RESEARCH ARTICLE

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

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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 ws 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-adaption 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

Asian Pacific J Cancer Prev, **14 (4)**, 2265-2268

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 intronic miRNA and its host gene have close relation. Intronic miRNA and their host gene usually coordinately 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

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adrenocortical cancer. We collected three kinds of data including relation of miRNAs and their targets, relation of TFs and miRNAs, relation of miRNAs and their hostgenes. We also manually collected differentially expressed elements and adrenocortical cancer-related elements from databases and literatures. We derived three level networks that are differentially expressed, related and global networks. Global network shows all experimentally validated pathways about genes and miRNAs, however, this network is so complex that we do not clearly find pathways associated with adrenocortical cancer. So we derived other two networks for further research. We separately extracted pathways about differentially expressed elements. We compared similarities and differences about these pathways in the three networks. The pathways about differentially expressed elements have the most important influence on progression of adrenocortical cancer. When these pathways are abnormal modulated, they will result in development of adrenocortical cancer. These key pathways will help us to understand pathogenesis of adrenocortical cancer and guide medical investigator to further research in adrenocortical cancer.

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 official symbol that are from National Center for Biotechnology Information (NCBI) database that can be accessed online at (http://www.ncbi.nlm.nih.gov/gene/) to unify all miRNAs and genes. These experimental data supply strongly evidence to support our study. For the second step, we collected human experimentally validated dataset about TFs and miRNAs from TransmiR (Wang et al., 2009), which is a manually extracted database between TF and miRNA. Data of TransmiR is extracted from public literatures and biological experiments. The third step, we manually extracted host gene of human miRNA from miRBase (Kozomara and Griffiths-Jones, 2011) and NCBI. We used official symbol and official ID to sign each host gene. The fourth step, we collected differentially expressed genes from KEGG pathway database (Kanehisa and Goto, 2000), Cancer Genetics Web that can be accessed online at (http://www.cancerindex.org/geneweb/index.html) and NCBI SNP database that can be accessed online (http://www.ncbi.nlm.nih.gov/snp/) as well as pertinent literatures. We collected adrenocortical cancer-related genes from Targeted Therapy Database (Mocellin et al., 2010) and pertinent literatures including these genes that affect tumor growth, migration, radial therapy and clinical outcome of human adrenocortical cancer. Additionally, we extracted popular TFs by P-match method (Chekmenev et al., 2005). We considered them as adrenocortical cancer-related genes and only focused on these TFs that are included in transmiR. We downloaded 1,000 nt promoter region sequences of targets that are targeted by differentially expressed miRNAs from UCSC database (Fujita et al., 2011). We used P-match method that combines pattern matching and weight matrix approaches to identify transcription factor binding sites (TFBSs) in 1,000 nt promoter region sequences and mapped TFBSs onto promoter region of targets. Matrix library of P-match is as well as sets of known TF-binding sites collected in TRANSFAC, so it provides the possibility to search for a large variety of different TF binding site. We used the vertebrate matrix and restricted high quality criterion for the matrix. The fifth step, we extracted differentially expressed miRNAs from pertinent literatures and mir2Disease (Jiang et al., 2009), which is a manually curated database about differentially expressed miRNAs in various human diseases. We manually mined pertinent literatures for extracting related miRNAs in adrenocortical cancer.

We used the following method to construct three level networks: the differentially expressed network, related network and global network. We extracted regulatory relations for TFs, miRNAs, targets and host gene from First step-result, Second step-result and Third step-result. After we combined their relations we derived the global network. We separately extracted differentially expressed elements and related elements from Fourth step-result and Fifth step-result, meanwhile, we separately mapped them onto global network. After we combined their relations we separately derived the differentially expressed network and related network. The complete data of differentially expressed network can be found in Table 1. The complete data of related network can be found in Table 2.

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.

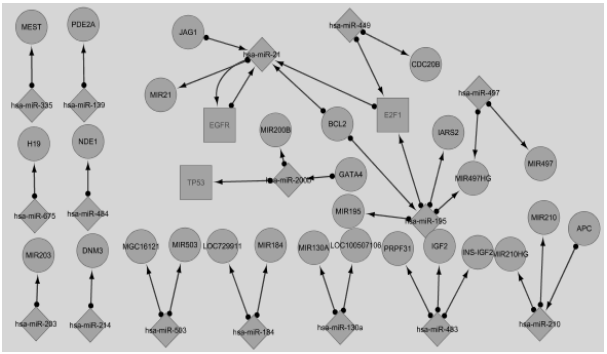

Figure 1. The Differentially Expressed Network

Table 1. Regulatory Relation Between miRNAs and E2F1

miRNAs that target gene	Differentially expressed network	hsa-miR-21
	Related network	hsa-miR-21
	Global network	hsa-miR-106a, hsa-miR-106b, hsa-miR-126, hsa-miR-149*, hsa-miR-17, hsa-miR-20, hsa-miR-20a, hsa-miR-21, hsa-miR-223, hsa-miR-23b, hsa-miR-330-3p, hsa-miR-34a, hsa-miR-93, hsa-miR-98
	Gene	E2F1
miRNAs that target gene	Differentially expressed network	hsa-miR-195, hsa-miR-449
	Related network	hsa-miR-195, hsa-miR-449
	Global network	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-449c, mir-15a, mir-15b, mir-16-1

