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A BRIEF REVIEW OF NUCLEAR-FACTOR-KAPPA-B IN SILICO STUDIES

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Abstract

This mini-review briefly summarises the use of computer-aided tools and research developments in nuclear-factor-kappa-b (NF-kB) within the last ten years. Computer-aided or in silico studies on the NF-kB complex give hope for developing potential therapeutic targets for various diseases involving the NF-KB protein. In silico investigations, as opposed to in vivo and in vitro research,

have the advantages of being more exact and time-efficient and eliminating the utilisation of humans and animals. It provides a framework for evaluating the activity of prospective therapies against molecular targets, predicting their activity based on a compound's structure even before it has been synthesised for additional in vitro and in vivo research. Targeting NF-kB protein is crucial as it can implicate many diseases, such as immune and inflammatory responses, involved in stress response, cellular growth, and apoptosis. These findings are critical for directing future research into the NF-kB protein, its function in human disorders, and prospective therapeutic targets.

Keywords

Inflammation, Nuclear Factor Kappa B, Signalling Pathway, Cancer, In Silico, Immune Response

1. Introduction

Ranjan Sen et al. (1986) discovered the nuclear factor kappa-B cells (NF-kB) as a transcription modulator of the immunoglobulin-k light-chain gene in Nobel laureate David Baltimore's lab. The NF-kB protein complex regulates DNA transcription, cytokine synthesis, and cell survival. NF-kB is found in almost all animal cell types and plays a role in cellular responses to cytokines, stress, free radicals, heavy metals, ultraviolet irradiation, oxidised low-density lipoprotein, and bacterial or viral antigens. The transcription factor NF-kB is necessary to control the immune response to infection. NF-kB malfunction has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and aberrant immunological development. This leads to the presence of constitutively active or dysregulated NF-kB protein in a variety of unique kinds of human tumours. Active NF-kB causes gene expression to be activated, maintaining the cell's growth and protecting it from situations that would otherwise cause apoptosis. Proteins that control NF-kB signalling are mutated or overexpressed in cancer, which impairs the coordination of the malignant cell with the rest of the body. This is evident in metastasis and the absence of tumour clearance due to the immune system's inefficiency (Hayden et al. 2006).

The NF-kB protein complex is typically found in the cytoplasm of cells. It is a protein complex comprising five proteins: NF-kB1, p50, p65, RelA, and c-Rel. These proteins are all related to each other, and they have similar structures. Each NF-kB protein has a central Rel homology domain also known as RHD, which oversees binding to DNA and triggers transcription. Other NF-kB protein domains participate in protein-protein interactions and regulate the complex's activity. In mammals, the NF-kB family consists of five transcription factors (TF), namely p65/RelA, RelB, c-Rel, p105/p50 (NF-kB1), and p100/p52 (NF-kB2) (Siebenlist et al. 1994).

NF-kB protein complex is released from the cytoplasm and translocates to the nucleus when cells are activated. In immunological response, inflammation, cell proliferation, and development, the NF-kB complex binds to DNA and promotes gene transcription. A p50/RelA heterodimer is the "canonical" NF-kB complex. While inactive, NF-kB is found in the cytosol complexed with the inhibitor of kappa B (IkB). Various external inputs can activate the enzyme IkB kinase (IKK) through integral membrane receptors. IKK then phosphorylates the IkB protein, which causes it to be ubiquitinated, separated from NF-kB, and subsequently destroyed by the proteasome. NF-kB is then activated and translocated into the nucleus, which binds to response elements, particularly DNA sequences. The DNA/NF-kB complex employs other proteins, such

as coactivators and RNA polymerase, to transcribe downstream DNA into mRNA. The mRNA is subsequently converted into protein, which alters cell function (Solt and May 2008).

NF-kB consists of two distinct signalling pathways, canonical (also called the classical pathway) and non-canonical (also referred to as the alternate pathway). The canonical pathway is activated when a ligand, such as TNF-alpha or ILK1 (interleukin 1), binds to the NF-kB surface, causing adaptors to be recruited. The IKK complex is drawn to these adaptors, phosphorylating I-kB and directing proteasomes to destroy it. Two catalytically active kinases, IKKa and IKKB, and a regulatory scaffold protein called the NF-kB essential modulator comprise the IKK complex (NEMO). IKK and NEMO are necessary for the p50/RelA and p50/c-Rel complexes to be activated, but IKK is generally inactive (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 illnesses can be caused by NF-kB pathway dysregulation. When the NF-kB canonical pathway is activated, pro-inflammatory cytokines such

as interleukin (ILK)-1, ILK-6, and tumour necrosis factor (TNF) are produced. The NF-kB protein complex also modulates inflammatory T-cell activation (Brasier 2010). Autoimmunity causes cell destruction and chronic inflammation due to a prolonged immunological response against self-antigens (Liu et al. 2017). Rheumatoid arthritis, inflammatory bowel disease, asthma, chronic obstructive lung disease, and diabetes are among the autoimmune and inflammatory disorders implicated by 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) used a computer modelling technique to examine synovial-tissue-based pharmaceutical targets and diagnostic biomarkers in rheumatoid arthritis. They discovered that inhibiting several prominent signalling pathways, such as LCK-CD4, VAV1-CD4, and MLT-ROR, could potentially serve as drug targets by developing a mathematical model with 30 two-gene and three-gene network interactions and analysing the effect of 92 different

perturbations on rate constants. The increased activation of the DEC2-IL1 transcription factor and the NF-kB pathway, which might be used as diagnostic indicators, was very intriguing.

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 it provides 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 give hope for developing potential therapeutic targets for various diseases involving the NF-KB signalling pathway. In silico investigations, as opposed to in vivo and in vitro research, have the advantages of being more exact and time-efficient and eliminating the utilisation of humans and animals. It provides a framework for evaluating the activity of prospective therapies against molecular targets, predicting their activity based on a compound's structure even before it has been synthesised for additional in vitro and in vivo research. 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 crucial as it can implicate many diseases described above. Before moving on to in vitro and in vivo investigations, in silico approaches 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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