Coding and non-coding RNAs transcribed at the RORA locus (ROR α) are differentially expressed in the whole blood of sepsis patients.

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Sepsis is a leading cause of mortality¹. We mined published datasets from the whole blood of patients with sepsis to identify differentially expressed genes in the septic state^{2,3}. We found changes in RORA expression as among the most significant quantitative differences in sepsis whole blood gene expression. Analysis of a separate dataset⁴ demonstrated significant repression of a long non-coding RNA produced at the RORA locus in the blood of patients with sepsis.

Keywords: sepsis, septic shock, RORA, lncRNA-RORA-2:1, non-coding RNAs in human disease.

Sepsis is a leading cause of mortality in the United States¹. There are zero FDA-approved targeted treatments for patients with sepsis and septic shock. To understand the transcriptional landscape of human blood during conditions of sepsis we used published and public microarray datasets to compare whole blood gene expression profiles of control subjects to that of pediatric and adult patients with sepsis²⁻⁴. We identified coding and non-coding transcripts produced from the RORA gene as among the most differentially expressed in the whole blood of patients with sepsis. RORA may be relevant to the biology of sepsis.

Methods

We used datasets GSE100159, GSE26440, and GSE145227 for this differential gene expression analysis.

GSE100509 was generated with whole blood using Illumina HumanWG-6 v3.0 expression beadchip technology with n=12 control subjects and n=33 sepsis patients. GSE26440 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with n=32 control subjects and n=98 sepsis patients. GSE145227 was generated with whole blood using Affymetrix Human Custom lncRNA Array with n=10 control subjects and n=12 sepsis patients. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether RORA expression was significantly different when comparing whole blood from control subjects and whole blood from patients with sepsis using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray datasets²⁻⁴ to identify genes whose expression was most different in whole blood of patients with sepsis when compared to control subjects.

RORA is differentially expressed in the blood of patients with sepsis.

We identified a RORA transcript as among the most differentially expressed when comparing global gene expression profiles of whole blood from control subjects and whole blood from patients with sepsis. When sorting each transcript detected by microarray based on change in expression between control subjects and septic patients, RORA ranked 89 out of 48803 total transcripts. Differential expression of RORA was statistically significant (Table 1; $p=5.7E-06$).

In an independent sepsis whole blood microarray dataset, we identified RORA as among the most differentially expressed when comparing global gene expression profiles of whole blood from control subjects and whole blood from patients with sepsis. When sorting each transcript detected by microarray based on change in expression between control subjects and septic patients in this dataset, RORA ranked 109 out of 54675 total transcripts. Differential expression of RORA was statistically significant (Table 1; $p=2.36E-11$).

RORA is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

We obtained exact mRNA expression levels for the differentially expressed RORA transcript to directly compare RORA expression in the whole blood between control and septic patients. RORA was expressed at lower levels in the whole blood of patients with sepsis; this difference was statistically significant (Figure 1A; $p<0.0001$). We calculated a fold change of 0.1799 ± 0.1153 in RORA expression when comparing the whole blood of patients with sepsis to that of control subjects (Table 1).

1 In a separate patient dataset, we confirmed lower expression of RORA expression in the whole
2 blood of patients with sepsis as compared to the whole blood of control, non-infected patients
3 (Figure 1). This difference again, was statistically significant (Figure 1B; $p < 0.0001$). We
4 calculated a fold change of 0.7819 ± 0.1207 in RORA expression when comparing the whole
5 blood of patients with sepsis to that of control subjects (Table 1).

7 A long non-coding RNA produced at the RORA locus, lncRNA-RORA-2:1, is differentially
8 expressed in the blood of patients with sepsis.

9 We integrated our mRNA expression analysis of whole blood in sepsis with analysis of a
10 third public microarray dataset, this detecting non-coding RNAs globally by array in the whole
11 blood of patients with sepsis. We identified a long non-coding RNA transcript, lncRNA-
12 RORA-2:1, that was among the most differentially expressed non-coding RNAs when
13 comparing the blood of control subjects and the blood of patients with sepsis. When sorting
14 each transcript detected by microarray based on change in expression between control
15 subjects and patients with sepsis, lncRNA-RORA-2:1 ranked 89 out of 95722 total transcripts.
16 Differential expression of lncRNA-RORA-2:1 was statistically significant (Table 2; $p = 5.7E-06$).

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19 lncRNA-RORA-2:1 is expressed at significantly lower levels in the blood of patients with sepsis
20 as compared to control subjects.