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

Genes that regulate miRNAs	Differentially expressed network	EGFR
	Related network	EGFR, ESR1, MIR21, NFKB1, REL, RELA
	Global network	EGFR, ESR1, MIR21, NFKB1, REL, RELA
miRNA		miR-21
Genes that regulate miRNAs	Differentially expressed network	MIR21
	Related network	BCL2, E2F1, E2F2, EGFR, JAG1, MSH2, MYC, TGFBR2, MIR21
	Global network	PPIF, RASGRP1, TOPORS, SPRY2, BASP1, CCR1, JMY, ANKRD46, DAXX, E2F1, E2F2, EGFR, EIF2S1, EIF4A2, ERBB2, FMOD, ISCU, SPATS2L, PDCD4

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.

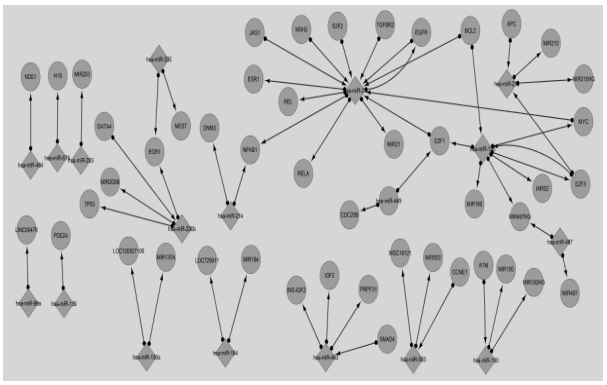

Figure 2. The Related Expressed Network

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 E2F1as 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 differentially expressed

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 nighteen 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

This work was supported by the National Natural Science Foundation of China (No. 60973091; 60905022).

References

Almeida MQ, Hoff AO (2012). Recent advances in the molecular pathogenesis and targeted therapies of medullary thyroid carcinoma. *Curr Opin Oncol*, **24**, 229-34.

Baskerville S, Bartel DP (2005). Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *RNA*, **11**, 241-7.

Betel D, Wilson M, Gabow A, et al (2008). The microRNA.org resource: targets and expression. *Nucl Acids Res*, **36**, D149-53.

Cao G, Huang B, Liu Z, et al (2010). Intronic miR-301 feedback regulates its host gene, ska2, in A549 cells by targeting MEOX2 to affect ERK/CREB pathways. *Biochem Biophys Res Commun*, **396**, 978-82.

Chekmenev DS, Haid C, Kel AE (2005). P-Match: transcription factor binding site search by combining patterns and weight matrices. *Nucleic Acids Res*, **33**, W432-7.

Fujita PA, Rhead B, Zweig AS, et al (2011). The UCSC Genome Browser database: update 2011. *Nucleic Acids Res*, **39**, D876-82.

Herrmann LJM, Heinze B, Fassnacht M, et al (2012). TP53 germline mutations in adult patients with adrenocortical carcinoma. *J Clin Endocrinol Metab*, **97**, E476-85.

Hobert O (2008). Gene regulation by transcription factors and microRNAs. *Science*, **319**, 1785-6.

Jiang Q, Wang Y, Hao Y, et al (2009). miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res*, **37**, D98-104.

Kanehisa M, Goto S (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res*, **28**, 27-30.

Kirschner LS (2002). Signaling pathways in adrenocortical cancer. *Ann N Y Acad Sci*, **968**, 222-39.

Kozomara A, Griffiths-Jones S (2011). miRBase integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res, **39**, D152-7.

Mocellin S, Shrager J, Scolyer R, et al (2010). Targeted Therapy Database (TTD): a model to match patient's molecular profile with current knowledge on cancer biology. *PLoS One*, **5**, e11965.

Papadopoulos GL, Reczko M, Simossis VA, et al (2009). The database of experimentally supported targets: a functional update of TarBase. *Nucleic Acids Res*, **37**, D155-8.

Patterson EE, Holloway AK, Weng J, et al (2011). MicroRNA profiling of adrenocortical tumors reveals miR-483 as a marker of malignancy. *Cancer*, **117**, 1630-9.

Rodriguez A, Griffiths-Jones S, Ashurst JL, et al (2004). Identification of mammalian microRNA host genes and transcription units. *Genome Res*, **14**, 1902-10.

Sidhu S, Sywak M, Robinson B, et al (2004). Adrenocortical cancer: recent clinical and molecular advances. *Curr Opin Oncol*, **16**, 13-8.

Wang J, Lu M, Qiu C, et al (2009). TransmiR: a transcription factor-microRNA regulation database. *Nucleic Acids Res*, **38**, D119-22