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Review Article

Structure-based drug design approach to target toll-like receptor signaling pathways for disease treatment

Mohammed Alaidarous
Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Al-Majmaah 11952, PO Box 66, Saudi Arabia

For correspondence:* ***Email:* *m.alaidarous@mu.edu.sa;* ***Tel:*** *+966164042900*

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Abstract

Toll-like receptor (TLR) signaling pathways are the first line of defence against many microbial organisms. The question of how TLRs recognize endogenous ligands remains controversial. Several studies have shown that TLRs are implicated in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. Therefore, in structure-based drug design, TLRs are now viewed as potential therapeutic targets in the treatment of autoimmune diseases. This review shows how proteins, specifically TLRs, are used as therapeutic targets to design inhibitors (drugs) using the structure-based drug design approach for disease treatment.

Keywords: *Structure-based drug design, Toll-like receptors, Autoimmune diseases, Endogenous ligands, X-ray crystallography, Homology modeling*

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INTRODUCTION

Structure-based drug design has been a thriving field since the human genome project was completed. Advanced developments in proteomics, transcriptomics and structural genomics helped in improving the computational tools used for structure-based drug design. Potential drug targets can now be mined via the use of bioinformatics tools. The genes for these targets can then be cloned, and protein products can be synthesized and purified for use. The three-dimensional structures of proteins used to determine potential drug targets are derived through a number of techniques, and the efficiency of these techniques has improved in recent years [1]. Below, the principles behind choosing drug targets and determining the three-dimensional structures of those targets will be reviewed. In addition, some receptor molecules from the TLR signaling pathways that have been targeted by drugs will also be reviewed.

Choosing a drug target

The process of designing a drug involves a series of steps. The first step is the identification of a drug target. This step is typically based on the biochemical and biological characteristics of the target molecule. A target molecule should be uniquely associated with a certain human disease or a disease-causing pathogen. For example, in targeting a pathogen, the molecule chosen from the pathogen as a target should be unique in that no other molecules in the pathogen can perform its function [2]. The chosen molecule should be essential, and its absence should guarantee the death of the disease-causing organism [2]. When these conditions are met, targeting of these molecules by drugs of interest will succeed. Typically, drugs

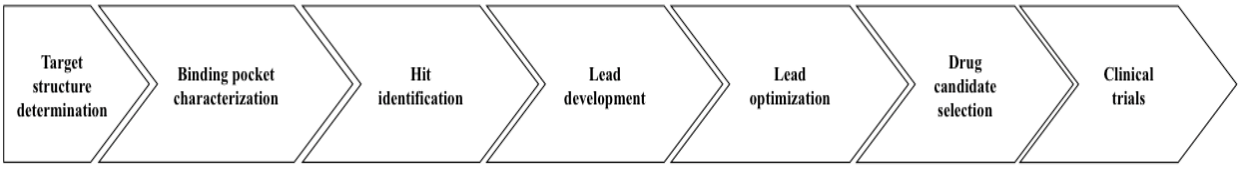

Figure 1: Structure-based drug design pipeline modified from Grey and Thompson [3].

Table 1: Examples of potential targets whose three-dimensional structures were determined using NMR, x-ray crystallography or homology modeling

Name of target	Technique	Reference
Human transforming growth factor alpha	NMR	[7]
HIV-1 reverse transcriptase	NMR	[8]
Vascular endothelial growth factor	NMR	[9]
Interleukin-13	NMR	[10]
Amyloidogenic beta 2-microglobulin	X-ray crystallography	[11]
Brucella methionyle-tRNA-synthetase	X-ray crystallography	[12]
Toll-interleukin 1 receptor (TIR) domain containing adaptor protein	X-ray crystallography	[13]
Human epidermal growth factor receptor 2	X-ray crystallography	[14]
Abl kinase	Homology modeling	[15]
Cyclin-dependent kinase 2	Homology modeling	[16]
Lymphocytic-specific kinase	Homology modeling	[17]
Tyrosine kinase	Homology modeling	[18]

of interest (or inhibitors) are made from protein molecules, but lately, RNA transcripts have been used as potential drug leads [2]. Examples of drugs and their target molecules will be discussed later in the review. Figure 1 shows a general pipeline for choosing a target for a structure-based drug design.

