Comparative transcriptional profiling of medullary and cortical thymic cell types identifies epigenetic and transcription factors that uniquely define the mTEC^{hi} and cTEC lineages

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The thymus has the unique ability to impart lymphocytes in trans the concept of self-tolerance by negative selection and to enforce positive selection of lymphocytes that express optimal T-cell receptors (1,2). The thymus possesses a cortex and a medulla (4), and epithelial cells known as mTEC and cTEC are intimately involved in these processes (5). Here I compared the transcriptomes of mTEC^{hi} and cTEC (6) using global differential gene expression analysis. I present a series of epigenetic molecules and transcription factors that represent the most significant differences in the global gene expression profiles between these two cell types.

Keywords: thymus, medullary epithelial cells, cortical epithelial cells, mTEC^{hi}, cTEC, Utf1, Foxe1, Hoxd11, Fezf2, Myb, Arid4a, Ascl4, Baz2a, RbmX, Rbm47, R3hcc1, Zfc3h1, Zcchc7, autoimmunity, transplant medicine

Introduction

The thymus has the remarkable ability to provide lymphocytes the instructions for two essential processes in the human immune system known as negative and positive selection (1, 2). In negative selection, developing lymphocytes are displayed self-antigen, peptides that represent antigens that otherwise would be restricted to the periphery, known as tissue-restricted antigen (TRA) by presentation on the class II major histocompatibility complex (MHC-II) on mTEC^{hi} cells (6). TRA expression in mTEC^{hi}, cells that express high levels of MHC-II, is in large part dependent on the autoimmune regulator Aire (7) and lymphocytes whose T-cell receptors (TCRs) recognize self-antigen displayed on mTEC^{hi} MHC-II are deleted, or negatively selected (8). This process enables the immune system to remove any lymphocytes that would otherwise go on to enter the periphery and attack self cells, tissues and organs rather than pathogens (8). Positive selection occurs in the cortex of the thymus in cTEC (9, 10) and in this process, TCR that display an optimal binding affinity to peptide-MHC complexes displayed on the cTEC surface are chosen to survive, or positively selected (11-13). Developing lymphocytes whose TCR display binding affinity that is relatively strong or relatively weak are either deleted or left to die in a process known as anergy or death by neglect (1, 2, 14, 15).

The combined processes of positive and negative selection, occurring by interactions with the mTEC and cTEC of the thymus, are essential for the generation of an adaptive immune system that has the ability to defend the human body against pathogens while also preventing dangerous autoimmune pathology (8, 16-18). Understanding the transcriptional behaviors of both mTEC and cTEC are critical for complete understanding of how positive and negative selection occur, for the discovery of novel pathways to combat infectious and autoimmune diseases, and for the generation of novel tools and techniques to treat transplant rejection (19-21). In this study, we compared the total gene expression profiles, or transcriptomes, of mTEC^{hi} and cTEC using a public dataset (3) to determine that most significant transcriptional changes between these two cell types. This analysis revealed that multiple transcription factors and epigenetic molecules were represented among the most differentially expressed

genes in the mTEC^{hi} and cTEC transcriptomes. Remarkably, multiple RNA-binding proteins were also found to be among the genes whose expression was most different between these two cell types.

Methods

Dataset GSE89965 (3) was utilized for this differential gene expression analysis, performed using GEO2R. This public dataset was generated by investigators (3) using microarray (Affymetrix Mouse Gene 1-ST array) analysis of Aire-GFP, EpCAM+Ly51+ cells (cTEC), and Aire-GFP Epcam+MHC-II(hi) cells (mTEC^{hi}); $n=4$ from mTEC^{hi} and $n=3$ from cTEC, from C57Bl/6 Adig mice 6 weeks of age (adult). No adjustment was made to p -values, and there was no log-transformation applied to the data. The NCBI generated category of platform annotation was used. An unpaired two-tailed t-test with Welch's correction was utilized to determine the statistical significance of the RNA expression values between each cell type.

Results

We performed global differential gene expression profiling of the mTEC^{hi} and cTEC transcriptomes (3) to understand in a systematic fashion the transcriptional behavior of these immunologically important thymic cells types and to determine the most significant transcriptional changes between medullary and cortical thymic epithelium, cells required for the processes of positive and negative selection (1, 2, 4, 5, 8-10).

A series of differentially expressed transcription factors that uniquely define the mTEC^{hi} and cTEC lineages.

Five transcription factors were among the most differentially expressed genes when comparing the transcriptomes of mTEC^{hi} and cTEC. Three were expressed at higher levels in mTEC^{hi}, while two were expressed at higher levels in cTEC. These included *Utf1*, *Foxe1*, *Fezf2*, *Hoxd11*, and *Myb*.

