Ribosomal proteins and ribosomal pseudogenes are differentially expressed by medullary and cortical epithelial cells of the thymus.

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The thymus is a unique organ with the ability to impart the concept of self-tolerance, or immunological privilege to developing lymphocytes (1, 2). It possesses anatomical compartmentalization in the form of a medulla and cortex (3). To understand the transcriptional behavior of the medullary (mTEC) and cortical epithelial cells (cTEC) of the thymus as they most differ from each other, we performed global differential gene expression profiling using a public microarray dataset of each cell type, isolated from the mouse thymus (4). These analyses revealed that eleven unique ribosomal proteins and pseudogenes were among the most differentially expressed genes when comparing the mTEC transcriptome with the cTEC transcriptome. These data suggest a potential cell-type specific role for these ribosomal subunits and pseudogenes in the epithelial cells of the thymus.

Keywords: thymus, medullary epithelial cells, cortical epithelial cells, cTEC, mTEC(hi), mTEC(lo), ribosome, ribosomal subunits, ribosomal pseudogene

Introduction

The thymus is a unique organ with the ability to educate developing lymphocytes what it means to be self by presenting them with antigen that is generally otherwise restricted to the periphery (peripheral tissue antigen) (1-3). Antigen presentation for lymphocyte tolerance instruction occurs in an anatomically regulated fashion, with thymic epithelial cells in the cortex and medulla, termed cTEC and mTEC (3), and is controlled in large part by expression of the Class II major histocompatibility complex peptide antigen receptor MHC-II (5,6). mTEC expressing high levels of MHC-II (mTEC^{hi}) also express relatively higher levels of the autoimmune regulator Aire, which induces promiscuous expression of tissue-restricted antigen for self-antigen presentation (5,6).

Here we used a public microarray dataset to perform global differential gene expression analysis with a cross-comparison of the mTEC^{hi}, mTEC^{lo}, and cTEC transcriptomes from adult murine thymus. This transcriptome study revealed that ribosomal protein subunits and ribosomal pseudogenes are among the most differentially expressed genes when comparing these cell types and suggests a potential role for cell-type specific ribosomal subunit function in immunologically active cells of the thymus.

Methods

GSE89965 was utilized for this differential gene expression analysis, performed using GEO2R. This public dataset was generated by the authors using microarray (Affymetrix Mouse Gene 1-ST array) analysis of Aire-GFP, EpCAM+Ly51+ cells (cTEC), Aire-GFP Epcam+MHC-II(hi) cells (mTEC^{hi}), and Aire-GFP Epcam+MHC-II(lo) cells (mTEC^{lo}); $n=4$ from mTEC^{hi} and mTEC^{lo}; $n=3$ 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. For statistical test to determine the significance of Rps2

pseudogene expression values across the three cell types, a one-way ANOVA with multiple comparisons was performed. Data are from three analyses; comparing mTEC^{hi} to mTEC^{lo}, comparing mTEC^{hi} to cTEC, and comparing mTEC^{lo} to cTEC.

Results

Multiple ribosomal subunits and ribosomal pseudogenes are differentially expressed when comparing the transcriptomes of mTEC^{hi} to cTEC.

We performed comparative global gene expression profiling to determine the most differentially expressed genes between the transcriptomes of mTEC^{hi} and cTEC using a public dataset of microarray expression data from flow cytometry sorted EpCAM+MHC-II+ and EpCAM+Ly51+ cells. This systems-level analysis revealed that among the messages whose expression was most different between the mTEC^{hi} and cTEC transcriptomes were six ribosomal subunits and five ribosomal pseudogenes.

