Complex Regulatory Network of MicroRNAs, Transcription Factors, Gene Alterations in Adrenocortical Cancer

Bo Zhang¹,², Zhi-Wen Xu¹,²*, Kun-Hao Wang¹,², Tian-Cheng Lu³, Ye Du⁴*

Abstract

Several lines of evidence indicate that cancer is a multistep process. To survey the mechanisms involving gene alteration and miRNAs in adrenocortical cancer, we focused on transcriptional factors as a point of penetration to build a regulatory network. We derived three level networks: differentially expressed; related; and global. A topology network was then set up for development of adrenocortical cancer. In this network, we found that some pathways with differentially expressed elements (genetic and miRNA) showed some self-adaptation relations, such as EGFR. The differentially expressed elements partially uncovered mechanistic changes for adrenocortical cancer which should guide medical researchers to further achieve pertinent research.

Keywords: Adrenocortical cancer - network - transcription factor - MicroRNA - target gene - host gene

Introduction

Adrenocortical carcinoma is a rare tumor that carries a very poor prognosis. Despite efforts to develop new therapeutic regimens to treat this disease, surgery remains the mainstay of treatment (Kirschner, 2002). When the disease is localized to the adrenal gland and readily amenable to surgical resection, reasonable 5-year survival rates are possible. Locally invasive disease carries a poorer prognosis, and metastatic disease is uniformly fatal within 1 year (Sidhu et al., 2004).

Experimental data indicated that differentially expressed genes and differentially expressed microRNAs (miRNAs) play key roles in development, metastasis and therapy of adrenocortical cancer, such as TP53 Germline Mutations in Adult Patients with adrenocortical carcinoma, show that according to the Chompret criteria for LFS, any patient with adrenocortical cancer (ACC), irrespective of age and family history, is at high risk for a TP53 germline mutation (Herrmann et al., 2012) and additional miRNAs associated with ACC, elucidated the functional role of four miRNAs in the pathogenesis of ACC cells (Patterson et al., 2011). Genes and miRNAs associated with adrenocortical cancer also act as roles in adrenocortical cancer.

Recent studies demonstrate low penetrance mutations leading to later tumour manifestation, we know that any patient with adrenocortical cancer (ACC), irrespective of age and family history, is at high risk for a TP53 germline mutation. Transcription factors (TFs) and miRNAs are prominent regulator for gene expression (Hobert, 2008). TFs are some special proteins that can activate or repress transcription by binding to cis-regulatory elements located in the upstream regions of genes. They alone or together with other proteins regulate gene expression at the transcriptional level. MiRNAs are small (21-24 nt) non-coding RNA molecules that influence gene expression at the post-transcriptional level. MiRNA participates in various biological processes, including proliferation, differentiation and apoptosis, etc.

A wide range of genes are targeted by miRNAs. These target genes (targets) are important to uncover the biological role of miRNAs. Numerous databases, including computational method (Betel et al., 2008) and experimentally validated databases (Papadopoulos et al., 2009), supply enough resource to study relations between miRNAs and their targets. MiRNAs locate inside of many genes that are named their host genes. Rodriguez et al. (2004) indicated that miRNAs are transcribed in parallel with their host transcripts and two different transcription classes of miRNAs (exonic and intronic) were identified (Rodriguez et al., 2004). Baskerville and Bartel (2005) indicated that non-coding RNA and its host gene have close relation. Intronic miRNA and their host gene usually coordinate express in biological progression. They usually act as potential partner to achieve biological function and affect the alteration of pathways (Cao et al., 2010). Medical researchers have discovered many differentially expressed elements (genes and miRNAs) in adrenocortical cancer. But most of their mechanisms are still unclear.

In the present study, we investigated the underlying network of miRNAs, targets of miRNAs, TFs, host gene of miRNAs and their control mechanisms in human
adapted by Bo Zhang et al. from the given text. The document discusses the development of a three-level network for adrenocortical cancer. The network includes differentially expressed elements, related elements, and host genes. The authors used various databases and literature to identify key pathways and regulatory elements involved in adrenocortical cancer. The network helps in understanding the pathogenesis of adrenocortical cancer and guides medical investigators for further research.

