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Characterization of genome-wide binding of NF-κB in TNFα-stimulated HeLa cells

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ABSTRACT

This study characterized the genome-wide binding of NF-κB RelA with ChIP-Seq and explored its effects on the gene transcription with DNA microarray. It was found that NF-κB showed several significant binding characteristics, including the inter- and intra-chromosomal differential high-fold enrichment binding, the dominant intronic binding to vast majority of target genes through multiple ChIP-seq peaks and κB sites, extensively binding to large number of genes in the human genome, and binding its target genes more broadly through noncanonical κB sites than canonical κB sites. These in vivo genome-wide binding characteristics exerted effects on the transcription of its direct target genes in genome, reflecting some important traits of this protein which acts as a stimulatory transcription factor involving in many biological processes and responding to various internal and external stimuli.

1. Introduction

NF-κB is a ubiquitous transcription factor existing in most cell types (Sen & Baltimore, 1986a). It regulates the transcription of a variety of genes, including cytokines and growth factors, adhesion molecules, immune receptors, and acute-phase proteins, and is thus involved in many biological processes, such as inflammation, immunity, cell activation, apoptosis, proliferation, differentiation, and survival (Natoli et al., 2005a; Sen, 2006; Sen & Baltimore, 1986a; Vallabhapurapu & Karin, 2009). The NF-κB/Rel family consists of 5 members, including RelA/p65, c-Rel, RelB, NF-κB1/p50 and NF-κB2/p52. NF-κB functions as heterodimer or homodimer (Ghosh et al., 1998), in which the heterodimer p50/p65 is the most common functional form in cells because only RelA contains a transactivation domain (Schmitz & Baeuerle, 1991). NF-κB is generally sequestered in the cytoplasm by inhibitory protein IκB as an inactivated form (Ghosh et al., 1998). When cells are stimulated by a wide variety of stimuli, such as TNF-α, LPS, virus and UV (Pahl, 1999), IκB is degraded and NF-κB translocates into nucleus (Beg & Baldwin, 1993; Karin, 1999), where it binds to its DNA targets (κB sites), and regulates transcription of its target genes. Studies have revealed that the function of NF-κB is considerably complex and diverse (Sen & Smale, 2010). Deciphering the complete function of NF-κB is still a challenge.

NF-κB is a sequence-specific DNA-binding transcription factor; the sequence-specific binding with DNA is the key step in its regulation of target gene expression. Therefore, investigation of its in vitro and in vivo DNA binding profile (DBP) is crucial for identifying its direct target genes (DTGs) in genome. In recent years, the in vitro DBP of NF-κB has been characterized with several high-throughput techniques, such as dsDNA microarray (Linnell et al., 2004; Matys et al., 2003; Nijnik et al., 2003; Udalova et al., 2002; Wang et al., 2003), and EMSA-Seq (Wong et al., 2011). This study revealed that NF-κB could bind both canonical and non-canonical motifs (Wong et al., 2011).

In recent years, the in vivo DBPs of NF-κB have also been investigated with several low- and high-throughput techniques based on chromatin immunoprecipitation (ChIP). These techniques include ChIP-PCR (Nowak et al., 2005; Saccani et al., 2001), ChIP-cloning (Antonaki et al., 2011), ChIP-chip (Martone et al., 2003; Schreiber et al., 2006), ChIP-PET (Lim et al., 2007) and ChIP-Seq (Kasowski et al., 2010). Using these techniques, the in vivo DNA-binding targets and many new target genes of NF-κB in various cells, such as U937 (Schreiber et al., 2006), HeLaS3 (Martone et al., 2003) and THP-1 (Lim et al., 2007), under different stimuli, such as LPS (Lim et al., 2007; Schreiber et al., 2006) and TNF-α (Martone et al., 2003), were identified. These in vivo studies identified distinct but overlapping sets of target genes of NF-κB in different cells under various stimuli. However, these studies mainly focused on identifying target genes

Abbreviations: ChIP, chromatin immunoprecipitation; ChIP-chip, ChIP in combination with DNA microarray; ChIP-PCR, ChIP in combination with polymerase chain reaction; ChIP-PET, ChIP in combination with paired end tag; ChIP-Seq, ChIP in combination with sequencing; DBP, DNA binding profile; DMEM, Dulbecco's Modified Eagle Medium; DTG, direct target genes; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EMSA-Seq, electrophoresis mobility shift assay in combination with sequencing; FBS, fetal bovine serum; FC, fold change; FE, fold enrichment; HC-peak, high-confidence peak; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa B; PCR, polymerase chain reaction; PIC, protease inhibitor cocktail; PMSF, phenylmethylsulfonyl fluoride; siRNA, small interfering RNA; TES, transcription end site; TNFα, tumor necrosis factor-alpha; TSS, transcription start site.

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of NF-κB, and did not described in vivo genome-wide binding characteristics of this factor and their effect on gene transcription.

In this study, we checked the genome-wide binding characteristic of NF-κB and its effects on the transcription of target genes of transcription factor in the human cervical carcinoma cells HeLa stimulated by tumor necrosis factor-alpha (TNFα) by using ChIP-Seq and genechip assays. It was found that NF-κB had several significant binding characteristics that exerted effects on the transcription of its target genes. These genome-wide binding characteristics reflected some important traits of this protein that acts as a stimulatory transcription factor involving in many biological processes and responds to various stimuli.

