Stage-selective differential expression of eukaryotic initiation factor (Eif) genes in the medullary epithelial cells of the murine thymus.

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The thymus is the site of positive and negative selection in mammals (1), the process by which developing lymphocytes are selected for or deleted based on their ability to encounter foreign or self antigen, respectively, with optimal binding affinity (2, 3). The thymus consists of a medulla and a cortex, with mTEC, or medullary thymic epithelial cells expressing high or low amounts of the class II major histocompatibility complex (mTEC^{hi} and mTEC^{lo}) (4, 5). To understand how the mTEC transcriptome changes over mammalian adult development, we performed global differential gene expression profiling using a public microarray dataset to compare the transcriptomes of mTEC at months 1, 3, and 6 (6). We found that multiple eukaryotic initiation factors, or Eif genes, were differentially expressed by mTEC^{lo} and mTEC^{hi}. These data suggest a unique requirement for specific eukaryotic initiation factors during translation of proteins in the mTEC^{lo} and mTEC^{hi} cell types of the thymus.

Keywords: thymus, mTEC, medullary thymic epithelial cells, translation, Eif, Eif2ak2, PKR, eukaryotic initiation factor, differential gene expression, systems-level analysis of the immunologically active cells of the thymus.

Introduction

The protection afforded to human beings by their immune systems is dependent on being able to recognize, at the molecular level, that all of the protein products of one's own self are "safe" and that all other antigens are foreign and potentially dangerous. The thymus has the ability to teach developing lymphocytes what it means to be self by presenting them with antigen that is generally otherwise restricted to the periphery (tissue restricted antigen) (1-3). Self-instruction by antigen presentation 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 can be sorted based on their expression of MHC-II as either mTEC^{hi} or mTEC^{lo}, and 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).

In this analysis we used a public microarray dataset (4) to compare the mTEC transcriptomes across three points in mammalian life, at 1 month, 3 months, and 6 months, by global differential gene expression analysis, to determine the most significant changes in the transcriptional behavior across developmental progression. This transcriptome study revealed that eukaryotic initiation factors are among the most differentially expressed genes when comparing these developmental time points in mTEC. The data allude to a potential cell-type specific function in the medullary epithelial cells of the thymus for these Eif genes in translation, a site of antigen presentation for instruction of tolerance, to avoid autoimmunity and immune responses to self-antigen.

Methods

GSE56928 (6) was utilized for this differential gene expression analysis, performed using GEO2R. This public dataset was generated by the authors using microarray (Affymetrix Mouse 430 2.0 array) of mTEC^{hi} and mTEC^{lo}, $n=2$ for each developmental time point (1 month, 3

month, and 6 months). The Benjamini and Hochberg method of p-value adjustment was utilized for p-value calculation by GEO2R, and auto-detect option was used for log-transformation applied to global expression data. The NCBI generated category of platform annotation was used. For statistical test to determine the significance of difference between average RNA expression values across each developmental time point for both mTEC^{hi} and mTEC^{lo}, an ordinary one-way ANOVA with multiple comparisons (Tukey's multiple comparisons test) was utilized (PRISM 8.1.2) (227).

Results

Multiple eukaryotic initiation factors are among the most differentially expressed genes in mTEC^{lo} cells of the mammalian thymus when compared across adult development

We performed global differential gene expression profiling using a public microarray dataset (6) containing transcriptome data from mTEC^{lo} at three developmental time points in mammalian life: 1 month, 3 months, and 6 months. This analysis revealed that 4 transcripts from Eif gene family were among the most differentially expressed in the mTEC^{lo} transcriptome when comparing mTEC^{lo} at these three developmental timepoints. These included Eif4g2, Eif2ak2, Eif4g1, and Eif5.

Out of 45101 total transcripts detected by the microarray dataset used for this analysis, Eif4g2 was the 81st most differentially expressed gene in the mTEC^{lo} transcriptome across the 1 month, 3 month, and 6 month time points of mammalian life (Table 1; $p=7.13\text{E-}06$), Eif2ak2 was the 224th most differentially expressed gene (Table 1; $p=2.91\text{E-}05$), Eif4g1 was the 236th most differentially expressed gene (Table 1; $p=3.07\text{E-}05$), and Eif5 was the 246th most differentially expressed gene in the mTEC^{lo} transcriptome when comparing global gene expression profiles at 1 month, 3 months, and 6 months (Table 1; $p=3.26\text{E-}05$).