**Materials and Methods**

On material collection and data processing, we collected experimentally validated dataset about human miRNAs and their targets from two databases (Tarbase 5.0 and miRtarBase) for the first step. We used public literatures and biological experiments. We separately extracted pathways about differently expressed elements in adrenocortical cancer. Combined action of these pathways will help us to understand pathogenesis of adrenocortical cancer and guide medical investigator to further research in adrenocortical cancer.

**Results and Discussion**

Differentially expressed network of adrenocortical cancer

Figure 1 shows many important regulatory relations about differentially expressed elements in adrenocortical cancer. This network is composed of three TFs (TP53, E2F1 and EGFR), targets of miRNAs, miRNAs and their hostgenes. Besides host gene, other nodes are all differential expression in adrenocortical cancer. The most significant pathways are about three TFs, for example hsa-miR-21 targets EGFR that regulates hsa-miR-21. E2F1 regulates hsa-miR-195 (hsa-miR-449) that target BCL2. Almeida and Hoff (2011) indicated that BCL2, E2F1, EGF, c-KIT, MYB, PRKCA, and CTNNB1 were overexpressed in the larger nodules at messenger and/or protein levels. They are all differentially expressed elements in adrenocortical cancer. Combined action of them shows the pathogenesis of adrenocortical cancer.
Complex Regulatory Network of MicroRNAs, Transcription Factors, Genes in Adrenocortical Cancer

Table 1. Regulatory Relation Between miRNAs and E2F1

<table>
<thead>
<tr>
<th>miRNAs that target gene</th>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-21</td>
<td>hsa-miR-21</td>
<td>hsa-miR-106a, hsa-miR-106b, hsa-miR-126, hsa-miR-149*</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-17</td>
<td>hsa-miR-20, hsa-miR-20a, hsa-miR-21, hsa-miR-223</td>
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</tr>
<tr>
<td>hsa-miR-23b</td>
<td>hsa-miR-330-3p, hsa-miR-34a, hsa-miR-93, hsa-miR-98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2F1</td>
<td></td>
<td>mir-106a, mir-18b, mir-19b-2, mir-20b, mir-25, mir-363, mir-92a-2, mir-93, mir-17, mir-18a, mir-19a, mir-19b, mir-20a, mir-92a, mir-106b, let-7i, mir-15b, mir-15a, mir-16, mir-195, mir-106b, mir-449a, mir-449b, mir-223, mir-449a, mir-449b, mir-15a, mir-15b, mir-16-1</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Regulatory Relation Between Genes and hsa-miR-21

<table>
<thead>
<tr>
<th>Genes that regulate miRNAs</th>
<th>Differentially expressed network</th>
<th>Related network</th>
<th>Global network</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>EGFR, ESR1, MIR21, NFKB1, REL, RELA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2F1</td>
<td>EGFR, ESR1, MIR21, NFKB1, REL, RELA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td></td>
<td>MIR21</td>
<td></td>
</tr>
<tr>
<td>BCL2, E2F1, E2F2, EGFR, JAG1, MSH2, MYC, TGFB2, MIR21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFIF, RASGRP1, TOPORS, SPRY2, BASP1, CCR1, JMY, ANKRD46, DAXX, E2F1, E2F2, EGFR, EIF2S1, EIF4A2, ERBB2, FMOO, ISCU, SPATS2L, PDCD4</td>
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</tr>
</tbody>
</table>

Adrenocortical cancer-related network

Figure 2 shows mass regulatory relations about genes and miRNAs in adrenocortical cancer. Naturally, the related network includes differentially expressed network. There are ten TFs (E2F1, E2F3, EGFR, EGR1, ESR1, MYC, NFKB1, REL, RELA and TP53), and 26 miRNAs as well as mass targets in related network. Figure 2 also shows additional pathways about genes and miRNAs, such as NFKB1 regulates hsa-miR-21 that targets MYC, hsa-miR-21 targets EGFR that regulates hsa-miR-21, TP53 regulates hsa-miR-200b that targets GATA4, E2F1 regulates hsa-miR-195(hsa-miR-449) that targets BCL2(E2F3), and EGR1 regulates hsa-miR-200b(hsa-miR-335) that targets GATA4.

