Negative regulation of eIF5a2 gene expression by Aire.

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The autoimmune regulator, Aire, functions in the process whereby promiscuous transcription of antigens that are otherwise only expressed in peripheral tissues is enabled in specific cells of the thymus for negative selection of self-reactive developing lymphocytes to prevent autoimmunity (1, 2). In this role, Aire is typically thought of as a positive regulator of gene transcription. Here, we found that Aire negatively regulates the expression of the A2 isoform of the eukaryotic translation initiation factor Eif5a (Eif5a2) in the thymus using two published datasets (3, 4). We identified Eif5a2 as among the most differentially expressed genes in the thymic medullary epithelial cells of Aire knock-out mice. Message for Eif5a2 was present in significantly higher quantities in the absence of Aire in mTEC. During translation initiation, eIF2 delivers the initiator methionyl-tRNA to the small ribosome subunit to form the ternary eIF2 - GTP - tRNA^{met} translation pre-initiation complex, and eIF5 acts as a GDP dissociation inhibitor (GDI), stabilizing binding of GDP to eIF2 and maintaining it in an inactive state (5). We suggest that Aire controls the expression of eIF5a2 in the thymus in order to limit its GDI function, de-restrict general protein synthesis and promote translation of tissue-restricted antigen in mTEC.

Keywords: thymus, mTEC, mTEC^{hi}, protein translation, translation initiation in the thymus, global differential gene expression analysis, systems biology of the immunologically active cells of the thymus.

Aire promotes promiscuous gene expression in the medullary epithelial cells of the thymus (mTEC) for negative selection of potentially self-reactive lymphocyte clones during lymphocyte development (1, 2). Aire exerts this function by positively regulating gene expression of tissue-restricted antigen. We studied Aire's function in the thymus in an unbiased manner by comparing the transcriptomes of mTEC from mice that possess specific deletions in the Aire gene along with the transcriptomes of mTEC from wild-type mice (3, 4). We found that the eukaryotic translation initiation factor Eif5a2 was among the genes whose expression changed the most in mTEC upon deletion of Aire. Eif5a2 expression significantly increased upon Aire deletion. eIF2 delivers the initiator methionyl-tRNA to the small ribosome subunit to form the ternary eIF2 - GTP - tRNA^{met} translation pre-initiation complex. Acting in a translational control role, eIF5 functions as a GDP dissociation inhibitor (GDI), stabilizing binding of GDP to eIF2 and maintaining it in an inactive state (5). We found that the A2 isoform (6) of the eukaryotic translation initiation factor, Eif5a2, was among the genes whose expression changed most significantly when comparing the transcriptomes of mTEC from wild-type and Aire knock-out mice. The expression of the RNA message for eIF5a2 was significantly higher in mTEC from Aire-deficient thymuses versus that of mTEC from the wild-type thymus. These findings demonstrate unprecedented negative regulation of translation components in the thymus of mice by Aire.

Methods

We used datasets GSE102527 (3) and GSE14365 (4) to perform this analysis, using GEO2R.

Koh et al. used Affymetrix Mouse Gene 2.0 ST Array to generate the GSE102527 microarray dataset. In this dataset, n=2 wild-type mTEC^{hi} and n=2 Aire knock-out mTEC^{hi}.

Hubert et al. Affymetrix Mouse Genome 430 2.0 Array Array to generate the GSE14365 microarray dataset. In this dataset, n=3 wild-type mTEC and n=2 Aire knock-out mTEC.

The "Benjamini and Hochberg" method of p-value adjustment was utilized for all analyses. Log-transformation was applied to all data. The NCBI category of platform annotation was utilized for all analyses.

1 An unpaired, two-tailed t test with Welch's correction was performed to evaluate the statistical
2 significance of difference in Eif5aa2 expression between Aire-sufficient and Aire-deficient genotypes
3 using PRISM (8.1.2 (227)).
4

5 **Results**

6 We compared the transcriptomes of mTEC and mTEC expressing high levels of the class II major
7 histocompatibility complex receptor (MHC-II) (mTEC^{hi}), from wild-type mice and from mice possessing
8 a specific deletion in the Aire gene. We performed this analysis using two separate published datasets (3,
9 4).
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11 Eif5a2 is one of the most differentially expressed genes in the Aire-deleted mTEC of the thymus