The expression of Eif4g2, Eif2ak2, Eif4g1, and Eif5 all increased from 1 month to 6 months and this change in RNA expression level was statistically significant (Figure 1; Eif4g2: $p=0.0006$; Eif2ak2: $p=0.0032$; Eif4g1: $p=0.0128$; and Eif5: $p=0.0175$).

Interestingly, though the expression of each of these genes increased from 1 month to 6 months in mTEC^{lo}, from 1 month to 3 months, the expression of all four Eif genes decreased in mTEC^{lo}, and in one case (Eif4g2), this change in expression level was statistically significant (Figure 1; $p=0.0271$).

Eif2ak2 and Eif3a eukaryotic initiation factors are among the most differentially expressed genes in mTEC^{hi} cells of the thymus when compared across mammalian development

We performed a similar global differential gene expression profiling using the same microarray dataset, now with transcriptome data from murine mTEC^{hi} at the same three developmental time points in mammalian life: 1 month, 3 months, and 6 months. Here, 2 transcripts from the Eif gene family were among the most differentially expressed in the mTEC^{hi} transcriptome when comparing mTEC^{hi} at these developmental stages. These included Eif2ak2 and Eif3a (Table 1).

Thus, Eif2ak2 was the only Eif gene that was differentially expressed transcriptome-wide, in both mTEC^{lo} and mTEC^{hi} when comparing their global gene expression profiles across development at 1 month, 3 month, and 6 months of mammalian life (Table 1).

Out of 45101 total transcripts detected, Eif2ak2 was the 26th most differentially expressed gene in the mTEC^{hi} transcriptome across the 1 month, 3 month and 6 month developmental time points (Table 1; $p=4.97E-06$) and Eif3a was the 42nd most differentially expressed gene in the mTEC^{hi} transcriptome when comparing global gene expression profiles across development at these three points in mammalian life (Table 1; $p=1.01E-05$).

The expression of both Eif2ak2 and Eif3a increased from 1 month to 6 months in mTEC^{hi}, and this change was statistically significant (Figure 2; Eif2ak2: $p=0.0020$ and Eif3a: $p=0.0063$). The expression of each of these Eif genes marginally increased from 1 month to 3 months but this change was not statistically significant (Figure 2).

Discussion

In this study, we performed a differential gene expression analysis of the medullary epithelial cells of the thymus, expressing either high or low levels of the class II major histocompatibility complex, at three time points in mammalian life: 1 month, 3 months, and 6 months. We found that mTEC^{hi} and mTEC^{lo} differentially express genes of the eukaryotic initiation factor (Eif) family. Four Eif genes were among the 250 most differentially expressed genes in the mTEC^{lo} transcriptome when comparing these cells across development: Eif2ak2, Eif4g2, Eif4g1, and Eif5, while only one Eif gene was among the 250 most differentially expressed genes in both the mTEC^{hi} and mTEC^{lo} transcriptomes: Eif2ak2.

The protein products of Eif genes are important for the process by which messenger RNA is converted into a protein, known as translation (9). This involves ribosomes; for proteins that are to be secreted in the extracellular space or to be embedded into the plasma membrane, translation occurs on ribosomes in the endoplasmic reticulum; for proteins that have a cytosolic localization, they are translated on ribosomes free in the cytosol (10-13). There are three main steps in translation: initiation, elongation, and termination. eIFs orchestrate the macromolecular assembly of mRNA, ribosome, and tRNAs that guide mRNA translation into protein: the initiator tRNA and the tRNA that codes for Methionine, generally the first amino acid of a protein sequence, in the initiation phase of translation (9, 13).

Eif2ak2 is also known as protein kinase R, or PKR (14-17). Eif2ak2 is the only Eif gene in this study that was differentially expressed at significant levels by both mTEC^{hi} and mTEC^{lo} from 1 to 6 months of thymic development. PKR is one of four kinases that is activated upon sensing of cellular stress (17). PKR has been identified as a scaffold, an important part of an innate immune macromolecular assembly known as the inflammasome, most classical of which has NLRP3 or cryopyrin as its core, or even perhaps a repressor of the innate immune response orchestrated by inflammasomes (14-19).