2. Results

2.1. ChIP-Seq reads and peaks

By using the Illumina GA IIx Analyzer, a total of 23,921,462 reads were obtained from the sequenced ChIP DNA, in which 87.4% was uniquely mapped to human genome (hg19). In the uniquely-mapped reads, 95.88% (20,053,436) were in pairs. After peak calling, 1,667,070 peaks were obtained. In these peaks, 76.57% (1,276,537) had a fold enrichment (FE) lower than 10, 20.81% (346,980) had a FE of 10 to 20, and only 2.61% (43,553) had a FE over 20. Among the peaks with FE over 20 (defined as high-confidence peak, HC-peak), 37,989 (87.22%) had a FE of less than 30, 790 had a FE of 40 to 49, and 205 had a FE of 50 to 99. Besides these peaks, there were 209 peaks with a FE of 60 to 999. Nine peaks had a FE over 1000. The highest FE was up to 20,758, which was located on chromosome 2, within introns of gene LOC285045 and the peak width was 785 bases. The width of HC-peaks averaged 1838 base pairs.

2.2. Peak distribution on chromosome

To characterize the chromosome distribution of ChIP peaks, the number of all peaks and the peaks with FE ≥10 and ≥20 present on each chromosome were counted, and the peak density per 100 kb of each chromosome was calculated. As a result (Fig. S1), when counting all peaks, the total number of peaks on each chromosome was nearly proportional to the length of the chromosome, and the peak density appeared to have no significant difference among chromosomes. However, when counting peaks with FE ≥ 10, the number of peaks was found to be not proportional to the length of chromosome, and the peak density showed obvious variation among chromosomes. This variation was further enlarged when only HC-peaks were counted (Fig. 1). It was found that NF-κB had the highest binding strength to chromosome 5, but the lowest binding strength to chromosome X.

To find the possible correlation between peak distribution and genes on chromosomes, the sum of FE values of all peaks and HC-peaks in every non-overlapping 1000-kb region of each chromosome was calculated, meanwhile, the transcription start site (TSS) in the corresponding region was counted. The data were schematically plotted on chromosomes in proportion. As a result, it was found that the distribution of HC-peaks was closely related to that of TSS on chromosomes (Fig. S2). However, the distribution of all peaks had no relationship with that of TSS (data not shown). This finding revealed that the high-affinity binding of NF-κB mainly occurred in the genomic regions with TSS. The analysis of peak distribution around TSS revealed that there was a significantly high enrichment of peaks in −200 bp upstream of TSS (Fig. 2). These data demonstrated that NF-κB more easily bound to promoter regions near TSS.

2.3. Correlation of peaks with genes

To investigate the correlation of peaks with the annotated genes, the genomic region from −100 kb upstream of TSS to +100 kb after transcription end site (TES) was defined as the gene region as described previously in a ChIP-PET study of NF-κB (Lim et al., 2007). The peaks that fell into the gene region of a gene were regarded as gene-related peaks. As a result (Fig. S3), when all peaks were repeatedly ascribed to genes, peaks covered all genes (29,158) in the human genome, and the HC-peaks covered 25,551 genes. When peaks were counted once only to their nearest gene, all peaks covered 27,642 genes and the HC-peaks covered 13,600 genes. The genes covered by HC-peaks were conservatively regarded as NF-κB-bound genes, in which 57% of genes were occupied by multiple peaks (≥3), and the gene TRIO was occupied by as many as 251 HC-peaks.

To find the location preference of peaks in the gene region, the genome was divided into 8 parts (Lim et al., 2007), including 5′ distal region, 5′ proximal region, first exon, other exons, first intron, other introns, 3′ region and intergenic region. All peaks and HC-peaks in these regions were counted. As a result, when peaks were repeatedly counted, peaks of two kinds were predominantly located in 5′ distal region and 3′ region (Figs. 3A and S4A); however, when peaks were counted only one time to their nearest gene, peaks of two kinds were predominantly located in introns (Figs. 3B and S4B). In comparison, the least peaks were located in exons. As an example, the HC-peaks in the gene region of the NFKBIA gene were showed (Fig. 3C). NFKBIA (coding IκB) is a known target gene of NF-κB that was also identified as a direct target gene of this transcription factor in this study.

2.4. Gene regulation in TNFα-stimulated HeLa cell

To find the effect of genomic binding of NF-κB on gene expression, the global gene expression was profiled with DNA microarray. As a result (Fig. 4), it was found that 434 and 790 genes were up- and downregulated 2.0 fold by TNFα stimulation, respectively, in which, 432 TNFα-upregulated genes were significantly downregulated by p65 siRNA and 582 TNFα-downregulated genes were significantly upregulated by p65 siRNA. These genes were thus regarded as genes that were regulated by RELA. To identify the direct target genes of NF-κB, these genes were compared with the genes bound by NF-κB identified with above ChIP-Seq assay. As a result, it was found that 266 activated and 318 repressed genes were shared by two groups of genes. These genes were regarded as direct target genes (DTGs) of NF-κB. Besides these high-confidence DTGs, many genes regulated by TNFα 1.5–2.0 fold but antagonized by p65 siRNA were also bound by NF-κB. These genes were regarded as the potential DTGs of NF-κB (genes and their detailed analysis see reference (Xing et al., 2013)).

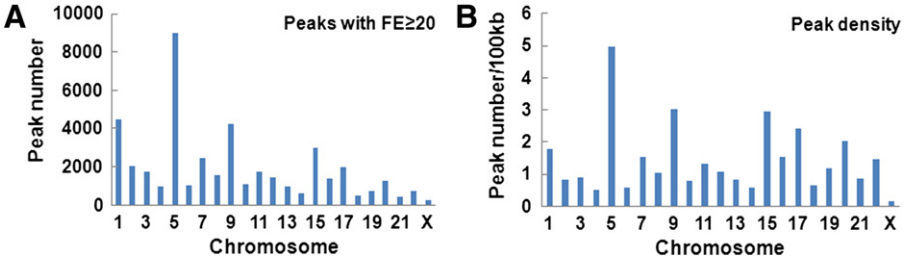

Fig. 1. Peak numbers and densities on chromosomes.