12 We found that the gene encoding the eIF5a2 eukaryotic translation initiation factor, Eif5a2, was
13 among the genes whose expression changed the most significantly upon deletion of Aire in mTEC from
14 the murine thymus. All transcripts of the transcriptome were ranked based on the level of change in gene
15 expression between wild-type and Aire-deleted mTEC (differential expression). In one dataset, Eif5a2
16 ranked 55 out of 33,732 total transcripts (3) in terms of global differential expression. This was
17 statistically significant (Table 1; p=8.6E-05). To validate these findings, we checked whether Eif5a2
18 would be considered significantly differentially expressed in an independent dataset. In these analyses,
19 the transcriptomes of mTEC^{hi}, not bulk mTEC, were compared between wild-type and Aire knock-out
20 mice. Eif5a2 in this dataset (4) was ranked 1097 out of 45,101 total transcripts. This means that Eif5a2
21 gene expression in Aire-deleted mTEC^{hi} changed in magnitude greater than 97.57% of the remaining
22 RNA messages in the transcriptome did. The global differential expression of Eif5a2 in this second
23 dataset was also statistically significant (Table 1; p=2.25E-02).
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Next, we obtained the exact mRNA expression values for Eif5a2 in the mTEC of both Aire-deficient and wild-type mice (3). We also performed a statistical test to measure whether the difference in mRNA expression levels between wild-type and Aire-deleted mTEC groups was considered statistically significant. Eif5a2 was expressed at higher levels in the mTEC of Aire knockout mice (Figure 1; 6.14 AU) than in the mTEC of wild-type mice (3.38 AU), and this difference in gene expression was statistically significant (Figure 1; $p=0.0045$).

Thus, we found that Eif5a2 was among the genes whose expression changed the most significantly out of all the transcripts expressed mTEC and mTEC^{hi}, and that Eif5a2 gene expression in Aire knock-out mTEC was significantly higher than in wild-type mTEC.

Discussion

We compared the transcriptomes of mTEC from wild-type and Aire-deficient mice using two independent published datasets (3, 4) to continue to understand the function of Aire at the transcriptional level in a global and unbiased manner and by genetic means. We found that mTEC lacking Aire expressed significantly higher amounts of mRNA for the eukaryotic translation initiation factor eIF5a2 and that Eif5a2 was one of the genes whose expression changed the most significantly considering the entire mTEC transcriptome. Eif5a2 is an isoform of the Eif5 eukaryotic translation initiation factor (6).

During the initiation of translation, or the step of protein synthesis where ribosomes are paired with mRNA messages that contain start codons to catalyze the synthesis of polypeptide chain of amino acids from of an mRNA template, eIF2 delivers the initiator methionyl-tRNA to the small ribosome subunit to form the ternary eIF2 - GTP - tRNA^{met} translation pre-initiation complex. While eIF5 acts as a GTPase activating protein, or GAP, inducing GTP hydrolysis and converting GTP to GDP, eIF5 also acts a GDP dissociation inhibitor (GDI), stabilizing binding of GDP to eIF2 and maintaining it in an inactive state (5). Thus, eIF5 not only catalyzes hydrolysis of eIF2 to its inactive GDP-bound state, it also stabilizes binding of eIF2 to GDP as a GDI, maintaining eIF2 in this inactive state (5). We suggest that by restricting the expression of eIF5a2, Aire subverts this translational control mechanism to support self-

antigen production during promiscuous gene expression by Aire in mTEC involved in negative selection.

Future studies can assess the role of Eif5a2 in protein synthesis during Aire-induced promiscuous gene expression and any impact it might have on recognition of self and negative selection using specific deletion of Eif5a2 in the mTEC of the thymus.

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Rank	ID	p-value	t	B	Gene	Gene name
GSE14365						
55	1434593_at	8.6E-05	1.24E+01	2.11491	Eif5a2	eukaryotic translation initiation factor 5A2
GSE102527						
1097	17396492	2.25E-02	3.11	-3.516636	Eif5a2	eukaryotic translation initiation factor 5A2

Table 1: Eif5a2 is one of the most differentially expressed genes in the Aire-deleted mTEC of the thymus.

The rank of differential expression, the transcript or probe ID, the p-value of differential expression, t, a moderated t statistic, B, the log-odds of differential expression between Aire-sufficient and Aire-deleted states, the log-fold change in expression between Aire-sufficient and Aire-deleted conditions, and the gene name is shown in this chart (all provided by GEO2R).

Figure 1: Negative regulation of Eif5a2 expression by Aire.

The mRNA expression level of Eif5a2 is shown in this bar graph, with the error bars denoting the standard error of the mean; $n=3$ for mTEC and $n=2$ for Aire -/- mTEC. A statistical test was performed to evaluate the significance of difference between Aire-sufficient and Aire-deleted conditions and the results of this test, a p-value, is shown on each graph.