Salam et al., 2024

Volume 10, pp. 01-11

Received: 23rd August 2024

Revised: 1st September 2024, 8th September 2024

Accepted: 26th August 2024

Date of Publication: 15th September 2024

DOI- https://doi.org/10.20319/lijhls.2024.10.0111

This paper can be cited as: Salam, D.S.D. A, Hwang, S.S. Chee, X.W. (2024). Mini-Review of Nuclear-Factor-Kappa-B in Silico Studies. LIFE: International Journal of Health and Life-Sciences, (10) 01-11

MINI-REVIEW OF NUCLEAR-FACTOR-KAPPA-B *IN SILICO* STUDIES

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Abstract

This mini-review provides a concise overview of the advancements in computer-assisted methodologies and research related to nuclear factor kappa B (NF-kB) over the past decade. Applying computer-aided or in silico methods to investigating the NF-kB complex offers

intriguing options for identifying treatment targets for various disorders involving the NF-kB protein. Compared to traditional in vivo and in vitro investigations, in silico research has multiple advantages, including improved precision, increased efficiency, and eliminating the requirement for human and animal participants. This method creates a framework for evaluating the efficacy of potential treatments against specific molecular targets, allowing for the prediction of their efficacy based on the structural properties of compounds before synthesis for subsequent in vitro and in vivo testing. Targeting the NF-kB protein is critical because it plays a role in many disorders involving immunological and inflammatory responses, stress responses, cellular proliferation, and apoptosis. These findings are essential for guiding future research into the role of the NF-kB protein in human disorders and identifying possible therapeutic targets.

Keywords:
Nuclear Factor Kappa B, In Silico, Signalling Pathway, Inflammation, Immune Response.

1. Introduction

In their 1986 study, Ranjan Sen and colleagues identified the nuclear factor kappa-B (NF-kB) as a transcriptional regulator of the immunoglobulin-k light-chain gene within the laboratory of Nobel Prize winner David Baltimore. The NF-kB protein complex is crucial for the regulation of DNA transcription, the production of cytokines, and the survival of cells. This protein complex is found in nearly all animal cells. It has a role in cellular responses to various stimuli, including cytokines, oxidative stress, free radicals, heavy metals, UV light, oxidised low-density lipoprotein, and bacterial and viral antigens. NF-kB is an essential transcription factor that modulates the immune response during infections. Dysregulation of NF-kB has been linked to cancer, inflammatory and autoimmune illnesses, septic shock, viral infections, and aberrant immune system development. This dysregulation often results in the persistent activation or abnormal regulation of NF-kB proteins in different types of human tumours. The active form of NF-kB promotes gene expression that supports cell proliferation and protects against conditions that typically induce programmed cell death (apoptosis). In cancer, mutations or overexpression of proteins that regulate NF-kB signalling disrupt the ability of malignant cells to interact appropriately with the surrounding tissue. This disruption is particularly evident in processes such as metastasis and the immune system's failure to effectively eliminate tumours (Hayden et al., 2006).

The NF-kB protein complex is predominantly located in the cytoplasm of cells and comprises five proteins: NF-kB1, p50, p65, RelA, and c-Rel. These proteins are interrelated

and share structural similarities. Each NF-kB protein comprises a core Rel homology domain (RHD) for DNA binding and transcription initiation. Additional domains within the NF-kB proteins promote protein-protein interactions and regulate the complex's function. In mammals, the NF-kB family contains five transcription factors: p65/RelA, RelB, c-Rel, p105/p50 (NF-kB1), and p100/p52 (NF-kB2) (Siebenlist et al. 1994).

