Chapter 3

Transcriptome Analysis of Systems Biology for Schizophrenia

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Abstract

Transcriptome analysis of postmortem brain samples provides more insights to evaluate biological dysfunctions by analysis of differential expression and genetic interactions in schizophrenia. The growing development of new technologies such as next-generation sequencing (NGS) helps to explore detailed and underlying molecular changes from global perspective of view, not only focus in single SNP variants. It is implicated that schizophrenia genetic and protein interactions may give rise to biological dysfunction not only in dopamine dysfunction but also in immune, energy metabolism, mitochondrial dysfunction and hemostasis. Epigenetic investigation of schizophrenia provides important information on how the environmental factors affect the genetic architecture of the disease. DNA methylation plays a pivotal role in etiology for schizophrenia. The schizophrenia differential methylation genes and differential expression genes were analyzed to find the potential protein complexes related to the etiology of schizophrenia from alteration of DNA methylation. The protein complexes and pathways involved in schizophrenia differential methylation network may be responsible for the etiology and potential treatment targets. It is implicated that the interaction between differential expression candidate genes and differential methylation genes may describe the global view of disease mechanisms and it has important roles in the pathogenesis for schizophrenia.

Keywords: systems biology, protein complexes, methylation, pathway enrichment analysis, network analysis, schizophrenia, epigenetics, mitochondria

1. Introduction

Schizophrenia is a debilitating brain disorder. It belongs to a group of multiple pathologies and is also known as a complex genetic disorder effected or stimulated by environmental

factors. Evidences of gene variations as risk factors of schizophrenia have been accumulated since 1938 [1]. Many studies have attempted to resolve the biological and genetic complexity of schizophrenia. However, the molecular mechanism of schizophrenia pathophysiology is far from clear, partly because the disease mechanism mainly locates in brain regions and sampling affected tissues are hence difficult.

The development of treatment is progressing slowly. Mostly antipsychotics are based on the dopamine, serotonin, and γ-Aminobutyric acid (GABA) theory. But none of the theories is conclusive for the disease mechanism. The major treatment of schizophrenia, called the antipsychotics, mostly block corresponding receptors in dopaminergic, serotoninergic, and GABAergic pathways. However, both traditional and atypical antipsychotics have predominant and unneglectable side effects such as involuntary movement disorders and metabolic syndrome. Besides, these medications may not get into the core targets of schizophrenia. Novel treatment strategies have long been anticipated to have more advanced and specific approaches and mechanisms.

New technologies such as next-generation sequencing (NGS), mRNA microarray, high-throughput single nucleotide polymorphism (SNP), and copy number variation (CNV) associations with diseases allowed us to propose novel candidate genes or molecular etiology of mental disorders [2]. Furthermore, the most comprehensive biological databases for schizophrenia genetic research including SZGene [3] and SZGene database (SZDB) [4] have been constructed. The SZGene database (last updated 23 December 2011) has listed 1727 studies investigating over 1008 candidate genes, 8788 polymorphisms, and 287 meta-analysis. In these extensive studies, one or more genetic markers in genes are hypothesized to be involved in the etiology of schizophrenia. SZDB (http://www.szdb.org/) is a comprehensive resource for SZ research which includes SZ genetic data, gene expression data, network-based data, brain expression quantitative trait loci (eQTL) data, and SNP function annotation information. It contains 9,444,230 SNPs with sample size of 82,315, including 35,476 schizophrenia cases and 46,839 controls. Recent NGS researches including comprehensive and collective postmortem brain sample data come from [5].

On the basis of current empirical evidence and mostly consensual assessments of informed opinion, it appears that the historical candidate gene literature did not yield clear insights into the genetic basis of schizophrenia [6].

