Vaccine Induced Differential Expression of miRNAs at Cytolytic Stage in Chickens Resistant or Susceptible to Marek's Disease

Huanmin Zhang, Qingmei Xie, Shuang Chang, Yanghua He, Catherine W. Ernst, and Jiuzhou Song
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Jan. 10, 2015

Lines 6, 7, and MD Vaccines

HVT or Rispens Commercial Dosages; Trial 1: 2000 PFU HVT or Rispens; Trial 2:

MicroRNA and Vaccination

This study was designed to:
– Profile miRNAs induced by MD vaccines in a MD resistant line (63) and a susceptible line (72) of chickens by deep-sequencing.
– Identify miRNAs differentially expressed between HVT and Rispens groups within each line.
– Identify miRNAs differentially expressed between the lines for each vaccine treatment.
– Predict target genes of the differentially expressed miRNAs.
– Explore genetic networks underlying differentially expressed miRNAs’ target genes.

Small RNA Sequence (PF) Reads

<table>
<thead>
<tr>
<th>Sample</th>
<th>Barcode</th>
<th>PF Reads</th>
<th>% of PF Reads</th>
<th>R1 % ≥ Q30</th>
<th>R1 Ave Q-Score</th>
<th>Insert Size (bp)</th>
<th>Yield (Gbp)</th>
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</thead>
<tbody>
<tr>
<td>6;Control</td>
<td>CGATGT</td>
<td>18,113,545</td>
<td>15.2%</td>
<td>95.8%</td>
<td>37.4</td>
<td>27</td>
<td>0.91</td>
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<td>6;HVT</td>
<td>TTAGGC</td>
<td>14,877,701</td>
<td>12.5%</td>
<td>96.6%</td>
<td>38.0</td>
<td>19</td>
<td>0.74</td>
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<tr>
<td>6;Risp</td>
<td>GCCAAT</td>
<td>26,256,599</td>
<td>22.8%</td>
<td>95.8%</td>
<td>37.4</td>
<td>36</td>
<td>1.31</td>
</tr>
<tr>
<td>7;Control</td>
<td>ACTTGA</td>
<td>27,906,779</td>
<td>23.6%</td>
<td>95.8%</td>
<td>37.5</td>
<td>30</td>
<td>1.61</td>
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<tr>
<td>7;HVT</td>
<td>GATCAG</td>
<td>16,325,605</td>
<td>13.7%</td>
<td>96.8%</td>
<td>38.0</td>
<td>32</td>
<td>0.82</td>
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<td>7;Risp</td>
<td>CTTGTA</td>
<td>14,659,345</td>
<td>12.3%</td>
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<td>1,303,711</td>
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<td>36.7</td>
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<tr>
<td>Total</td>
<td></td>
<td>119,443,285</td>
<td>5.98</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

miRNA Sequence Data Analysis

– miRDeep*, version 3.1 using default setting of parameters except:
  – Gallus Gallus 4.0 genome indices were used;
  – Input adaptor sequence (illumine): TGGATTCTCGGGTGCCAAGGAACTCCAGTCAC.
– Input file format: FastQ.
– Reads with ≥ 20 phred score (99% base call accuracy) was classified as good reads.

miRNA Sequence Data Analysis

– Reads aligned to > 100 loci were also filtered out.
– Reads mapped to tRNA, snoRNA and piRNA loci were also excluded.
– PF reads were normalized with TPM.
– miRNA isoforms are those variants that share 90% sequence homology with a mature miRNA.
– Target genes of both known and novel miRNAs are predicted using the TargetScan algorithm and are ranked by confidence score.
– Pathways were predicted using DAVID.

An et al., 2013. Nucleic Acids Res. 41(2):727
Levis et al., 2005. Cell 120(1):15
miRNA Profiles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Line 6</th>
<th>Line 7</th>
<th>In Common</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Known</td>
<td>Novel</td>
</tr>
<tr>
<td>Control</td>
<td>418</td>
<td>149</td>
<td>269</td>
</tr>
<tr>
<td>HVT</td>
<td>372</td>
<td>155</td>
<td>217</td>
</tr>
<tr>
<td>Rispens</td>
<td>400</td>
<td>144</td>
<td>256</td>
</tr>
</tbody>
</table>

In Common: C&H (Unique: C/H) 271 (148/101) 142 129 140 128 105
In Common: C&R (Unique: C/R) 271 (127) 137 134 259 134 116 136 107
In Common: H&R (Unique: H/R) 244 (10) 138 106 229 141 88 129 31

Lines 6, HVT/Control
19 Up- and 39 Down-regulated Expression of miRNAs
(Log2 Fold Change > 1.5 & P < 0.05)

Lines 6, Rispens/Control
15 Up- and 2 Down-regulated Expression of miRNAs
(Log2 Fold Change > 1.5 & P < 0.05)

Lines 7, HVT/Control
17 Up- and 0 Down-regulated Expression of miRNAs
(Log2 Fold Change > 1.5 & P < 0.05)