Ribosomal protein S18, Rps18 was ranked 40 out of 34760 transcripts detected by microarray in this dataset (Table 1; $p=0.000003$); ribosomal protein L23, Rpl23 was ranked 179 ($p=0.0000192$), ribosomal protein large P0, Rplp0 was ranked 190 (Table 1; $p=0.0000203$); ribosomal protein S7 was ranked 192 (Table 1; $p=0.0000206$); ribosomal protein L6 was ranked 215 ($p=0.0000254$); ribosomal protein S10 was ranked 227 (Table 1; $p=0.0000268$); and ribosomal protein L3, Rpl3 was ranked 250 (Table 1; $p=0.0000305$).

The ribosomal protein L7a pseudogene, or Rpl7a (Gm5766) was ranked 66 out of 34760 transcripts detected (Table 1; $p=0.00000544$); the ribosomal protein large P0 pseudogene Rplp0 (Gm5779) was ranked 103 (Table 1; $p=0.0000941$); the ribosomal protein S2 pseudogene, Rps2(Gm10653) was ranked 168 (Table 1; $p=0.0000173$), and ribosomal protein S23 pseudogene, Rps23 was ranked 267 (Table 1; $p=0.0000326$).

The expression of all of these genes was significantly higher in mTEC^{hi}, except for the Rps2 pseudogene, which was expressed at significantly higher levels in cTEC (Figure 1B, $p=0.0025$). For all genes expressed at significantly higher levels in mTEC^{hi}, p was calculated to

be less than or equal to 0.0001 except for Rpl6 $p=0.0002$), the Rpl7a pseudogene ($p=0.0039$), and the Rpl3 pseudogene ($p=0.0004$).

Overall, five ribosomal subunits and four ribosomal subunit pseudogenes were differentially expressed at the global level in mTEC^{hi} when comparing its transcriptome to that of cTEC, and their expressional levels were significantly higher in mTEC^{hi} than in cTEC.

Specific ribosomal subunits are differentially expressed, and at significantly higher levels in the mTEC^{lo} transcriptome when compared to cTEC.

We continued our systems-level study of mTEC and cTEC transcriptional behavior by comparing microarray data from mTEC^{lo} and cTEC ($n=4$ for mTEC^{lo} and $n=3$ for cTEC).

Here, four ribosomal subunits were significantly differentially expressed between mTEC^{lo} and cTEC at the global level, and three were expressed at significantly higher levels in mTEC^{lo}. Rps9 (rank 20 out of 34760), Rps13 (rank 95), and Rpl23a (rank 226) were expressed at significantly higher levels in mTEC^{lo}, (Figure 2; Rps9: $p=0.0007$; Rps13: $p=0.0015$; and Rpl23a: $p=0.0028$) while Rps10a (rank 208) was expressed at significantly higher levels in cTEC (Figure 2; Rps10a: $p=0.0031$).

The only pseudogene among the two hundred and fifty most differentially expressed genes in this analysis, ribosomal protein S2 pseudogene Rps2, was expressed at significantly higher levels in cTEC (Figure 2; $p=0.0091$).

The Rps2 pseudogene is differentially expressed when comparing the transcriptomes of mTEC^{hi} to mTEC^{lo}, and at significantly higher levels in mTEC^{lo}

When comparing the transcriptomes of mTEC^{hi} and mTEC^{lo}, the only ribosomal subunit or subunit pseudogene that was differentially expressed and within the top 250 most differentially expressed genes globally was the Rps2 pseudogene (Table 1; $p=0.000133$). Rps2 pseudogene was the 176th most differentially expressed gene between the transcriptomes of mTEC^{hi} and mTEC^{lo} (Table 1). The expression of the Rps2 pseudogene was significantly higher in mTEC^{lo} (Figure 3; $p=0.0004$)

Rps2 pseudogene is differentially expressed in the epithelial cells of the thymus and graded across cell type.

The only gene that was differentially expressed at the threshold of 250/ 34760 total transcripts detected by the microarray dataset utilized in this study was the Rps2 pseudogene (Figure 4). The expression of the Rps2 pseudogene was significantly higher in cTEC than in mTEC^{lo} and mTEC^{hi}, and was significantly higher in mTEC^{lo} than in mTEC^{hi}.

