AIRE controls the expression of IFIT3 in the thymus.

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Comparing the transcriptomes of a thymic cell line engineered to over-express the autoimmune regulator gene AIRE with that of its parental cell line using a public dataset (1) revealed that IFIT1, IFIT2 and IFIT3 were among the genes whose expression changed most significantly upon expression of AIRE, with a substantial increase in IFIT1, IFIT2, and IFIT3 expression. Analysis of a separate dataset showed that in the thymuses of mice engineered to lack a functional copy of the AIRE gene (2), IFIT3 expression was greatly reduced. After either deletion or ectopic expression of Aire, IFIT3 was identified as one of the most differentially expressed genes. IFIT3 expression was found to co-segregate with low rather than high expression of the class II antigen presentation complex MHC-II (3). We conclude that AIRE exerts control over the gene expression of IFIT3 through an as-of-yet unidentified mechanism not likely to reflect AIRE's role as a transcriptional inducer of gene expression of peripheral tissue antigens.

Keywords: AIRE, thymus, negative selection, IFIT3, IFIT2, IFIT1, differential gene expression, systems biology of the immunologically active cells of the thymus.

Introduction

AIRE is critical for the process by which tissue-restricted antigens (TRAs) are expressed in the thymus in order to select for and delete clones that might later recognize these TRAs (2). This process, known as negative selection (4), is essential to mitigate the risk that one's immune system will recognize self-antigen as foreign and cause an aberrant autoimmune reaction. We mined multiple datasets that focused on the autoimmune regulator, AIRE (1, 2, 3). One dataset examined the consequence of enforced expression of AIRE in a thymic cell line (1), while contrarily another assessed the consequence of deletion of the Aire gene in the thymus (2). Thymic cells that over-expressed Aire manifested significantly higher levels of IFIT3, while cells possessing a deletion of the Aire gene manifested over 75% reduction in IFIT3 levels. In both cases, IFIT3 was among the most differentially expressed genes after manipulation of Aire levels. Aire exerts control over IFIT3 expression through mechanisms that are not yet defined.

Methods

GSE35244 (1) was utilized for this analysis, along with GEO2R. Investigators used Affymetrix Human Genome U133 Plus 2.0 Array to create GSE35244.

GSE92654 (2) was also utilized for this analysis along with GEO2R. Investigators used Affymetrix Mouse Gene 1.0 ST Array to create GSE92654.

GSE84956 (3) was also utilized for this analysis along with GEO2R. Investigators used Illumina MouseRef-8 v2.0 expression beadchip to create GSE84956.

The “Holm” method of p-value adjustment was utilized for all three analyses. Log-transformation was applied to all data. The NCBI category of platform annotation was utilized for all analyses. A statistical test to evaluate the significance of difference in mRNA expression values was performed using a two-tailed, unpaired t-test with Welch’s correction using PRISM software (8.1.2) (227).

Results

We performed whole-transcriptome profiling of cells that have been manipulated *in vitro* or *in vivo* to over-express or possess deletions of the AIRE gene, respectively.

Using the TEC 1A3 thymic epithelial cell line, the transcriptomes (1) of cells that were engineered to over-express AIRE using a Flp-in recombinase-mediated over-expression scheme were subject to global differential gene expression profiling. We found that IFIT3 was, out of 54676 total transcripts produced by these cells, the 35th most differentially expressed gene upon over-expression of AIRE. This was statistically significant (Table 1; $p=2.02\text{E-}05$). Surprisingly, we also found IFIT2 and IFIT1 among the top 1% of the most differentially expressed genes in TEC1A3 cells upon expression of AIRE. IFIT2 ranked 32 and IFIT1 ranked 53 with respect to rank of differential expression globally (Table 1; $p=2.6\text{E-}05$ and $p=4.68\text{E-}05$, respectively).

We next extracted the exact mRNA expression values of IFIT2, IFIT3 and IFIT1 in both the parental TEC 1A3 cell line as well as the TEC 1A3 AIRE Flp-in variant that over-expressed AIRE. We also performed a statistical test to evaluate the significance of the difference in expression between the control and AIRE-induced states. Upon ectopic expression of AIRE, the mRNA of all three differentially expressed IFIT genes increased dramatically (Figure 1; IFIT2: $p=0.0013$; IFIT1: $p=0.0005$; IFIT3: $p=0.0002$).

Next, using transcriptome data (2) from mice that had been engineered to harbor deletions in the endogenous AIRE gene, the transcriptomes of cells of the thymus that had been isolated from these AIRE knock-out mice were subject to differential gene expression profiling. We found that in the thymus, IFIT3 was among the top 1% of genes that were most differentially expressed upon deletion of AIRE *in vivo*. IFIT3 was the 204th ranked gene with respect to global differential expression in AIRE-deleted cells of the medullary epithelium, out of 54675 total transcripts. This was statistically significant. (Table 1; $p=1.53\text{E-}07$). Upon deletion of AIRE in the thymus, the expression of IFIT3 substantially and significantly decreased (Figure 2; $p<0.0001$).

The medulla of the thymus is the site of negative selection in humans and mice (4, 5, 6). Cells of the epithelium of the medulla of the thymus (mTEC) can be distinguished based on their expression of MHC-II into cells known as mTEC^{hi} and mTEC^{lo} (7); MHC-II is the receptor by which cells of the thymus display self-antigen known as tissue-restricted antigen or peripheral tissue antigens to developing lymphocytes in order to delete lymphocytes that have the ability to recognize self during development (5, 9). Thus, we compared the transcriptomes of mTEC^{hi} and mTEC^{lo} to determine if IFIT3 was differentially expressed in mTEC^{hi} cells, as this would suggest that although IFIT3 did not possess the usual characteristics of a tissue-restricted antigen (TRA), that its expression might still be controlled in a similar manner as TRA. We found that though in fact, IFIT3 was among the most differentially expressed genes when comparing the transcriptomes of mTEC^{hi} and mTEC^{lo}, IFIT3 was expressed at significantly higher levels in mTEC^{lo} rather than mTEC^{hi} (Figure 3; $p=0.0032$). This suggests that IFIT3 expression is controlled by AIRE but that it is not expressed in a manner typical to tissue-restricted antigens whose expression is controlled by AIRE, which are preferentially expressed in mTEC^{hi} (8, 9, 10).