Obtaining the structure of the target molecule

The second step involves obtaining the structural details of the target molecule. These structural details can be obtained by using structural determination techniques such as nuclear magnetic resonance (NMR), X-ray crystallography or homology modeling. The first two methods require the expression and purification of the target molecule, after which sophisticated equipment and laboratory procedures are used to derive the three-dimensional structure. For X-ray crystallography, the sample must be crystallized while NMR uses the liquid phase of the sample [4,5]. When homology modeling is used, the DNA sequence of the target molecule is used to identify homologous molecules with high levels of DNA sequence similarity and available three-dimensional structures. Those three-dimensional structures are then used to determine the likely structure of the chosen target molecule [4,5]. For example, MODELLER is a famous computational software program used for homology-based protein structural modeling [6]. Table 1 shows examples of potential targets whose structures were determined using NMR, X-ray crystallography or homology modeling.

The identification of the target molecule binding site is the next step in this process. This is a site at which a ligand can bind to a target molecule and thus alter its function. A huge number of chemical molecules obtained from available databases are then docked into the target molecule binding site using docking software. Chemical compounds with the higher scores are then optimized by the addition of small groups, such as carbonyl and benzene rings, to achieve the maximum interaction scores. Successful chemical compounds (drugs) may be synthesized and then characterized in a laboratory for use in *in vivo* studies before entering into clinical trials [19].

Protease inhibitors

A number of protease inhibitors have been designed using the structure-based drug design pipeline shown in Figure 1. Examples include Agenerase, which has the commercial names Amprenavir and Viracept and is also known as Nelfinavir [20]. Other agents produced via structure-based drug design include Relenza (Zanamivir), which was designed to inhibit the enzyme neuraminidase; Minitab mesylate (commercially known as Glivec), which was designed to inhibit Abelson tyrosine kinase, and Tomudex, which was designed to inhibit thymidylate synthase [21]. Other inhibitors have been developed against a number of enzymes,

such as tyrosine phosphatase, β-lactamase, carbonic anhydrase and DNA gyrase. Furthermore, Iressa and Tarvesa are used to inhibit tyrosine kinase. Both drugs were used for lung cancer treatment, and Tarvesa is also used to treat pancreatic cancer [22]. In addition, Isoniazid is used in the treatment of *Mycobacterium tuberculosis* and was discovered via structure-based design methods. This drug inhibits the InhA's ability to facilitate the synthesis of mycolic acid, an important component of the *Mycobacterium tuberculosis* cell wall [23]. Carfilzomib and Bortezomib are proteasome inhibitors used to treat refractory multiple myeloma [24]. Lamivudine and Telbivudine inhibit the action of reverse transcriptase, a very important enzyme in hepatitis B virus (HBV) DNA replication [25]. Entecavir, an inhibitor of viral polymerase, is also used in HBV treatment [26].

Toll-like receptors (TLRs)

Toll-like receptors are a group of innate immunity receptors found in the cells of the immune system, such as the epithelial and endothelial cells of mucosal linings, macrophages, neutrophils, dendritic cells and B-cells [27]. These toll-like receptors recognize signature elements in pathogens, such as the bacterial lipopolysaccharide, single-stranded RNA, lipoproteins and unmethylated CpG DNA [27]. This immunity shields the body against pathogenic microorganisms by initiating inflammation to enable the body to elicit the correct immune response [28]. However, unregulated innate immune responses can lead to autoimmune disorders, in which the body attacks itself, causing organ damages [27]. In humans, various families of TLRs have been identified and due to their importance in defense against pathogens, they have been viewed as potential drug targets in the treatment of diseases such as autoimmune disorders [29].

TLR agonists

These are substances that work in synergy with TLRs to enhance their functioning. Imiquimod is a drug used for the treatment of diseases that target TLR signaling pathways [30]. Specifically, it targets the TLR7–MYD88-dependent pathway and causes the secretion of the proinflammatory cytokine IFN. In addition, Imiquimod is used against viral particles and tumor cells. Therefore, it is quite crucial in cancer therapy. It also induces the release of tumor necrosis factor (TNF) and interleukin-6 (IL-6). Imiquimod has been proven to cause the destruction of cancerous cells on the skin. Hence, it is used in the treatment of genital warts, melanomas and hepatitis C [31]. Other imdazoquinolines are used in the treatment of chronic lymphocytic lymphoma [32]. Single-stranded RNA agonists are used to target TLR7 and TLR8. 7-deazaguanosine is used to activate certain TLRs by replacing a guanosine residue in an RNA ligand and thus inducing responses mediated by T-cells that target tumors [33]. All these molecules activate TLR pathways to facilitate the activation of dendritic cells. These dendritic cells then reduce the suppressive abilities of the T-cells, resulting in an antitumor response [30-33].