The schizophrenia-associated studies have difficulties to conclude a simple disease etiology. The important candidate genes varied in literature reviews with low reproducibility. Our group has expanded the association interaction of these candidate genes by constructing genetic association network. It could represent the whole interaction paths by its association trace. Till today, pathway analyses did not enrich smaller ISC p values in hypothesis-driven candidate genes, nor did a comprehensive evaluation of meta-hypotheses driving candidate gene selection. The hypothesis-driven candidate genes studied in the literature are not found enriched for the common genetic variation involved in the etiology of schizophrenia [7]. Functional enrichment analysis is used to identify groups of genes or protein complexes which are differentially expressed in a large set of gene classes. They may have association with disease phenotypes. Statistical approaches help to identify significantly enriched groups of candidate gene. NGS and microarray genome-wide association study (GWAS) results could

identify hundreds or thousands of candidate genes for analysis. Specific groups of multiple genes may link to a specific biological pathway, and the simultaneous change in expression level within genes may lead to the difference in phenotypic expression.

2. Next-generation sequencing analysis for mental disorders

2.1. Tissue sample

Different tissue samples obtained from cell culture, blood, postmortem brain, and even cerebrospinal fluid (CSF) have been used to understand the pathology of schizophrenia. The identities of the most significantly dysregulated genes were mostly distinct for each tissue; however, the findings also indicated common biological functions and regulatory pathways or complexes. For example, increased levels of cytokines and correlated N-methyl-D-aspartate (NMDA) receptor change could be found in the peripheral blood and cerebrospinal fluid of schizophrenic patients [8, 9]. In addition, the phenotypic insights of iPSC models in schizophrenia include transcriptional dysregulation, oxidative stress synaptic dysregulation, and neurodevelopmental abnormalities [10], which might be associated and compatible with the antioxidative activity of antipsychotics such as olanzapine and clozaril [11].

In recent years, the postmortem brain tissues from schizophrenia subjects have been extensively studied, which serve as a vital component for illustrating the molecular change of schizophrenia. There are some researches using CSF as a target for analysis of specific gene expression such as immune system or cytokines [8].

2.2. STEA and schizophrenia

Schizophrenic transcriptome enrichment analysis (STEA) can be used to understand the network and pathways for schizophrenia from a global and comprehensive approach. Schizophrenia is a multi-genetic and inheritable disorder. Its onset and etiology involves many genes with interaction of multiple pathways, as well as the interaction of methylation genes with environmental factors or epigenetic insult. For instance, epigenetic changes, like DNA methylation and histone modification, are affected by the environment factors such as stress, chemical, and oxidative reaction.

DNA methylation is the most well-studied epigenetic change and was recently analyzed using STEA in relation to schizophrenia-associated phenotype. Researchers ranked top candidate genes for their correlation between methylation patterns and differential expression level in each of the phenotypes. This system biology approach might prove promising to look for an enrichment of genes and important implications for the disease mechanism that are predicted to be targeted in the progression of the disease.

2.3. Next-generation sequencing

As the high-throughput DNA sequencing technologies are becoming more affordable, the application of these technologies is expected to discover new genomic variations associated within a wide variety of mental disorders. They are also regarded as the key to comprehending those of multivariate genetic origins. The use of next-generation sequencing technologies

is expected to facilitate the discovery of schizophrenia candidate genes. In comparison with traditional sequencing, the use of NGS is regarded as an ideal to discover genetic mutations and differential gene expression.

Next-generation sequencing (NGS) is a revolutionized sequencing technique; it makes 6–20 million reads from human genome into pieces at unprecedented speeds to discover novel biological applications. The use of NGS has made possible to identify genetic mutations in complex diseases. NGS is contributing to a new understanding of these diseases, albeit from a different perspective, and thus a new type of research consent is needed. There are different NGS platforms including SOLiD, Illumina, GS Junior System, Pacific Biosciences, and more. NGS data in psychiatric genetic researches face challenges of drastic developments in the understanding mechanisms of schizophrenia. It helps to explore the complex disease from global view aspect, not only in specific SNPs or genetic variants but also the genetic interactions and corresponding networks and pathways.