Discussion

In this study we used three separate datasets containing transcriptome data to assess in a systematic and unbiased manner the role of AIRE on gene expression in cells of the thymus *in vitro* and *in vivo*. We found that IFIT1, IFIT2, and IFIT3 were among the genes most differentially expressed by cells over-expressing AIRE *in vitro*. IFIT3 expression was dramatically reduced in the thymic epithelial cells of AIRE-deleted mice. Moreover, IFIT3 was differentially expressed when comparing the transcriptomes of mTEC^{lo} and mTEC^{hi} but its expression was higher in mTEC^{lo} and not mTEC^{hi}.

The fact that control of IFIT3 expression does not correlate or co-segregate with MHC-II expression is evidence that IFIT3 is not a typical gene that AIRE controls the expression of for presentation by MHC-II for the process of negative selection.

IFITs are genes that possess anti-viral activity against a broad host of viruses (11). IFIT1 functions in an anti-viral manner by inhibiting the translation of viral proteins by binding to the 5' end of viral RNAs through a 5' triphosphate group on the viral RNA (PPP-RNA) (12, 13) which prevents recruitment of the ribosomal machinery for protein translation (14). West Nile Virus deficient in the ability to 2'O-methylate the 5' cap of viral RNAs was specifically compromised by IFITs, demonstrating that viruses subvert IFIT anti-viral function by modifying their own RNAs with the 2'O-methyl mark at the 5' cap of their RNAs (13). A more recent study showed that this anti-viral function of IFIT1 required IFIT3 and that IFIT3 enhanced the stability of the IFIT1 protein (15).

Other mechanisms aside from inhibition of the translation of viral proteins have been implicated as a function of IFIT proteins. An innate immune pathway that initiates interferon signaling through TBK1 and IRF3 is triggered by sensing of RNA viruses by the protein MAVS. One study showed that IFIT3 bridged the gap between MAVS and TBK1/IRF3 after activation of MAVS by pattern recognition of intracellular RNA viruses (16).

In summary, we found that AIRE controls the expression of IFIT3 and potentially of IFIT1 and IFIT2 as well. IFIT1, IFIT2, and IFIT3 all interact with each other. IFIT2 and IFIT3 homodimers preferentially disassociate to form IFIT2/3 heterodimers that can interact with IFIT1 (15). Control of IFIT3 or other IFIT genes by AIRE has not been previously reported. We cannot rule out a role for IFIT3 as a TRA/PTA but our analysis here argues against this. Further studies should assess how and why AIRE controls IFIT3 as well as IFIT1 and IFIT2, by assessing the function of IFIT3 and other IFITs in the thymus, by analysis of the IFIT3 locus and by assessment of the effect of AIRE deletion on IFIT gene product function in the thymus *in vivo*, respectively.

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Figure 1: IFIT2, IFIT3, and IFIT1 are among the most differentially expressed genes in the transcriptomes of TEC 1A3 cells engineered to over-express AIRE.

The levels of IFIT2, IFIT3 and IFIT1 are graphically represented as a bar graph, with the error bars denoting the standard error of the mean (left) and as dots with the horizontal black line representing the mean value (right). A statistical test was performed to evaluate the significance of the difference in IFIT gene expression between control and AIRE-expressing groups; the result of this test is shown as a p-value on each graph.

Figure 2: IFIT3 is one of the most differentially expressed genes in the thymic medullary epithelial cells of mice engineered to harbor a deletion in the AIRE gene.

The level of IFIT3 is graphically represented as a bar graph, with the error bars denoting the standard error of the mean (left) and as dots with the horizontal black line representing the mean value (right). A statistical test was performed to evaluate the significance of the difference in IFIT gene expression between control and AIRE-deficient groups; the result of this test is shown as a p-value on the graph.

Figure 3: When mTEC are sorted based on their levels of MHC-II, IFIT3 does not segregate with MHC-II expression.

The level of IFIT3 is graphically represented as a bar graph, with the error bars denoting the standard error of the mean (left) and as dots with the horizontal black line representing the mean value (right). A statistical test was performed to evaluate the significance of the difference in IFIT gene expression between control and AIRE-deficient groups; the result of this test is shown as a p-value on the graph.

Table 1

Rank	ID	p-value	t	B	Gene	Gene Name
GSE35422						
32	226757_at	2.02E-05	1.39E+01	3.090374	IFIT2	interferon induced protein with tetratricopeptide repeats 2
35	229450_at	2.6E-05	1.32E+01	2.929866	IFIT3	interferon induced protein with tetratricopeptide repeats 3
53	203153_at	4.68E-05	1.18E+01	2.52674	IFIT1	interferon induced protein with tetratricopeptide repeats 1
GSE92654						
204	10462618	1.53E-07	-11.951772	7.97739	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
GSE84956						
168	ILMN_1230458	1.5E-07	-1.53E+01	8.212292	IFIT3	interferon-induced protein with tetratricopeptide repeats 3

Table 1: IFIT genes are differentially expressed in cells of the thymus.

The rank of global differential expression, ID (probe or transcript ID), p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between control and AIRE-manipulated groups (or between mTEC^{hi} and mTEC^{lo}), as well as the gene name and gene symbol are shown in the table above.