It is clear is that PKR is a critical component of the interferon signaling pathway and can be induced by the presence of double stranded RNA to shutdown protein synthesis in order to prevent viral protein synthesis. Many viruses encode factors that interfere with PKR for this specific reason (20-23). What is also clear is that PKR phosphorylates eIF2a to prevent assembly of the 43S initiation complex (17). Though it was earlier thought to mediate apoptosis by its activation of interferon signaling, PRK/Eif2ak2 has more recently been shown to repress apoptotic signaling after such inflammasome activation (16). Eif2ak2 has also more recently been identified as a regulator of pyroptosis, an unconventional form of cell death, and it does this by regulating activating caspase-1 (15). Thus, Eif2ak2 has two roles in cell death pathways, apoptotic via its function in the interferon signaling pathway and through its kinase activity (24), and pyroptotic through its function as an activator of caspase-1, independent of its kinase activity (15).

The significant differential expression of Eif2ak2 in a stage-specific fashion in mTEC could suggest either that antiviral signaling is progressively activated in the mammalian thymus as adulthood progresses, or that antiviral signaling is important for the integrity of medullary epithelial cells of the thymus for reasons that are unclear. The activation or modulation of cell death pathways of two types are also implicated by the increased expression of Eif2ak2 in the medullary epithelial cells of the thymus but the significance of this is not clear and could potentially involve the process of thymic involution, wherein the thymus progressively shrinks through the course of adult life (25).

In summary, comparing the transcriptomes of mTEC across three points in development revealed that genes of the eukaryotic initiation factor, or Eif family were differentially expressed and among the most differentially expressed in the entire mTEC transcriptome. One Eif gene, Eif2ak2 also known as PKR, was differentially expressed in a stage-specific fashion and significantly so in both mTEC^{hi} and mTEC^{lo}. Future study is required to understand the immunologic and/or developmental significance of Eif gene expression in mTEC function.

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Figure 1: Multiple eukaryotic initiation factors are among the most differentially expressed genes in mTEC^{lo} cells of the mammalian thymus when compared across adult development.

The expression of eukaryotic initiation factor genes Eif2ak2, Eif4g2, Eif4g1, and Eif5 in mTEC^{lo} is graphically represented, with the mean value of RNA expression of each gene at each time point shown as a horizontal black bar. A statistical test to evaluate the significance of the difference of RNA expression level between each developmental time point was performed using an ordinary one-way ANOVA with multiple comparisons (Tukey's multiple comparisons test) and the *p*-value for each comparison is denoted on each graph.

Figure 2: Eif2ak2 and Eif3a eukaryotic initiation factors are among the most differentially expressed genes in mTEC^{hi} cells of the mammalian thymus when compared across adult development.

The expression of eukaryotic initiation factor genes Eif2ak2 and Eif3a in mTEC^{hi} is graphically represented, with the mean value of RNA expression of each gene at each time point shown as a horizontal black bar. A statistical test to evaluate the significance of the difference of RNA expression level between each developmental time point was performed using an ordinary one-way ANOVA with multiple comparisons (Tukey's multiple comparisons test) and the *p*-value for each comparison is denoted on each graph.

Rank	Gene Symbol	Gene Name	p-value	F	ID
81	Eif4g2	eukaryotic translation initiation factor 4, gamma 2	7.13E-06	7.24E+01	1458431_at
224	Eif2ak2	eukaryotic translation initiation factor 2-alpha kinase 2	2.91E-05	4.98E+01	1422005_at
236	Eif4g1	eukaryotic translation initiation factor 4, gamma 1	3.07E-05	4.91E+01	1427037_at
246	Eif5	eukaryotic translation initiation factor 5	3.26E-05	4.84E+01	1456256_at
Rank	Gene Symbol	Gene Name	p-value	F	ID
26	Eif2ak2	eukaryotic translation initiation factor 2-alpha kinase 2	4.97E-06	8.67E+01	1422005_at
42	Eif3a	eukaryotic translation initiation factor 3, subunit A	1.01E-05	7.15E+01	1416661_at

Table 1: Multiple eukaryotic initiation factors are differentially expressed in the medullary epithelial cells of the thymus.

The rank of differential expression relative to 45101 total transcripts detected and measured, *p*-value of differential expression relative to the mTEC^{hi} and mTEC^{lo} transcriptomes, F statistic, (all determined by NCBI GEO2R) and Affymetrix probe ID for each transcript are displayed.