2.4. The new research models for schizophrenia

The thorough understanding of the potential etiology and pathology of schizophrenia is essential to rapidly improve its diagnoses and more effective therapies. By the understanding of epigenetic changes, gene-gene interaction network using systems biology approaches makes it possible to approach the mechanism of schizophrenia. New analytical technologies such as next-generation sequencing, IPSC neuron model, SZDB, expression pattern analysis, and protein-protein interaction analysis are promising approaches to provide novel insights of pathology which may lead to new treatment strategies for schizophrenia [12]. These approaches may lead to the discovery of underlying epigenetic and genetic factors for schizophrenia and may thereby identify corresponding complexes or pathways and reveal novel therapeutic targets for this devastating disorder [13].

The next-generation data of RNA sequences by big data analysis could bring new insights into the comprehensive and global view and revealed more detailed transcriptional alteration in schizophrenia. Recent developments of DNA sequencing technology and whole genome studies implicated that mutations play a vital role in the genetic architecture of schizophrenia and implicated in several molecular pathways, including chromatin regulation, activity-regulated cytoskeleton, postsynaptic density, and N-methyl-D-aspartate (NMDA) receptor, which are associated with schizophrenia [14]. Schizophrenia-network pathway complex analysis (SCZ-NPCA) has included enrichment analysis which demonstrates the role of the implicated pathways in schizophrenia, such as transcription activity, signaling pathway, cancer-related pathway, tumor suppression, coagulation, insulin secretion, cell cycle, cell differentiation, and apoptosis [5].

2.5. Epigenetics and DNA methylation profiles in schizophrenia

External factors such as environmental stress are known to cause the onset of schizophrenia. Exposure to stress induces stable changes by transcriptional dysfunction, resulting in aberrant changes of genetic expression, neural circuit functions, and ultimately behavior changes and disease symptoms [15]. Epigenetic factors such as aberrant DNA methylation have important

roles in regulating gene expression [16]. Epigenetic changes may be one of the pivotal features of many human mental disorders.

Methylation of genomic DNA could mediate gene expression. Although there are no specific methylated gene patterns identified in schizophrenia, there are significant associations between promoter CpG islands (CGI) hypermethylation with gene repression and CGI hypomethylation with increased gene expression [17]. CGIs have been suggested to suppress gene expression by blocking the promoters. Recent researches focus on methylation array in postmortem brain studies such as hypermethylation of *RELN*, hypermethylation and downregulated transcription of *SOX10*, and hypomethylation of *MB-COMT* in promoter [18]. Methylation gene discovery in schizophrenia including COMT, REELIN, dopaminergic, serotonergic, and GABAergic pathways shows differential methylation profiles in schizophrenia [19]. Global hypomethylation has also been noted in schizophrenia patients in experiments with larger sample sizes [20].

Previous studies were mostly done on mouse models or stem cell lines [21, 22]. Nonetheless, a vast amount of methylation arrays of postmortem human brains have been released recently [23, 24]. These latest advances may implicate the importance of methylation patterns in schizophrenic patients. Previous researches of genetic methylation of mental disorders focus on the descriptive finding of differential methylation patterns. But how differentially methylated susceptible genes affect the expression levels of target genes remains to be further systematically analyzed. The relationship between the genetic differential methylation levels and differential expression patterns was explored in schizophrenia. They could be identified as disease biomarkers.

Recent researches gradually focus on novel methylation profile of susceptible genes by network biology analysis. There have been many studies focusing on the discovery of differential expression of schizophrenic candidate genes and the construction of PPI networks and related pathways for the hope of a better understanding of schizophrenia [5, 25–28]. Differentially expressed disease genes from postmortem brain samples of schizophrenia reveal the overall relationships between maker genes and schizophrenia. The constructed disease network and underlying pathways, protein complexes, provided the potential treatment strategy for schizophrenia. It could be proposed as potential targets for developing new treatments due to their functional and topological significance [26].

Analyses of DNA methylation identified potential biological processes that regulate gene expression and contribute to disease mechanisms. We constructed the differential methylation and expression networks to interactions of methylated genes. Therefore, large-scale analyses for differential methylation of schizophrenic susceptible genes were conducted and integrated with the differential expression data of schizophrenic susceptible genes to build the methylation-to-expression genetic network. The network explored the epigenetic mechanism of schizophrenic methylation networks, differential methylation pathways, complexes, and corresponding biological functions. The genetic, epigenetic, and transcriptomic information was integrated to give a comprehensive overview of schizophrenia.

The schizophrenic differential methylation network (SDMN) was constructed for the comprehensive view of methylation profile in schizophrenia. The SDMN was generated by query-query protein-protein interaction (QQPPI) [29] and genetic interactions in Pathway

Commons database of schizophrenic differential methylation genes (SDMGs) [30]. Pathway Commons database [30] which collects BIND [31], BioGRID [32], CTD [33], DIP [34], HPRD [35], HumanCyc [36], IntAct [37], KEGG [38], NetPath [39], PANTHER [40], PhosphoSitePlus [41], PID [42], Reactome [43], SMPDB [44], TRANSFAC [45], MiRTarBase [46], DrugBank [47], Recon 2 [48], and WikiPathways databases [49] contain 34,661 molecular pathways.

The regulatory relations for genetic interactions or the potential pathways may be responsible for disease mechanism of schizophrenia. The differentially expressed genes in the BA22 brain samples of schizophrenia are proposed as schizophrenia candidate marker genes (SCZCGs) [5]. For the exploration of modulation and regulation relations between schizophrenic hypermethylated promotors and differential expression genes, we analyzed hypermethylation promotors and extended protein-protein interactions (PPIs) of SCZCGs to their level one interactions to construct the hypermethylation to differential expression networks (HyDEN).

There are 688 (39.6%) genes (16 hypermethylated/672 hypomethylated, ratio 2.38%) differentially methylated in promotor regions from total 1,869 schizophrenic differentially methylated genes. 639 (36.9%) genes (24 hypermethylated/615 hypomethylated, ratio 3.90%) are differentially methylated in intron. 481 (27.7%) genes (23 hypermethylated/458 hypomethylated, ratio 5.02%) are differentially methylated in exon region. The Venn diagram revealed the most differential methylation genes appear in promotor regions (39.6%) and least differentially methylated in exon regions (27.7%) of the schizophrenic methylation profile on specific gene location. It is indicated that the highly differential methylation in promotor regions may be one of the etiologies for schizophrenia. Recent researches focus on evidences of epigenetic profile of common genetic variants in schizophrenia. In the epigenetic profile of DNA methylation, the phenomena of predominant hypomethylation in promotors were noted in schizophrenia.

The ten schizophrenic hypermethylation genes discovered by SDMN are founded to be associated with biological functions such as cell structure, energy metabolism, mitochondrial function, GABA metabolism, signaling transduction, and zinc finger functions. The influence of schizophrenic hypermethylation genes may play a vital role in the etiology of schizophrenia. The previous studies have validated the relationships between the hypermethylation genes and schizophrenia, yet, little is known about how methylation profile modulates the disease phenotype. By the analysis of SDMN, we could investigate the relationship between the hypermethylation genes and epigenetic mechanism in which the future experimental validation was needed. It may be one of the important disease mechanisms which are responsible for pathogenesis of schizophrenia.

2.6. Potential complexes and pathways for schizophrenia

How does the disease affect human body? Or how does schizophrenia affect the performance of brain? Pathway analysis is the building process of identifying protein interactions, associated annotation, and domain knowledgebase [50]. The pathway enrichment analysis was performed with PID [42], Reactome [51], Cell-Map [30], and HumanCyc [52] databases to obtain the potential pathways for the pathophysiology of schizophrenia. Pathways reported

to be associated with pathogenesis of schizophrenia include apoptosis [53], immune system [54], TNF signaling pathways [55], hemostasis [56], p53 pathway [57], BARD1 signaling pathway [58], ceramide signaling pathway [59], ErbB2 signaling pathway [60], and androgen receptor pathway [61] and HDAC signaling pathway [62, 63].

Recent research focuses on the methylation gene groups interact with differential expression gene groups to explore the integrated biological pathways designated to reveal disease pathways discovered in systems biology research which may implicate the mechanism of schizophrenia. External factors such as environmental stress are known to cause the onset of schizophrenia. Exposure to stress induces stable changes by transcriptional dysfunction, resulting in aberrant changes of genetic expression, neural circuit functions, and ultimately behavior changes and disease symptoms [15].

Epigenetic factors such as aberrant DNA methylation have important roles in regulating gene expression [16]. Epigenetic changes may be one of the pivotal features of many human mental disorders. The pathway enrichment analysis may indicate the biological functions influenced by SDMGs. It could reveal the potential disease mechanism and novel therapeutic strategy for schizophrenia.

There are corresponding pathways found in enrichment analysis from SDMGs which may implicate the underlying disease mechanisms and characteristics for schizophrenia under the regulatory role of SDMGs. Top-ranked pathways with FDR p-value <0.05 are TGF beta receptor, pyrimidine metabolism, metabolic pathways, Wnt pathway, folate biosynthesis, nicotinate and nicotinamide metabolism, and purine metabolism.

In order to understand the involved protein complexes in schizophrenia of how SDMGs interact with the expression level of SCZCGs, we searched the comprehensive resource of mammalian protein complexes (CORUM) [64] for the potential protein complexes responsible for the regulation and epigenetic mechanism in schizophrenia. The clique and complex analysis was performed with data of the CORUM database which has a collection of experimentally verified mammalian protein complexes to reveal the corresponding clique complexes. The gene groups from SDMGs and SCZCGs were analyzed and searched against CORUM to find the potential protein complexes related to the etiology of schizophrenia from alteration of DNA methylation. The most important protein complexes involved in SDMGs and SCZCGs may include Nop56p-associated pre-rRNA complex, ribosome-related subunit, mitochondrial respiratory chain complex I, TATA-binding protein-free TAF-II-containing complex (TFTC complex), and P300/CBP-associated factor complex (PCAF complex).

The biological functions of those complexes are associated with ribosome biosynthesis, mitochondrial dysfunction, and pre-rRNA processing. However, the top-ranked complexes represented in SDMGs include SRB/MED-containing cofactor complex (SMCC complex), mediator complex, Nop56p-associated pre-rRNA complex, CDC5L complex, CF IIAm complex, and 55S mitochondrial ribosome complex [65]. These complexes are translated by aberrant SDMGs to perform specific protein functions, which might be the potential molecular mechanism in epigenetic regulation for schizophrenia. The inheritable alterations of these complexes might explain the roles of hereditary factors in the etiology of schizophrenia with DNA methylation

[66]. In cancer etiology, the promoter hypermethylation also plays a major role by aberrant transcription of critical regulator genes such as tumor suppressor genes with the implications for the hypomethylation factors in the novel treatment strategy of cancer [67].

Epigenetic mechanism produces DNA methylation which alters gene expression without altering underlying DNA sequence. *Epigenetic changes may be passed on for multiple generations by cell division* [68]. Evidences of linkage analysis in schizophrenic family suggest a hereditary susceptibility [69]. The methylation of DNA confers long-term epigenetic silencing which could be reprogrammed by demethylation of DNA repair [70]. It is implicated that the epigenetic change, especially from the differentially expression genes, regulates the methylation of SDMGs and the production of corresponding protein complexes.

Recent study suggests that the hypomethylated genes are predominant in schizophrenia. Reducing hypomethylation of SDMGs or SCZCGs could be a novel therapeutic treatment method for schizophrenia. There might be the protective factors as per the etiology of cancer [25], in which most promotors are hypermethylated. Some hypermethylating agent, such as vitamin B1, induces upregulation of methyltransferase and reversion of hypomethylation as an adjuvant treatment in schizophrenia [71]. It has postulated that deficiency of vitamin B1 may result in genetic methylation and biochemical lesion relating to neurotransmitter metabolism in the brain, leading to psychotic manifestations [72].

2.7. Mitochondrial dysfunction in schizophrenia by genetic interaction network

By the analysis of transcriptome profiles in postmortem brain tissues and interactions between differential expression candidate genes, the novel finding of potential complexes and pathways could facilitate the investigation of potential schizophrenic pathoetiology. Recent researches focus on theories related to the hypofunction of mitochondria which may contribute to the pathogenesis of schizophrenia, especially negative symptoms such as anhedonia, lack of emotional expression, flattening, poor social and interpersonal activities, and poor self-care. Some advanced techniques propose the replacement of mitochondria; even restoration of mitochondrial function might be potential treatment for alleviation of the negative symptoms of schizophrenia. Since the mitochondria are responsible for vital biological processes such as energy metabolism, calcium buffering, and apoptosis, it indicates the importance of mitochondria dysfunction in the manifestation of schizophrenia [73].

The genetic profile of mitochondria and energy metabolism in the analysis of brain samples may contribute to reveal the novel insight to the etiology of schizophrenia [73, 74]. The genetic interactions and intermediate mediators among mitochondrial genes and many underexpressed SCZCGs indicate the genetic predisposition of mitochondria dysfunction in schizophrenia. The genetic interactions between mitochondria and schizophrenia may be revealed by the DRD2-NDUFS7 and the FLNA-ARRB2 interactions [5].

In SDMG, NDUFA10 has been found to be associated with the abnormalities of mitochondrial function in schizophrenia [75]. It plays a key role in respiratory electron transport chain responded to the exposure of antipsychotics [76]. NDUFA10 mutation causes mitochondrial complex I deficiency. It is associated with the progressive neurodegenerative disease such as

Leigh syndrome [77], which possibly shares the same etiopathogenesis with schizophrenia [78]. The mechanism involving NDUFA10 could be novel targets for schizophrenic therapeutic treatments. BARD1, RBMS1, PRKAB1, UBE2L3, SCO2, PIN4, MRPL43, BAG6, NDUFB11, CAPN1, STAT3, MPST, TCOF1, and SEC24C are all under-expressed genes which interact with the respiratory chain complex I in mitochondria.

3. Novel treatment strategy of antipsychotics

If the SCZCGs are responsible for the gene targets of disease mechanism of schizophrenia, the associated complexes or drugs derived from SCZCGs may contribute to novel treatment for schizophrenia whether they were traditional or atypical antipsychotics related. From the analysis of important cliques in SCZCGs, some of the drugs derived from clique analysis and mapped to the gene targets from DrugBank such as lovastatin and retinoid acid.

Lovastatin, a cholesterol-lowering agent is targeted by HDAC2. It was also the principal statin produced from *Monascus purpureus* derived from red yeast rice [79]. It has been implicated that statins target many of the pathways to neuroprogression in schizophrenia [80]. Adapalene, Tazarotene, and Tamibarotene are retinoids which involved RARA gene with multiple functions including eye vision, immune function, and activation of tumor suppressor genes. Retinoic acid has been reported for the treatment of schizophrenia. More and more evidence regarding retinoid dysregulation in schizophrenia implicated targeting retinoid receptors may be a novel approach to treat schizophrenia [25, 81].

Although there is not yet a clear and well-evidenced disease mechanism for schizophrenia, the current findings may contribute to novel indications or drug repurposing for schizophrenia. However, further evaluation and validation are needed in the near future.

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