The NF-kB protein complex is released from the cytoplasm and translocates to the nucleus upon cellular activation. In immune responses, inflammation, cell proliferation, and development, the NF-kB complex binds to DNA, enhancing gene transcription. The "canonical" NF-kB complex is characterised as a p50/RelA heterodimer. When inactive, NF-kB exists in the cytosol in association with the inhibitor of kappa B (IkB). External stimuli can activate IkB kinase (IKK) via integral membrane receptors. The IKK complex then phosphorylates the IkB protein, which is ubiquitinated, dissociated from NF-kB, and degraded by the proteasome. This step activates NF-kB, translocating to the nucleus and binding to particular DNA response regions. The DNA/NF-kB complex recruits other proteins, such as coactivators and RNA polymerase, to aid in the transcription of downstream DNA into mRNA, which is then translated into protein, affecting cellular function (Solt and May 2008)

NF-kB signalling operates through two distinct pathways: canonical (or classical) and non-canonical (or alternative) pathways. The canonical route is activated when a ligand, such as TNF-alpha or interleukin 1 (IL-1), binds to the NF-kB receptor and recruits adaptor proteins. This recruitment draws the IKK complex, which phosphorylates IkB and targets it for destruction by proteasomes. The IKK complex is made up of two active kinases, IKKa and IKKB, and a regulatory scaffold protein called the NF-kB essential modulator (NEMO). Both IKK and NEMO are required for the activation of the p50/RelA and p50/c-Rel complexes; however, IKK is usually dormant (Deptala et al. 1998).

Meanwhile, the non-canonical route regulates the activation of p100/RelB complexes, which occurs when lymphoid organs that produce B and T cells develop. This route involves using an IKK complex, either IKKa or IKKB, but not NEMO. NIK (NF-kB-inducing kinases) is activated when a receptor binds to its target. This phosphorylates the p100 IkB domain, starting an IKKa complex that releases p52 (RelB). The heterodimer subsequently translocates to the nucleus, activating target genes (Bonizzi et al. 2004).

There are some main differences between the two pathways. The canonical pathways involved in the degradation of IkB respond to numerous stimuli, which are rapid and transient. Meanwhile, the non-canonical pathway does not include the degradation of IkB. It is slow and

persistent, responding to specific signals such as tumour necrosis factor (TNF) and only specific functions. Many inflammation-related disorders, immunological responses, and cancer emerge due to the NF-kB signalling system failing to operate appropriately (Wu and Miyamoto 2007). Activation of the NF-kB pathway is tightly controlled and regulated as the pathway itself involves numerous networks, consequently indicating that the control of the pathway is more complex than simply a protein-protein interaction. Several studies (Begalli et al. 2017; Yu et al. 2020) suggested that inhibiting and activating the NF-kB pathway might be a viable targeted treatment for various disorders, including cancer. Hence, inhibiting NF-kB and this signalling pathway is exciting.

2. Inflammatory and Immune Response

Inflammatory and autoimmune diseases can be caused by NF-kB pathway dysfunction. When the NF-kB canonical pathway is engaged, pro-inflammatory cytokines such as interleukin (ILK)-1, ILK-6, and TNF-α are generated. The NF-kB protein complex also regulates inflammatory T-cell activation (Brasier 2010). Autoimmunity destroys cells and induces chronic inflammation due to a protracted immune response to self-antigens (Liu et al. 2017). Rheumatoid arthritis, inflammatory bowel disease, asthma, chronic obstructive pulmonary disease, and diabetes are among the autoimmune and inflammatory illnesses linked to NF-kB signalling pathways. Kanan et al. (2021) employed Quantitative Structure-Activity Relationship (QSAR) models to test for anti-inflammation medicines that target the NF-B/IBa and p50/p65 (RelA) heterodimer complex as a potential COVID-19 therapeutic target. The inflammatory response in SARS-CoV-2 was thought to be dominated by NF-kB activation. Using the QSAR model, they could forecast the toxicity of over 220,000 drug-like compounds and narrow down 382 candidate molecules for additional molecular dynamics simulations and free energy calculations. On the other hand, Li and colleagues (2021) explore the effects of B-caryophyllene (BCP), a natural sesquiterpene, on anti-inflammatory signalling pathways focusing on acute gouty arthritis treatment. They used bioinformatics methods such as GeneCards, String database, GO functional enrichment analysis, and molecular docking experiments to address adverse reactions of currently available arthritis treatment. They found that BCP is highly connected to the NF-kB and Toll-like receptor pathways. Also, molecular docking experiments are used for comparative inhibitor study of NF-κB complex as anti-Type 2 Diabetes Mellitus compounds (Hikmaranti et al. 2020). Four natural chemicals in walnuts, urolithin A and B, gallic acid, and ellagic acid, show the ability to dynamically bind to the active site of NF-B with varying affinities. Because it has the most significant binding energy

