al. (2001). The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat. Med. 7, 1028-1034.