Utf1, undifferentiated embryonic cell transcription factor 1, was the 2nd most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.42E-08$). Utf1 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 1; $p<0.0001$).

Foxe1, forkhead box E1, was the 12th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=9.17E-07$). Foxe1 expression was significantly higher in cTEC than in mTEC^{hi} (Figure 1; $p=0.0001$).

Fezf2, Fez family zinc finger 2, was the 26th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.51E-06$). Fezf2 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 1; $p=0.0001$).

Hoxd11, homeobox D11, was the 64th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=5.19E-06$). Hoxd11 expression was significantly higher in cTEC than in mTEC^{hi} (Figure 1; $p=0.0015$).

Myb, myeloblastosis oncogene, was the 15th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.34E-05$). Myb expression was significantly higher in mTEC^{hi} than in cTEC (Figure 1; $p=0.0005$).

A series of epigenetic molecules that are among the most significantly differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

Six epigenetic molecules were represented among the messages whose expression was most different in the global transcription profiles of mTEC^{hi} and cTEC. All but one were expressed at higher levels in mTEC^{hi}. These included Med12l, Rbpj, Sirt1, Baz2a, Ascl4, and Arid4a.

Med12l, mediator complex subunit 12-like, was the 54th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=4.37E-06$). Med12l expression was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p<0.0001$).

Rbpj, recombination signal binding protein for immunoglobulin kappa J region, was the 74th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC

transcriptomes (Table 1; $p=6.08E-06$). Rbpj expression was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p=0.0003$).

Sirt1, sirtuin 1, was the 125th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.16E-05$). Sirt1 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p=0.0006$).

Baz2a, bromodomain adjacent to zinc finger domain, 2A, was the 76th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=6.27E-06$). Baz2a expression was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p=0.0025$).

Ascl4, achaete-scute family bHLH transcription factor 4, was the 152nd most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.5E-05$). Ascl4 expression was significantly higher in cTEC than in mTEC^{hi} (Figure 2; $p=0.0026$).

Arid4a, AT rich interactive domain 4A (RBP1-like), was the 172nd most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.81E-05$). Arid4a expression was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p=0.0001$).

Multiple RNA-binding proteins uniquely define the mTEC^{hi} lineage and are among the most differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

Interestingly, five separate RNA binding proteins were among the most differentially expressed genes when comparing transcriptomes of mTEC^{hi} and cTEC. All but one were expressed at significantly higher levels in mTEC^{hi}. These included RbmX, Rbm47, R3hcc1, Zfc3h1, and Zcchc7.

RbmX, RNA binding motif protein, X chromosome, was the 61st most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1;

p=5.03E-06). Rbm47 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 3; p<0.0001).

Rbm47, RNA binding motif protein 47, was the 92nd most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; p=8.33E-06). Rbm47 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 3; p=0.0002).

R3hcc1, R3H domain and coiled-coil containing 1, was the 137th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; p=1.3E-05). R3hcc1 expression was significantly higher in cTEC than in mTEC^{hi} (Figure 3; p<0.0001).

Zfc3h1, zinc finger, C3H1-type containing, was the 148th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; p=1.44E-05). Zfc3h1 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 3; p<0.0001).

Zcchc7, zinc finger, CCHC domain containing 7, was the 221st most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; p=2.6E-05). Zcchc7 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 3; p=0.0003).

Two zinc-finger containing proteins uniquely define the mTEC^{hi} and cTEC lineages and are among the most differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

Of the messages whose expression level was most different between the transcriptomes of mTEC^{hi} and cTEC, two zinc finger proteins were represented, and one was expressed at higher levels in mTEC^{hi} while the other was expressed at higher levels in cTEC.

Zfp395, zinc finger protein 395, was the 17th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; p=1.22E-06). Zfp395 expression was significantly higher in cTEC than in mTEC^{hi} (Figure 4; p=0.0012).

Zfp397, zinc finger protein 397, was the 35th most differentially expressed gene globally when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.57E-06$). Zfp397 expression was significantly higher in mTEC^{hi} than in cTEC (Figure 4; $p<0.0001$).

Discussion

In this study, we compared the mTEC^{hi} and cTEC transcriptomes (3) using global differential gene expression profiling. We discovered five transcription factors, Utf1, Foxe1, Fezf2, Hoxd11 and Myb, six epigenetic and DNA-binding molecules Med12l, Rbpj, Sirt1, Baz2a, Ascl4 and Arid4a, five RNA-binding proteins, Rbm47, R3hcc1, Zfc3h1 and Zcchc7, and two zinc-finger containing proteins, Zfp395 and Zfp397, among the genes whose expression was most different when considering the total transcriptomes of both cell types.