Discussion

To better understand the transcriptional behavior of the epithelial cells of the thymus, in the cortex and the medulla, we compared the transcriptomes of cTEC, mTEC^{lo} and mTEC^{hi} (expressing low or high levels of the MHC Class II receptor), isolated from the mouse thymus (4) and asked which RNA messages were most significantly different, globally, in terms of their expression levels. We found that multiple ribosome subunits, both large (Rpl) and small (Rps) (7) were among the most differentially expressed genes in these thymic cell types.

The Rps2 pseudogene was the one ribosomal-type gene that was differentially expressed across all three comparisons at the threshold of our analysis (two hundred and fifty out of 34760). The expression of the Rps2 pseudogene may serve some critical function in these cell types that is appropriately graded, or it may be a reflection of some other cell-type specific process that displays a cell-type specific frequency or quality. Cowan et al. recently reported that Myc controls thymus size in the fetal thymus by enhancing ribosomal biogenesis in fetal TEC (8). This study did not isolate or study cells based on expression of MHC-Class II, nor did it report any role for or pattern of expression of ribosomal pseudogenes in the TEC cells of the thymus as we do here.

In conclusion, transcripts encoding ribosomal protein subunits and non-coding ribosomal pseudogene transcripts are significant components of the transcriptome of thymic epithelial cells, in both the medulla and cortex. Most of these ribosomal subunits were expressed at significantly higher levels in the medulla, except for Rpl10a. Global differential expression of ribosomal protein S and ribosomal protein L subunits could potentially reflect

cell-type specific functions for these ribosomal subunits in antigen presentation during Aire-dependent education during of self during lymphocyte development. A function for ribosomal subunit pseudogenes (9) in the thymus is not clear but may similarly reflect some mechanisms required for regulation of gene expression during Aire-dependent training of tolerance by the medullary epithelial cells of the thymus by the Class II MHC antigen receptor, and should be the focus of future study.

References

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Figure 1: Multiple ribosomal subunits and ribosomal pseudogenes are differentially expressed, and at significantly higher levels in the mTEC^{hi} transcriptome when compared to cTEC.

Figure 1A: The RNA expression value of six ribosomal subunits is shown with each dot representing one pool of thymic cells isolated from mice ($n=4$ for mTEC^{hi} (blue) and $n=3$ for cTEC (red)). The black bar represents the mean value. The statistical significance of the difference in expression values between the two categories was calculated using a two-tailed unpaired t-test with Welch's correction, and displayed on each graph as a p-value.

Figure 1B: The RNA expression value of five ribosomal subunit pseudogenes is shown with each dot representing one pool of thymic cells isolated from mice ($n=4$ for mTEC^{hi} (blue) and $n=3$ for cTEC (red)). The black bar represents the mean value. The statistical significance of the difference in expression values between the two categories was calculated using a two-tailed unpaired t-test with Welch's correction, and displayed on each graph as a p-value.

Figure 2: Multiple ribosomal subunits are differentially expressed, and at significantly higher levels in the mTEC^{lo} transcriptome when compared to cTEC.

Figure 2A: The RNA expression value of four ribosomal subunits is shown with each dot representing one pool of thymic cells isolated from mice (n=4 for mTEC^{lo} (blue) and n=3 for cTEC (red)). The black bar represents the mean value. The statistical significance of the difference in expression values between the two categories was calculated using a two-tailed unpaired t-test with Welch's correction, and displayed on each graph as a p-value.

Figure 2B: The RNA expression value of five ribosomal subunit pseudogenes is shown with each dot representing one pool of thymic cells isolated from mice (n=4 for mTEC^{lo} (blue) and n=3 for cTEC (red)). The black bar represents the mean value. The statistical significance of the difference in expression values between the two categories was calculated using a two-tailed unpaired t-test with Welch's correction, and displayed on each graph as a p-value.