21 Finally, we sought to determine, regardless of how large the magnitude of difference in
22 lncRNA-RORA-2:1 expression was relative to the magnitude of difference of the non-coding
23 RNAs in the whole blood transcriptome, whether the difference in whole blood lncRNA-
24 RORA-2:1 expression was statistically significant when comparing patients with sepsis and
25 control patients. lncRNA-RORA-2:1 was expressed at lower levels in the whole blood of
26 patients with sepsis and this difference was statistically significant (Figure 2; $p = 0.0002$). We
27 calculated a fold change of 0.7819 ± 0.1207 in lncRNA-RORA-2:1 expression when comparing
28 the whole blood of patients with sepsis to that of control subjects (Table 2).

Thus, using three independent published and public microarray datasets, we identified coding and non-coding transcripts produced at the RORA locus as among the most differentially expressed in the whole blood during sepsis in adults and in children.

Discussion

Sepsis is a leading cause of mortality in the United States and there are limited targeted therapeutic options for sepsis and septic shock¹. To understand how gene expression was most significantly different in the whole blood of patients with sepsis, we integrated data analysis from multiple public and published microarray datasets²⁻⁴. We identified coding and non-coding RNAs produced at the RORA locus, lncRNA-RORA-2:1, as among the most differentially expressed transcripts in the blood of patients with sepsis.

Retinoic-acid-receptor-related orphan nuclear receptor alpha was first identified as rearranged in retinoic acid-sensitive promyelocytic leukemias⁵. Early indications that RORA might have important functions in blood cells was that expression of a dominant negative RORA mutant inhibited retinoic acid-induced differentiation of the HL60 cell line into the neutrophil lineage⁶. Moreover, expression of this RORA mutant in IL3-dependent FDCP mix A4 cells lead to a developmental switch, wherein the differentiation of these cells to the neutrophil and monocyte lineages was shifted towards the basophil and mast cell lineages⁶. Separate studies indicated that RORA is important for the allergic response and for the development of natural killer cells⁷. An established and critical function of RORA is in the lineage commitment of T_H17 lymphocytes⁸. Retinoic acid functions in part through activation of RORa, acting as an inhibitor of Nrf2⁹. Outside of the cells of the blood, RORA is involved in transcriptional regulation of the circadian clock¹⁰. RORA activation of Bmal1, a transcriptional activator of the circadian clock oscillator, in the suprachiasmatic nucleus functions in balance with a repressor of Bmal1, Rev-Erb α , to ensure regular circadian clock function, a key regulator of locomotor activity¹⁰.

1 We found significant differential and decreased expression of non-coding and coding
2 RNA transcripts from the RORA locus in the whole blood of patients with sepsis, both pediatric
3 and adult, using published and public microarray data. A recent study identified a non-coding
4 RNA transcript produced at the RORA locus, lncRNA-RORA-2 as possessing diagnostic value
5 in pediatric sepsis; it is unclear whether this non-coding transcript is the same as the one
6 identified here as differentially expressed in whole blood from patients with sepsis¹¹.
7 Conditional depletion and deletion of RORA in individual hematopoietic cell types can provide
8 answers as to whether modulation of RORA or lncRNA-RORA-2:1 expression has any effect on
9 septic shock outcomes experimentally.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
109	ILMN_1867517	2.36E-11	-8.7335375	15.755466	0.1799 ± 0.1153	RORA	RAR related orphan receptor A
137	226682_at	2.61E-21	-11.385077	37.879265	0.2141 ± 0.2027	RORA	RAR related orphan receptor A

Table 1: RORA is differentially expressed in the blood of patients with sepsis.

The rank of differential expression, the probe ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change (FC) of RORA in whole blood from sepsis patients relative to that in controls, the gene and gene name are listed in this chart. Table 1 contains data from microarray experiments GSE100159 (probe ID ILMN_1867517) and GSE26440 (probe ID 226682_at).

Figure 1: RORA is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

The mRNA expression of RORA in whole blood from control subjects (left) and in whole blood from patients with sepsis (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in RORA expression between control subjects and patients with sepsis, a p-value, listed above.

Rank	ID	p-value	t	B	Fold change	Transcript
89	TC1500001671.oe.1	5.7E-06	-5.89	4.057331	0.7819 ± 0.1207	lnc-RORA-2:1

Table 2: A long non-coding RNA produced at the RORA locus is among the most differentially expressed RNAs in the blood of patients with sepsis.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change (FC) of lncRNA-RORA-2:1 in whole blood from sepsis patients relative to that in controls, and transcript name are listed in this chart. n/d: not detected.

Figure 2: lncRNA-RORA-2:1 is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

The mRNA expression of lncRNA-RORA-2:1 in whole blood from control subjects (left) and in whole blood from patients with sepsis (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in lncRNA-RORA-2:1 expression between control subjects and patients with sepsis, a p-value, listed above.