Global network of adrenocortical cancer

The global network includes more comprehensive regulatory relations including all relations. It is an experimentally validated network in human body. It includes differentially expressed network and related network.

Comparison and analysis of differentially expressed genes

Regulatory network of adrenocortical cancer is so complex that we could not understand well. So we extracted and compared all pathways of differentially expressed elements (genes and miRNAs). We classed nodes according to regulatory relation of adjacent nodes in three levels networks for comparing and analyzing each differentially expressed gene’s interacting features. Among these genes, five genes (E2F1, FGFR, TP53) show special feature that each gene regulates miRNA and it is targeted by the miRNA. The complete data can be found in supplementary material 9. We firstly focused on the TFs. The first class of TF has six kinds of adjacent nodes (three kinds of successors and three kinds of predecessors). This class of TF includes E2F1, FGFR and TP53. We only discussed E2F1s following part.

Table 1 shows E2F1, predecessors of E2F1 and successors of E2F1 as well as their regulatory relations.

There is one miRNA target E2F1 that regulates two miRNAs in differentially expressed network. One miRNA target E2F1 that regulates two miRNAs in related network. Sixteen miRNAs target E2F1 that regulates thirty-three miRNAs in global network. These predecessors indirectly influence successors by E2F1. We found that hsa-let-7a targets E2F1 that regulates hsa-let-7a in Table 1. They form a self-adaption relation. The expression of another will be changed, when either of them is differential expression. Supplementary material 9 shows that E2F1 indirectly influences other genes expression by some miRNAs, such as E2F1 regulates hsa-miR-195 that targets BCL2 and E2F3. Some TFs also indirectly influence E2F1 by some miRNAs, for example EGFR regulates hsa-miR-21 that target E2F1. These relations show that there are many complex relations among E2F1 miRNAs and other genes. We focused on the rest of genes that do not regulate any miRNA. Some genes only targeted by some miRNAs, but they do not regulate any miRNA. It is suggested that they maybe the last actor in adrenocortical cancer.

Comparison and analysis of differentially expressed miRNAs

As similar as differentially expressed genes, we compared and analyzed each differnetially expressed miRNA...
miRNA by the same method. We only focused on hsa-miR-21 as following part, predecessors of hsa-miR-21 and successors of hsa-miR-21 as well as their regulatory relations. EGFR regulates hsa-miR-21 that targets differentially expressed genes in Table 2. There are six genes regulates hsa-miR-21 that targets eight genes in related network. There are six genes regulate hsa-miR-21 that targets eighteen genes in global network. We omitted some targets in Table 2. We found that EGFR and hsa-miR-21, MIR21 and hsa-miR-21 form two self-adaption relations in Table 2. Figure 1 shows hsa-miR-21 also indirectly influences other miRNAs by some TFs, for example hsa-miR-21 targets E2F1 that regulates hsa-miR-195 and hsa-miR-449. Some miRNAs also indirectly influence hsa-miR-21 by some TFs, These relations show hsa-miR-21 also has many regulatory relations with genes and other miRNAs.

Analysis of host genes and miRNAs in adrenocortical cancer

Host gene and its miRNA show some important features in this study. Though these host genes are not differential expression in adrenocortical cancer, we considered them as differentially expressed genes when their miRNAs are differential expression. Figure 1 shows some pathways about host genes and miRNAs, Figure 2 shows IARS2 includes hsa-miR-195 that targets BLC2. We found that some host genes and their miRNAs show the feature, which is a host gene includes several miRNAs that alone or together target genes.

In conclusions, we collected a great many of experimentally validated relations about genes and miRNAs. We derived three level networks to find important pathways in adrenocortical cancer and found a topological network about development of adrenocortical cancer. Some pathways about differentially expressed elements showed special feature. Our study partly uncovered regulatory relations about development of adrenocortical cancer and supplied comprehensive data associated with adrenocortical cancer. They will guide medical investigator and biologist to further achieve pertinent research in adrenocortical cancer. In the following work, we will consider interaction of proteins and regulatory pattern (up-regulation and downregulation) into our network. They will derive a more comprehensive and extensive network about adrenocortical cancer. Carcinogenesis and therapy of adrenocortical cancer will get advanced understanding in future.

Acknowledgements

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