(-228,9 kcal/mol), ellagic acid is the most stable molecule and has the most activity blocking the NF-B pathway of the four. Ellagic acid, an active polyphenol molecule, is an antidepressant that suppresses NF-kB activation and translocation from the cytoplasm to the nucleus, lowering the pathophysiological consequences of type 2 diabetes mellitus. Meanwhile, Latawa et al. (2021) employed a computational modelling approach to investigate potential pharmaceutical targets and diagnostic biomarkers related to synovial tissue in rheumatoid arthritis. Their findings indicated that targeting several key signalling pathways, including LCK-CD4, VAV1-CD4, and MLT-ROR, could be promising for drug development. This was achieved by creating a mathematical model incorporating 30 interactions involving two-gene and three-gene networks, alongside an analysis of the effects of 92 distinct perturbations on rate constants. Notably, the heightened activation of the DEC2-IL1 transcription factor and the NF-kB pathway emerged as exciting candidates for diagnostic markers.

3. Cancer

For many years, researchers have hypothesised a link between inflammation and cancer. The missing connection between these two processes might be NF-kB. Mechanisms via which NF-kB activation can contribute to developing leukaemia and lymphoma. Inflammatory stimuli stimulate NF-kB, and constitutive NF-kB activity has been associated with cancer. In myeloid and lymphoid cells, NF-kB can be activated in response to growth factors and cytokines and the production of certain viral oncoproteins (Barnabei et al., 2021). For many years, researchers have hypothesised a link between inflammation and cancer. The missing connection between these two processes might be NF-kB. Mechanisms via which NF-kB activation can contribute to developing leukaemia and lymphoma. Inflammatory stimuli stimulate NF-kB, and constitutive activation occurs. Shankar et al. (2019) present an intriguing computational analysis and in vitro investigation to evaluate the mechanical route components of the PI3K-Akt and NF-kB signalling pathways in prostate cancer. Their findings show that an IKK complex component can coordinate the PI3K-Akt and NF-kB pathways. This chemical has the potential to be a therapeutic target for prostate cancer. Another interesting study is the combinational approach of EZH2 and NF-kB inhibitors for treating prostate cancer cells (Jin et al. 2021). Through KEGG analysis, the feedback stimulation of NF-kB signalling in prostate cancer cells caused by EZH2 inhibition generated several EZH2 substrate genes in NF-kB and TNFA signalling pathways. Apart from the prostate cancer studies, Murwanti et al. (2020) performed molecular docking analyses to elucidate the binding poses of protein-ligand interactions between NF-kB (p105) and curcumin in a study on triple-negative breast cancer.

It was shown that the curcumin aromatic ring interacts with NF-kB p105 at the Rel homology domain region through hydrogen bonds. Hence highlighting the curcumin potential to be developed as a chemotherapeutic treatment targeting NF-kB in triple-negative breast cancer patients.

4. Neurodegenerative

NF-kB signalling pathway is also implicated in the neurological system, particularly in the cortex and hippocampus areas, which are essential in human learning and memory. Defects in NF-kB signalling can produce pro-inflammatory mediators such as TNF-a, causing inflammation within the nervous system and leading to disorders such as Alzheimer's and Parkinson's (Kaltschmidt et al. 2022). Hira et al. (2020) researched to weigh the efficacy of aldosterone antagonists (eplerenone) in a streptozotocin-induced Alzheimer's disease model. In silico modelling was used to examine the chemical behaviour of substances that inhibit acetylcholinesterase using induced fit docking. Behavioural paradigms such as passive avoidance, raised plus maze, Morris water maze, open field, and balancing beam were used to test Alzheimer's anti-impact. They discovered that eplerenone can be utilised to enhance memory in dementia and Alzheimer's disease patients by correcting streptozotocin-induced memory impairment in mice.

