HNMT is differentially expressed in monocytes from patients with sepsis.

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We probed published and public microarray datasets^{1,2} to discover the most significant gene expression changes in the blood and blood cells of patients with sepsis. We identified significant differential expression of the histamine N-methyltransferase HNMT in monocytes and whole blood from patients with sepsis.

Keywords: sepsis, septic shock, histamine, HNMT, histamine N-methyltransferase, targeted therapeutics in septic shock.

1 Septic shock is a leading cause of mortality in the United States and worldwide³. We
2 used published and public microarray datasets^{1,2} to identify differentially expressed genes in the
3 blood of patients with sepsis. We identified HNMT as among the genes most differentially
4 expressed in blood and monocytes in the septic state.
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6 **Methods**

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9 We utilized microarray datasets GSE100159¹ and GSE46955² (platform GPL6104) for
10 this differential gene expression analysis of blood cells during sepsis. GSE100159 was
11 generated with whole blood using Illumina HumanWG-6 v3.0 expression beadchip technology
12 with n=12 whole blood from control subjects and n=33 whole blood from sepsis patients.
13 GSE46955 was generated using Illumina humanRef-8 v2.0 expression beadchip technology with
14 n=3 purified monocytes from healthy controls and n=3 purified monocytes from sepsis patients.
15 The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential
16 expression but raw p-values were used for assessment of statistical significance of global
17 differential expression. Log-transformation of data was auto-detected, and the NCBI generated
18 category of platform annotation was used. A statistical test was performed to evaluate whether
19 HNMT expression was significantly different when comparing whole blood or monocytes from
20 control subjects and whole blood or monocytes from patients with sepsis using a two-tailed,
21 unpaired t-test with Welch's correction. GEO2R provides information on differential expression
22 for every transcript measured by microarray but exact mRNA expression values only for the top
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250 most differentially expressed genes. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published and public microarray datasets to identify differentially expressed genes in patients with sepsis, in the whole blood and in monocytes.

HNMT is differentially expressed in monocytes from patients with sepsis

We identified histamine N-methyltransferase (HNMT) as among the most differentially expressed genes in the sepsis transcriptome. When sorting each transcript measured by significance of change in expression between monocytes from control subjects and monocytes from patients with sepsis, HNMT ranked 63 out of 22184 total transcripts, or differential expression greater than 99.7% of the transcripts measured globally (Table 1). Differential expression of HNMT in monocytes from patients with sepsis was statistically significant (Table 1; $p=7.58E-09$).

HNMT is differentially expressed in whole blood from patients with sepsis

We probed datasets to determine if we could observe differential expression of HNMT gene in any independent microarray data. We observed significant differential expression of HNMT in whole blood from patients with sepsis in a second microarray dataset (Table 2). When sorting each transcript measured by significance of change in expression between whole blood from control subjects and whole blood from patients with sepsis, HNMT ranked 5481 out of

48803 total transcripts, or differential expression greater than 88.7% of the transcripts measured globally (Table 2). Differential expression of HNMT in the whole blood of patients with sepsis was statistically significant (Table 2; $p=1.11\text{E-}03$).

HNMT is expressed at significantly higher levels in monocytes from patients with sepsis

We obtained exact mRNA expression values for the differentially expressed HNMT transcript in monocytes from patients with sepsis and from control subjects to directly compare HNMT expression. We also performed a statistical test to determine if this difference was significantly different. HNMT was expressed at higher levels in monocytes from patients with sepsis and this difference was statistically significant (Figure 1; $p=0.0001$). We calculated a 1.2012 ± 0.0112 fold change in HNMT gene expression when comparing monocytes from patients with sepsis to monocytes from healthy controls (Table 1).

Discussion

Metabolism of histamine can proceed through two mechanisms; N(tau)-methylation using histamine N-methyltransferase (HNMT) or oxidative deamination using diamine oxidase^{4,5}. In conjunction with S-adenosylmethionine (SAM), HNMT catalyzes the conversion of histamine to N-methylhistamine^{4,5}. Methylhistamine is thought to be negligibly active^{4,6}; methylation of histamine is thus considered to be inactivating.

Histamine is present at increased levels in the plasma of patients with sepsis and in experimental sepsis models⁷⁻⁹. One study showed that plasma histamine increased from day 1 to

day 4 in patients with sepsis. In 9 out of 20 patients with sepsis, plasma concentration of histamine reached 1 nanogram per microliter (1 ng/uL); 0 out of 20 control patients achieved the 1 ng/uL plasma histamine threshold⁷. In patients not surviving sepsis (9/20 patients), plasma histamine was significantly higher than in patients surviving sepsis (11/20 patients)⁷. Finally, all patients with a low Elebute and Stoner Sepsis Score (<20 points) and plasma histamine concentration less than 1 ng/uL on day 1 survived, while all patients with a high sepsis score (>20 points) and plasma histamine concentration greater than 1 ng/uL expired⁷. Thus, increased plasma histamine was associated with sepsis outcome and severity.

Histamine receptor blockade pharmacologically has been shown to be efficacious in experimental models of sepsis^{10,11}. In animals with septic shock treated with 3-10mg/kg, severe blood pressure drops and reduced urinary output were prevented; this benefit was evident even when administered after shock¹⁰. In endotoxin-induced septic shock in mice, fatality could be prevented in 90%-100% of mice by pre-treatment with the antihistamine drug hydroxyzine hydrochloride at 5mg/kg; with diphenhydramine, 60% of endotoxin-induced sepsis fatalities could be prevented¹¹. Thus, in mice, administration of antihistamines appears to confer some level of survival benefit even when the drug is provided after endotoxin delivery.

We found here that changes in expression of the histamine N-methyltransferase HNMT gene in monocytes from patients with sepsis were among the most significant transcriptome-wide. It is unclear how increases in expression of an enzyme that inactivates histamine translate to experimental therapeutic benefit of histamine receptor antagonism but antihistamine interventions in patients with sepsis or patients at high-risk of developing sepsis may be worthy of clinical follow-up.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
63	ILMN_1751789	7.58E-09	1.22E+01	10.780848	1.2012 ± 0.0112	HNMT	histamine N-methyltransferase

Table 1: HNMT is differentially expressed in monocytes from 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 of HNMT in monocytes from patients with sepsis compared to monocytes from control subjects, the fold change in HNMT expression in monocytes from patients with sepsis, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
5481	ILMN_1790881	1.11E-03	-3.4780012	-1.274463	HNMT	histamine N-methyltransferase

Table 2: HNMT is differentially expressed in whole blood from 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 of HNMT in whole blood from patients with sepsis compared to whole blood from control subjects, gene and gene name are listed in this chart.

Figure 1: HNMT is expressed at significantly higher levels in monocytes from patients with sepsis.

The mRNA expression of HNMT in monocytes from control subjects (left) and in monocytes 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 HNMT expression between control monocytes and monocytes from patients with sepsis, a p-value, listed above.