Lines 7, Rispens/Control
13 Up- and 44 Down-regulated Expression of miRNAs
(Log2 Fold Change > 1.5 & P < 0.05)

Line 6 / Line 7, HVT
0 Over- and 71 Under-Expressed miRNAs
(Log2 Fold Change > 1.5 & P < 0.05)
Over- and Under-expressed miRNAs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>miRNAs</th>
<th>Target Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtotal</td>
<td>Known</td>
<td>Novel</td>
</tr>
<tr>
<td>Line 6, HVT/Contr</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Line 6, Risp/Contr</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Line 7, HVT/Contr</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Line 7, Risp/Contr</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Lines 6/7, HVT</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Lines 6/7, Risp</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
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Numbers of Differentially Expressed miRNAs and target Genes

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</tr>
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</table>

### Line 6, HVT/Control Target Genes

1. Cell adhesion molecules PW
2. Cytokine-cytokine receptor interaction PW
3. ECM-receptor interaction PW
4. ERBB signaling PW
5. Focal adhesion PW
6. Lysine degradation PW

### Line 6, Risp/Control Target Genes

1. Endocytosis PW
2. Gap junction PW
3. MAPK signaling PW
4. Melanogenesis PW
5. Oocyte meiosis PW
6. Ubiquitin mediated proteolysis PW
7. Wnt signaling pathway
Line 7, HVT/Control Target Genes
(Log, Fold Change ≥ 2; In contrast to Rispens)

1. Adherens junction
2. Endocytosis
3. Focal adhesion
4. MAPK signaling
5. O-Glycan biosynthesis
6. P53 signaling
7. Phosphatidylinositol signaling system
8. Progesterone-mediated oocyte maturation
9. Tight junction
10. Vascular smooth muscle contraction
11. Wnt signaling

Numbers of Differentially Expressed miRNAs and target Genes
(Log, Fold Change ≥ 2)

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<tr>
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<tr>
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<td>28</td>
<td>3</td>
</tr>
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<td>6/7, Risp</td>
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<td>2</td>
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</tbody>
</table>

Line 7, Risp/Control Target Genes
(Log, Fold Change ≥ 2; In contrast to HVT)

1. Adherens junction
2. Adipocytokine signaling
3. Calcium signaling
4. ErbB signaling
5. Focal adhesion
6. Gap junction
7. Insulin signaling
8. Keratan sulfate biosynthesis
9. MAPK signaling
10. Melanogenesis
11. N-Glycan biosynthesis

Numbers of Differentially Expressed miRNAs and target Genes
(Log, Fold Change ≥ 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>miRNAs</th>
<th>Target Genes</th>
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<tbody>
<tr>
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<tr>
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<td>28</td>
<td>3</td>
</tr>
<tr>
<td>6/7, Risp</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

Line 6/7, HVT Target Genes
(Log, Fold Change ≥ 2; In contrast to Rispens)

1. Adherens junction
2. Adipocytokine signaling
3. Endocytosis
4. ErbB signaling
5. Focal adhesion
6. Gap junction
7. Glycolysis
8. Gsk3 signaling
9. Hedgehog signaling
10. Insulin signaling
11. Jak-STAT signaling
12. MAPK signaling
13. Melanogenesis
14. Phosphatidylinositol signaling
15. Progesterone-mediated oocyte maturation
16. Regulation of actin cytoskeleton
17. Tight junction
18. Vascular smooth muscle contraction
19. Wnt signaling

Numbers of Differentially Expressed miRNAs and target Genes
(Log, Fold Change ≥ 2)

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<tr>
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</table>
**Line 6/7 Risp Target Genes**

1. Cell adhesion molecules
2. Endocytosis
3. Focal adhesion
4. Keratan sulfate biosynthesis
5. MAPK signaling
6. mTOR signaling
7. N-Glycan biosynthesis
8. Notch signaling
9. Phosphatidylinositol signaling
10. Tight junction
11. Wnt signaling

**Summary (Profiles)**

- Identified over 400 miRNAs in each of the 2 lines in the control groups; 280 of the 400 plus miRNAs are in common between the lines.
- HVT induced miRNAs in both lines, but about 100 more miRNAs in line 6 than line 7.
- Rispens induced approximately similar numbers of miRNAs in lines 6 (400) and 7 (359).

**Summary (Differentially expressed within a line)**

- HVT-induced 58 miRNAs differentially expressed in line 6 compared to its control.
- Rispens-induced 17 miRNAs differentially expressed in line 6.
- HVT-induced 17 miRNAs differentially expressed in line 7 compared to its control.
- Rispens-induced 57 miRNAs differentially expressed in line 7.

**Summary (Differentially expressed between the lines 6/7)**

- HVT-induced 71 miRNAs differentially expressed between lines 6 and 7;
  - 3,714 target genes
  - May be involved in 19 pathways
- Rispens-induced 50 miRNAs differentially expressed between lines 6 and 7;
  - 2,175 target genes
  - Maybe involved in 11 pathways