Transcriptomic evidence for dramatic functional transition of the Malpighian Tubules after a blood meal in the Asian tiger mosquito Aedes albopictus

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Abstract: Vector-borne diseases transmitted by mosquitoes are important for global health, because cause hundreds of thousands of deaths and sickening hundreds of millions people each year. The Asian tiger mosquito (Aedes albopictus), is enhancing this risk in the world and the United States. Ae. albopictus is vector of diseases such as West Nile fever, dengue fever, chikungunya fever, and Eastern Equine Encephalitis. To date, this research has used single-end RNA-Seq to demonstrate that a blood meal affects the expression of more than 1800 non-redundant transcripts and 15 metabolic pathways in Malpighian tubules of the Asian tiger mosquito, in a manner that suggests a potential functional transition in the epithelium from one dedicated to salt and water excretion to one dedicated to the metabolism and excretion of metabolites derived from a blood meal.

Introduction: The Asian tiger mosquito Ae. albopictus is one of the most invasive mosquito species in the world, and in the US there is an alarming expansion rate (Figure 1). Also this species is vector of important medically arboviruses such as dengue fever and chikungunya fever. Historically mosquitoes have been controlled through the use of pesticides that target the nervous system, however resistance to these chemicals is limiting their efficacy and there is an urgent need to develop new insecticides.

Malpighian tubules are the ‘kidneys’ of mosquitoes; they play a critical role in the acute processing of blood meals (within 1-2 h after engorgement) by excreting the excess salt and water that are ingested. However, our understanding of how the Malpighian tubules contribute to the chronic processing of blood meals (3-24 h after engorgement) is not well understood. Given that we have recently shown that the aforementioned acute diuretic mechanisms of mosquitoes are valuable new physiological targets for insecticides (Raphemont et al 2013), elucidating the roles of the Malpighian tubules in the chronic processing of blood meals may reveal additional mechanisms to target for the development of new insecticides to combat resistance.

This study tests the hypothesis that a blood meal causes significant changes to the expression of genes in the Malpighian tubules after adult female, Aedes albopictus mosquitoes consume a blood meal.

The goal of the study was to characterize the chronic changes in the gene expression that occur in the Malpighian tubules of Aedes albopictus using RNA-Seq during the first 24 h after females mosquitoes consumes a blood meal.

The significance of our study is that it will reveal which genes and metabolic pathways are involved in the chronic processing of blood meals by Malpighian tubules and help identify new potential insecticide targets.

Material and methods:

Ae. albopictus (ALBOPICTUS, MRA-804, deposited by Sandra Allan) were reared following the protocols described by Raphemont et al 2013.

The experimental design consisted of two treatments 1) Malpighian tubules from blood fed females (BF), and 2) Malpighian tubules from non-blood fed females (NBF). In both treatments, females were dissected at 3 h, 12 h and 24 h after treated.

Each treatment/time point had three biological replicates. A biological replicate consisted of RNA isolated from Malpighian tubules of 40 females.

With the RNA isolated, cDNA libraries were synthesized (fragments ~270 bp), pooled and sequenced (single-end reads 50 bp in length) using illumina® platform and protocols.

Raw reads were processed and analyzed using bioinformatics tools combined into the online-based software “MCIC-Galaxy pipeline”. Aedes aegypti transcriptome was used as annotation reference. Subsets of transcripts differentially expressed were submitted to functional pathway analysis using ‘DAVID v6.7 annotation clustering module’.

Manual searches were performed looking for genes that are known to be involved in urine production and post-prandial processes in Malpighian tubules.

Results:

Over 232 single-end raw reads (~13 million per sample) were aligned onto Aedes aegypti transcriptome, generating over 42 millions reads successfully mapped. A principal component analysis (PCA) of the sequencing libraries revealed global changes at the transcriptome level in treatments and time points (Figure 2).

The differential gene expression analysis revealed more than 2600 transcripts with significant up- or down-regulated (Table 1).

Table 1. Number of transcripts whose expression is significantly affected by blood feeding compared to non-blood-fed controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>All time points</th>
<th>Up-regulated</th>
<th>Down-regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 h BF</td>
<td>564</td>
<td>207</td>
<td>192</td>
</tr>
<tr>
<td>12 h BF</td>
<td>564</td>
<td>207</td>
<td>192</td>
</tr>
<tr>
<td>24 h BF</td>
<td>564</td>
<td>207</td>
<td>192</td>
</tr>
<tr>
<td>Total</td>
<td>1324</td>
<td>665</td>
<td>669</td>
</tr>
</tbody>
</table>

All the transcripts up- or down-regulated at two consecutive time points were submitted to perform functional clustering analysis using DAVID. A total of 340 up-regulated and 329 down-regulated transcripts met this criteria, revealing nine functional groups among the up-regulated and six among the down-regulated transcripts (Table 2).

Major findings:

1) The up-regulated functional pathways (e.g., oxidative phosphorylation, ATPase activity, glycolysis and sugar/inositol transporters) suggest a decrease in ATP synthesis and active transport by Malpighian tubules, which may reflect a decrease in diuresis after a blood feeding. Consistent with this notion, several transcripts encoding ion transporters and aquaporins were also down-regulated after blood feeding (Figure 3).

2) The up-regulated functional pathways (e.g., thioredoxin, glutathione S-transferase) suggest that the Malpighian tubules an increase in the capacity for the biodegradation of metabolites associated with a blood meal, such as toxic heme metabolites. Consistent with this notion, several transcripts encoding metabolite transporters (within the ATPase/AAA+ functional I pathway) were also up-regulated after blood feeding (Figure 3).

Conclusions and future directions:

1) Our study provides the first insights into the putative functional roles of mosquito Malpighian tubules in the chronic processing of blood meals.

2) Our molecular results suggest that the Malpighian tubules undergo a dramatic functional transition after blood feeding; i.e., they appear to change from an epithelium dedicated to salt and water balance to one dedicated to metabolite detoxification and excretion.

3) Functional assays are required to confirm a functional transition of the Malpighian tubules.

4) If the functional transition is confirmed then pharmacological inhibitors of metabolic enzymes and metabolite transporters should be explored as new potential insecticidal agents that could disrupt the renal processing of blood meals in mosquitoes.

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Literature cited:

