Multiple Sclerosis and Related Disorders (2014) 3, 94–106

Available online at www.sciencedirect.com

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Molecular network of ChIP-Seq-based NF-κB p65 target genes involves diverse immune functions relevant to the immunopathogenesis of multiple sclerosis

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Received 18 December 2012; received in revised form 24 April 2013; accepted 30 April 2013

KEYWORDS
ChIP-Seq;
GenomeJack;
Molecular network;
Multiple sclerosis;
Next generation sequencing;
NF-κB

Abstract
Background: The transcription factor nuclear factor-kappa B (NF-κB) acts as a central regulator of immune response, stress response, cell proliferation, and apoptosis. Aberrant regulation of NF-κB function triggers development of cancers, metabolic diseases, and autoimmune diseases. We attempted to characterize a global picture of the NF-κB target gene network relevant to the immunopathogenesis of multiple sclerosis (MS).
Methods: We identified the comprehensive set of 918 NF-κB p65 binding sites on protein-coding genes from chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) dataset of TNFα-stimulated human B lymphoblastoid cells. The molecular network was studied by a battery of pathway analysis tools of bioinformatics.
Results: The GenomeJack genome viewer showed that NF-κB p65 binding sites were accumulated in promoter (35.5%) and intronic (54.9%) regions with an existence of the NF-κB consensus sequence motif. A set of 52 genes (5.7%) corresponded to known NF-κB targets by database search. KEGG, PANTHER, and Ingenuity Pathways Analysis (IPA) revealed that the NF-κB p65 target gene network is linked to regulation of immune functions and oncogenesis, including B cell receptor signaling, T cell activation pathway, Toll-like receptor signaling, and apoptosis signaling, and molecular mechanisms of cancers. KeyMolnet indicated an involvement of the complex crosstalk among core transcription factors in the NF-κB p65 target gene network. Furthermore, the set of NF-κB p65 target genes included 10 genes among 98 MS risk alleles and 49 molecules among 709 MS brain lesion-specific proteins.
Conclusions: These results suggest that aberrant regulation of NF-κB-mediated gene expression, by inducing dysfunction of diverse immune functions, is closely associated with development and progression of MS.

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http://dx.doi.org/10.1016/j.msard.2013.04.005

1. Introduction

The transcription factor nuclear factor-kappa B (NF-κB) acts as a central regulator of innate and adaptive immune response, stress response, cell proliferation, and apoptosis (Barnes and Karin, 1997; Hayden et al., 2006). Deregulation of NF-κB function triggers development of cancers, metabolic diseases, and autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis (RA) (Yan and Greer, 2008; Gregersen et al., 2009). The NF-κB family proteins consist of five members, such as RelA (p65), RelB, c-Rel, NF-κB1 (p105), and NF-κB2 (p100) (Gilmore, 2006). The latter two are proteolytically processed into p50 and p52, respectively. All the members share the Rel homology domain (RHD) acting for DNA binding and dimerization. The NF-κB family proteins constitute either homodimers or heterodimers, except for RelB that exclusively forms heterodimers. The p50-RelA heterodimer represents a predominant NF-κB dimer in various cell types. The NF-κB dimers interact with specific DNA sequences named the κB site located on promoters to activate or repress transcription of target genes. Only p65 and c-Rel act as a potent transcriptional activator, whereas p50 and p52 homodimers generally repress transcription (Rothwarf and Karin, 1999). Optimal induction of NF-κB target genes requires phosphorylation of p65 within its transactivation domain in response to distinct stimuli by various kinases (Viatour et al., 2005).

NF-κB activity is regulated tightly at multiple levels (Viatour et al., 2005; Gilmore, 2006). In unstimulated cells, NF-κB proteins exist in an inactive state, being sequestrated in the cytoplasm via non-covalent interaction with the inhibitor of NF-κB (IκB) proteins, such as IκBα, IκBβ, IκBγ, and IκBε. Viral and bacterial products, cytokines, and oxidative stress activate the specific IκB kinase (IKK) complex, composed of two catalytic kinase subunits called IKKα and IKKβ and a regulatory subunit called NF-κB essential modulator (NEMO). IκB proteins, when phosphorylated by the IKK complex, are ubiquitinated, and processed for 26 S proteasome-mediated degradation, resulting in nuclear translocation of NF-κB dimers. The NF-κB signaling cascade is categorized into canonical and non-canonical pathways (Oeckinghaus et al., 2011). The canonical pathway is activated by various proinflammatory cytokines, such as tumor necrosis factor-alpha tumor necrosis factor-alpha (TNFα) and interleukin-1 (IL-1), transduced by both IKKβ and NEMO that chiefly mediate phosphorylation of IκBα, followed by nuclear translocation of p65-containing NF-κB heterodimers. The non-canonical pathway, activated by CD40 ligand, B-cell activating factor (BAFF), and lymphotoxin-beta (LTβ), requires IKKα-mediated phosphorylation of p100 dimerized with RelB, which are processed to form the p52-RelB complex.

MS is an inflammatory demyelinating disease of the central nervous system (CNS) white matter, presenting with a relapsing-remitting (RR) and/or progressive clinical course. It is mediated by an autoimmune process triggered by a complex interplay between genetic and environmental factors, leading to development of autoreactive T helper type 1 (Th1) and type 17 (Th17) lymphocytes (Comabella and Khoury, 2012). Several lines of evidence indicate that aberrant regulation of NF-κB signaling pathway plays a central role in acute relapse of MS. By gene expression profiling, we identified 43 differentially expressed genes in peripheral blood $CD3^+$ T cells between the peak of acute relapse and the complete remission of RRMS (Satoh et al., 2008). We found that the molecular network of 43 genes showed the most significant relationship with transcriptional regulation by NF-κB. Our observations are supported by several studies that verified an aberrant expression of NF-κB signaling molecules in peripheral blood mononuclear cells (PMBC) during MS relapse (Achiron et al., 2007; Lindsey et al., 2011). Intravenous methylprednisolone pulse (IVMP) immediately reduces the levels of activated p65 in PBMC of MS patients (Eggert et al., 2008). Furthermore, interferon-gamma (IFNγ, a prototype Th1 cytokine, is identified as one of NF-κB target genes (Sica et al., 1997), while interferon-beta (IFNβ, the first-line medication for RRMS, attenuates proinflammatory responses by inhibiting the NF-κB activity in lymphocytes (Martín-Saavedra et al., 2007). Mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1), a key regulator of NF-κB activation, positively regulates the encephalitogenic potential of inflammatory Th17 cells (Brüstle et al., 2012). To elucidate the precise role of NF-κB in MS relapse, it is highly important to thoroughly characterize NF-κB target genes involved in the immunopathogenesis of MS.

A number of previous studies identified hundreds of NF-κB target genes, including those involved in not only inflammatory and anti-apoptotic responses, but also anti-inflammatory and proapoptotic responses (Pahl, 1999). Importantly, NF-κB target genes often activate NF-κB itself, providing a positive regulatory loop that amplifies and perpetuates inflammatory responses (Barnes and Karin, 1997). However, it remains unclear how many of previously identified genes actually represent direct targets for NF-κB-mediated transcriptional activation.

Recently, the rapid progress in the next-generation sequencing (NGS) technology has revolutionized the field of genome research. As one of NGS applications, chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) provides a highly efficient method for genome-wide profiling of DNA-binding proteins, histone modifications, and nucleosomes (Park, 2009). ChIP-Seq endowed with an advantage of higher resolution, less noise, and greater coverage of the genome, compared with the microarray-based ChIP-Chip method, serves as an innovative tool for studying the comprehensive gene regulatory networks. However, since the NGS analysis produces extremely high-throughput experimental data, it is often difficult to extract the meaningful biological implications. Recent advances in systems biology enable us to illustrate the cell-wide map of the complex molecular interactions by using the literature-based knowledgebase of molecular pathways (Satoh, 2010). The logically arranged molecular networks construct the whole system characterized by robustness, which maintains the proper function of the system in the face of genetic and environmental perturbations. Therefore, the integration of high dimensional NGS data with underlying molecular networks offers a rational approach to characterize the network-based molecular mechanisms of gene regulation on the whole genome scale.

In the present study, to characterize a global picture of the NF-κB target gene network, we investigated the NF-κB p65 ChIP-Seq dataset of TNFα-stimulated human B lymphoblastoid cells. The dataset was retrieved from the public