There is a small agent known as "852A" that is used as an agonist of TLR7. It activates dendritic cells to trigger the release IFNα, a molecule with antitumor activity. In addition to cancer treatment, 852A is used in treating chronic lymphocytic leukemia. 852A causes cells displaying leukemic antigens to become more susceptible to cytokines [34].

Alternative anticancer drugs called CpG-based oligonucleotides are used to target the TLR9 pathway. These molecules are referred to as immune modulatory oligonucleotides. They are similar to bacterial DNA and hence act as ligands for TLR9 [35]. Agatolimod was developed to tackle hepatitis B infections, allergies, renal cancer and asthma [36]. CpG 7909 is used to treat patients with chronic lymphocytic leukemia. These oligonucleotides have shown elevated responses when used in combination with other drug agents that are already on the market [37].

Immunomodulation adjuvants MGN-1703 and MGN-1706, which contain noncoding DNA, were used as TLR9 agonists and thus function as anticancer agents. MGN-1703 is effective against colorectal and prostate cancer during the preclinical stages. IPH 3102 was developed for the treatment of breast cancer because it triggers NF-κB and INF-1, leading to the destruction of breat cancer cells [38].

ISS1018 is a type of compound known as an immunostimulatory sequence. Such sequences stimulate the TLR9 pathway, eliciting responses on the part of T-cells and the production of memory cells due to the activation of type 1 T helper (Th1) cells. ISS1018 triggers the release of immunoglobulin and interferon (IFNα) by B-cells and also causes dendritic cells to release TNFα, interleukin-12 and interferon-β [39]. Immunoregulators, such as IRS-954, inhibit cascade activated by TLR7 and TLR9 to treat systemic lupus erythematosus [40].

TLR antagonists

TLR antagonists are used to suppress the signaling of TLR pathways in cases of overactive immune systems. These antagonists are used in the treatment of autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus. Currently, these diseases are treated with antimalarial compounds such as hydroxychloroquine which acts as a TLR9 antagonist [41]. IRS-954 is a molecule that slows down the production of IFNα, thus combating systemic lupus erythematosus [40]. IMO3100 is used to treat multiple sclerosis psoriasis in addition to systemic lupus erythematosus and rheumatoid arthritis. It works by blocking TLR7 and TLR9 [42]. In addition, IMO2125 stimulates TLRs and is used in the treatment of hepatitis C infections, melanoma and other cancers. Azithromycin is used in the treatment of cystic fibrosis. This drug agent suppresses the activation of NF-κB, resulting in the release of inflammatory cytokines in the trachea, and it also significantly reduces the levels of TNF in the epithelial cells of the airways [39,43].

Ibudilast (Av411) has been found to suppress pain and treat the withdrawal symptoms associated with drug addiction. It slows down the production of TNFα and IL-6 [44]. IMO8400 is used in the treatment of dermatomyositis because it reduces the excessive stimulation of various TLRs [45]. Eritoran is another antagonist that is currently being investigated for use in the treatment of sepsis. It has been found to disable the responses that arise due to lipopolysaccharides' activation of TLRs [46]. Belimumab has been used to improve the conditions of those suffering from systemic lupus erythematosus. It is a human monoclonal antibody that prevents the activation of soluble B-cells, resulting in the programmed cell death of autoreactive B lymphocytes [47].

CONCLUSION

Structure-based drug design provides an excellent platform for the identification of novel inhibitors of target molecules to fight diseases. With the continuous advancement of X-ray crystallography, NMR and molecular homology modeling, new and important molecules will be targeted to fight diseases. Significant challenges involved in dealing with target molecules, such as membrane proteins, must be addressed in future studies. Current efforts in structure-based drug design will undoubtedly lead to the development of significant therapeutics to combat diseases.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The author declares that this work was done by him and all liabilities pertaining to claims relating to the content of this article will be borne by him.

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