Many of these molecules are not well-characterized. Understanding the function of these molecules in health and how they might malfunction in disease is important for future work that might manipulate these gene products through small molecules, antibodies or gene therapy in order to specifically target autoimmune pathologies and transplant rejection. Generation of mouse models with specific knockouts of these differentially expressed genes in the thymus using B5t-Cre (22-24) should be prioritized in order to begin to understand the function of these gene products in the thymus *in vivo* such that the relevant pathways can be targeted for generation of novel, targeted therapeutics to treat autoimmunity and transplant rejection.

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Figure 1: A series of differentially expressed transcription factors that unique define the mTEC^{hi} and cTEC lineages.

The expression of each differentially expressed gene is represented graphically, with the mean RNA expression value denoted by a horizontal black bar: mTEC^{hi}, $n=4$; cTEC, $n=3$. The statistical significance of the difference between the mean values between each cell type was calculated using a two-tailed unpaired t-test with Welch's correction, shown as a p -value on each graph.

Figure 2: A series of epigenetic molecules that are among the most significantly differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

The expression of each differentially expressed gene is represented graphically, with the mean RNA expression value denoted by a horizontal black bar: mTEC^{hi}, $n=4$; cTEC, $n=3$. The statistical significance of the difference between the mean values between each cell type was calculated using a two-tailed unpaired t-test with Welch's correction, shown as a p -value on each graph.

Figure 3: Multiple RNA-binding proteins uniquely define the mTEC^{hi} lineage and are among the most differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

The expression of each differentially expressed gene is represented graphically, with the mean RNA expression value denoted by a horizontal black bar: mTEC^{hi}, $n=4$; cTEC, $n=3$. The statistical significance of the difference between the mean values between each cell type was calculated using a two-tailed unpaired t-test with Welch's correction, shown as a p -value on each graph.

Figure 4: Two zinc-finger containing proteins uniquely define the mTEC^{hi} and cTEC lineages and are among the most differentially expressed genes when comparing the mTEC^{hi} and cTEC transcriptomes.

The expression of each differentially expressed gene is represented graphically, with the mean RNA expression value denoted by a horizontal black bar: mTEC^{hi} $n=4$; cTEC, $n=3$. The statistical significance of the difference between the mean values between each cell type was calculated using a two-tailed unpaired t-test with Welch's correction, shown as a p -value on each graph.

Table 1

Rank	p-value	t	B	Gene Symbol	Gene Name	ID
2	2.42E-08	-40.141	-3.31	Utf1	undifferentiated embryonic cell transcription factor 1	10558580
12	9.17E-07	21.421	-3.32	Foxe1	forkhead box E1	10504728
17	1.22E-06	20.386	-3.33	Zfp395	zinc finger protein 395	10415991
26	1.51E-06	-19.653	-3.33	Fezf2	Fez family zinc finger 2	10417620
35	2.57E-06	-17.905	-3.33	Zfp397	zinc finger protein 397	10454298
54	4.37E-06	-16.324	-3.34	Med12l	mediator complex subunit 12-like	10492231
61	5.03E-06	-15.927	-3.34	Rbmx	RNA binding motif protein, X chromosome	10604735
64	5.19E-06	15.836	-3.34	Hoxd11	homeobox D11	10472953
74	6.08E-06	-15.406	-3.35	Rbpj	recombination signal binding protein for immunoglobulin kappa J region	10521913
76	6.27E-06	-15.324	-3.35	Baz2a	bromodomain adjacent to zinc finger domain, 2A	10367122
92	8.33E-06	-14.577	-3.35	Rbm47	RNA binding motif protein 47	10530269
125	1.16E-05	-13.755	-3.36	Sirt1	sirtuin 1	10369738
137	1.3E-05	13.475	-3.36	R3hcc1	R3H domain and coiled-coil containing 1	10421188
148	1.44E-05	-13.240	-3.36	Zfc3h1	zinc finger, C3H1-type containing	10366409
152	1.5E-05	13.146	-3.36	Ascl4	achaete-scute family bHLH transcription factor 4	10365469
172	1.81E-05	-12.707	-3.37	Arid4a	AT rich interactive domain 4A (RBP1-like)	10396205
205	2.34E-05	-12.149	-3.37	Myb///Myb	myeloblastosis oncogene///myeloblastosis oncogene	10368199
221	2.6E-05	-11.925	-3.38	Zcchc7	zinc finger, CCHC domain containing 7	10504491

Table 1: Transcription factors, epigenetic molecules, DNA and RNA binding factors that are among the most differentially expressed genes when comparing the transcriptomes of mTEC^{hi} and cTEC.

Rank order with respect to differential expression globally, p-value, t (a moderated t-statistic), B, the log-odds that the gene is differentially expressed between each group, gene symbol, gene name and ID are listed (all provided by NCBI GEO2R).