5. Other Diseases

A lesser-known disease that could arise from the effect of pro-inflammatory cytokines generated due to dysregulation of the NF-kB signalling pathway is depression. People with depression are usually associated with loss of appetite and energy and a tendency to be suicidal, generally due to tissue damage resulting in an unpleasant sensory and emotional experience. Hence, seeking excellent and effective analgesics is vital for treating depression disorder. *In silico* exploration of bioactive phytochemicals found in E. pappillosum, a traditional medicine used for hysteria treatment, was done to predict the pharmacological activities for novel therapeutic applications and showed some promising results (Uddin et al. 2021). Similarly, molecular docking studies of Citrus maxima bioactive compounds such as hesperidin, naringenine, and naringin employing NF-kB validated their efficacy in mouse lipopolysaccharide-induced illness behaviour (Nandeesh et al. 2018). Citrus fruit phenolic fraction reduced IL-6 and NF-kB, demonstrating protective benefits from antioxidant and anti-inflammatory actions.

Stroke is another brain disease associated with the NF-kB signalling pathway due to its irregularity-inducing inflammation. Repurposing drugs using in silico studies is also faster and more cost-effective than a comparative study that could be done with thousands of medications, such as in the study of Ali et al. (2020). They found a potential therapeutic approach of established drugs, namely atorvastatin, cephalexin, and mycophenolate, to investigate the neuroprotective effects against the NF-kB compared to caeffic acid phenethyl ester (CAPE), a standard NF-kB inhibitor. In the same way, Jiang et al. (2019) identified tilianin as a potential mechanism to treat oxygen-glucose deprivation effects that result in cerebral ischemia or stroke. Tilianin is a natural flavonoid that has shown positive results in cardiovascular disease and exerted neuroprotective effects in stroke.

6. Future Perspectives

Several methods for inhibiting NF-kB activation include early-stage signal blocking, interference within the cytoplasmic phase, and NF-kB nuclear translocation inhibition (Gilmore and Herscovitch 2006). The discovery of NF-kB inhibitors is noteworthy because they provide a unique treatment method for various disorders, including inflammatory diseases and viral infections. *In silico* studies or computer-aided research have been used for many practical applications, especially targeting the NF-kB complex. Some of the tools included for experiments conducted by computer are data analysis, data mining, machine learning, finding homology models, quantitative structure-activity relationships (QSAR), and network analysis. *In silico* studies offer hope for discovering possible treatment targets for a variety of disorders affecting the NF-KB signalling system. In comparison to *in vivo* and *in vitro* research, *in silico* investigations are more precise and time-efficient, because they do not require the use of humans or animals. It provides the foundation for assessing the efficacy of potential therapeutics against molecular targets, predicting activity based on a compound's structure even before it is synthesised for further *in vitro* and *in vivo* testing. One of the *in silico* studies (Sung and Simon 2004) showed that targeting the NF-kB signalling pathway required careful selection due to the complex network structure of the pathway and the interconnected dynamic interactions between the molecules and types of inhibitors. Unlike the simplistic view of static inhibition, they found that upstream events inhibition resulted in similar inhibition dynamics; meanwhile, direct inhibition produced distinct dynamics via the quantitative dynamic model of various inhibitor types.

7. Conclusion

In summary, targeting the NF-kB protein complex is critical since it can be implicated in several disorders listed above. Before proceeding to *in vitro* and *in vivo* studies, *in silico* methods were preferred for predicting NF-kb targeted treatment. Apart from reducing the time and cost of research studies, *in silico* experiments decrease the use of animal models and human cohorts, which is the highlight of the ethical research debate. Future NF-kB protein complex studies should explore and adopt various ways, perhaps in silico, to understand the complex nature of signalling pathways and their roles in numerous diseases.

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