Figure 3: The Rps2 pseudogene is differentially expressed when comparing the transcriptomes of mTEC^{hi} to mTEC^{lo}, and at significantly higher levels in mTEC^{lo}.

The mean expression level of the Rps2 pseudogene is marked with a black bar (n=4 for mTEC^{hi} (red), and n=4 for mTEC^{lo} (blue)). The statistical significance of the difference in expression values between the two categories was calculated using a two-tailed unpaired t-test with Welch's correction, and displayed on the graph as a *p*-value.

Figure 4: The Rps2 pseudogene is differentially expressed when comparing the transcriptomes of mTEC^{hi} to mTEC^{lo}, and at significantly higher levels in mTEC^{lo}

The mean expression level of the Rps2 pseudogene is marked with a black bar (n=4 for mTEC^{hi} (blue), and n=4 for mTEC^{lo} (red) and n=3 for cTEC). The statistical significance of the difference in expression values between the two categories was calculated using a one-way ANOVA with multiple comparisons, and displayed on the graph as a *p*-value.

Table 1

A	Rank	Gene Symbol	Gene Name	p-value	t	B	ID
	40	Rps18	ribosomal protein S18	0.000003	-17.4263101	-3.34	10581535
	66	Gm5766///Rpl7a	ribosomal protein L7a pseudogene///ribosomal protein L7A	0.00000544	-15.7084304	-3.34	10603172
	103	Gm5779///Rplp0	ribosomal protein, large, P0 pseudogene///ribosomal protein, large, P0	0.0000941	-14.2664069	-3.35	10581027
	168	Gm10653///Rps2	ribosomal protein S2 pseudogene///ribosomal protein S2	0.0000173	12.8112523	-3.37	10402314
	179	Rpl23	ribosomal protein L23	0.0000192	-12.5773205	-3.37	10545125
	190	Rplp0	ribosomal protein, large, P0	0.0000203	-12.4583289	-3.37	10524718
	192	Rps7	ribosomal protein S7	0.0000206	-12.4228698	-3.37	10607391
	215	Rpl6	ribosomal protein L6	0.0000254	-11.970194	-3.37	10525187
	227	Rps10	ribosomal protein S10	0.0000268	-11.8564181	-3.38	10449370
	250	Gm5879///Rpl3	predicted gene 5879///ribosomal protein L3	0.0000305	-11.5869932	-3.38	10600324
	267	Gm8624///Rps23	ribosomal protein S23 pseudogene///ribosomal protein S23	0.0000326	11.4523442	-3.38	10406541
B	Rank	Gene Symbol	Gene Name	p-value	t	B	ID
	20	Rps9	ribosomal protein S9	0.00010265	-9.343295	-3.72	10549588
	95	Rps13	ribosomal protein S13	0.00057798	-6.775544	-3.79	10567163
	126	Gm10653///Rps2	ribosomal protein S2 pseudogene///ribosomal protein S2	0.00083082	6.319075	-3.81	10402314
	208	Rpl10a	ribosomal protein L10A	0.00134125	5.75362	-3.83	10407535
	226	Rpl23a	ribosomal protein L23A	0.00148276	-5.640193	-3.84	10598103
C	Rank	Gene Symbol	Gene Name	p-value	t	B	ID
	176	Gm10653///Rps2	ribosomal protein S2 pseudogene///ribosomal protein S2	0.000133	7.795409	-3.05	10402314

Table 1: Multiple ribosomal subunits and ribosomal pseudogenes are differentially expressed in the mTEC and cTEC transcriptomes.

Shown is the rank of differential expression relative to the 34760 total transcripts detected in each analysis, gene symbol, gene name, p-value with respect to global differential expression between each comparison, t, a moderated t-statistic, B, the log-odds probability of differential expression in the given comparison, and NCBI ID (all provided by GEO2R).

Table 1A: mTEC^{hi} vs cTEC

Table 1B: mTEC^{lo} vs cTEC

Table 1C: mTEC^{hi} vs mTEC^{lo}