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Network biology in development of monoclonal antibody therapeutics

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ABSTRACT

Monoclonal antibodies (mAbs) are large glycoproteins that recognize and remove/neutralize a specific target. Inflammation and inflammatory diseases are often treated with mAb-based therapeutics. Mathematical modeling is widely used in development of mAbs. Bioinformatics and structural modeling is used for humanization of mAbs and PK/PD modeling is extensively used in preclinical and clinical development. The objective of this commentary is to introduce systems biology-based modeling that can accelerate and improve development of mAbs.

1. Introduction

Antibodies, also called immunoglobulins, are large glycoproteins that recognize and remove/neutralize a specific target called antigen. Antigens can be foreign 'objects', such as bacteria and viruses, or the body's own molecules, such as cytokines, interleukins, and receptors. All antibodies are composed of two identical heavy chains and two identical light chains as a basic functional monomer unit. The antigen-binding (target-binding) region of an antibody, referred to as the fragment antigen binding (Fab) region; it recognizes a specific part of an antigen. Part of the heavy chain of an antibody is referred to as the fragment crystallizable (Fc) region. Fc region characterizes subclasses of antibodies and enables binding to Fc receptors (FcR). FcR are located on a number of cells in the immune system, including B lymphocytes, dendritic cells, and natural killer cells. Antibodies bind to FcR only when they are attached to an antigen. Binding of antibodies to FcR activates biological processes for clearance of antigens or stimulates lysis of target cells through phagocytosis or antibody-dependent cell-mediated cytotoxicity. Antibodies, their structure, subclasses, and function have extensively been studied [14,40,45,46].

An immune response against an antigen results in a collection of antibodies with different specificity and affinity [17]. This collection of antibodies (defined as polyclonal antibodies), targets the same antigen but via different binding sites (called epitopes) on the antigen. In contrast, monoclonal antibodies (mAbs) are not only specific for the same antigen, but also for the same epitope; they recognize the exact same part of an antigen. Production of mAbs is possible due hybridoma technology [24]. This technology includes immunization of animals, mostly rodents, with an antigen and leads to activation of B cells that produce antibodies against that antigen. The antibody-producing B cells are then isolated and co-cultured with an immortal myeloma cell line to create hybridoma, a fusion of an antibody producing B-cell and a myeloma cell [28]. These hybridoma cells are subsequently cloned and produce identical mAb. Since mAbs are derived from a single progenitor cell, they are homogeneous with respect to isotype, epitope, affinity, and specificity.

Immunization of mice with a human antigen results in a mAb that binds to that specific human antigen but has a mouse backbone. In fact, the first mAb approved for use in humans was a mouse mAb muromonoab-CD3 that targets human CD3 [18]. Administration of mouse mAbs to humans results in an immune response against these antibodies, which leads to their toxicity and limited efficacy in humans. In addition, these mAbs have a very short half-life due to weak interactions with human FcR [36]. This was the fate of muromonoab-CD3 and other early mouse mAbs for use in humans [18,26]. Hence, a number of approaches have been developed to render animal mAbs less immunogenic. These approaches include development of chimeric, humanized, and human antibodies [2]. A chimeric antibody is composed of human constant regions and animal variable regions. In a humanized antibody, 90–95% of the antibody is human, and 5–10% is animal. Human antibodies are fully derived from human germline sequences.

Development of mAbs for therapeutic use starts with selection of the target, which requires often requires extensive validation to understand the most effective way to modulate its activity. Candidate mAbs are generated using hybridoma technology [24]. If a human

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mAb does not bind to the mouse ortholog of the human target, a surrogate antibody is used to facilitate target validation and proof of concept studies. Selection of the lead for both surrogate and human mAbs requires employment of screening technologies and assays to identify desired favorable biochemical and biophysical properties [48]. While humanization, engineering of the Fc region, selection of a relevant species for preclinical safety studies, and exploratory PK/PD studies are carried on, manufacturability, selection of potential biomarkers, and clinical development plans are conducted. This process is described in detail in the literature [31,35,52,16]. Although guidelines and texts for development of mAbs are available, each target and its mAb are unique and require case-by-case approach.

In recent years, mAbs have become essential in the treatment of inflammation and inflammatory diseases. Four of ten top-selling mAbs in 2012 were for treatment of chronic inflammatory conditions [20]. The mechanistic properties of mAb for the treatment of inflammation and inflammatory diseases include blocking cytokines (such as TNF-α) and several interleukins (such as IL-12/23, IL-17, and IL-6) [25,55]. Quantitative pharmacology and model-based approaches are currently being applied in development of mAbs for the treatment of inflammation and inflammatory diseases; they include structural biology, bioinformatics/computational biology (humanization/chimerization efforts), and PK/PD modeling (preclinical and clinical development). For example, interspecies scaling of mAbs [29] is indispensable for determination of the first-in-human dose [33]. Moreover, indirect response modeling provides insights into determination of efficacious dose and dose escalations for clinical studies [44].

Approved mAb and ongoing clinical studies offer a foundation for development of new mAbs for the treatment of inflammation and inflammatory diseases. Quantitative models are emerging as a powerful and useful tool in drug development; thus, the use of these models and related methods have been studied and reviewed extensively [53,51,30,19]. Although an array of quantitative modeling approaches has been developed on biological systems for inflammation and inflammatory diseases, they were rarely used in a model-based drug development. These aforementioned modeling approaches commonly referred to as systems biology, include both theoretical as well as "big data"-based modeling. A thorough analysis of the gap between academic understanding and industry use of these models was published in 2011 [1]. In this publication, the causes of this gap are identified and a high-level framework to integrate systems biology-based models into drug development is presented. Five main types of systems biology-based models are described in this publication: heuristic, semi-mechanistic, mechanistic, network, and multi-scale systems pharmacology models. The focus of this commentary is to provide an insight into benefit of network biology-based models in development mAb for treatment of inflammation and inflammatory diseases.

2. Omics studies

It is impossible to address network biology-based models without addressing their relations to omics studies. Omics studies is a generic term referring to the genome-scale data sets that are emerging from high-throughput tools and technologies [22]. Examples of omics studies include whole-genome sequencing data (genomics), microarray-based genome-wide expression profiles (transcriptomics), and large-scale expression of proteins (proteomics). New specialized terms of omics studies are emerging as more scientific disciplines are interested in the systems biology such as pharmacogenomics or immunoproteomics. Although omics-based studies are widely implemented in understanding diseases and in mathematical models to predict clinical outcome, these studies are yet to be translated into drug development and clinical studies [32].

One modeling approach of omics studies is to use clustering and statistical methods to identify important genes related to a disease or different treatments for a disease. Koczan et al. showed that a subset of genes identified through transcriptomics data can identify patients with rheumatoid arthritis who respond to a treatment with anti-TNF-α mAb (etanercept) [23]. These important genes (or proteins) are then mapped onto known functional groups and interaction networks. Finally, the significance of enrichment of functional groups or interaction networks with the important genes is evaluated statistically. Calvano et al. analyzed changes in gene expression patterns in peripheral blood leukocytes in human subjects receiving a bacterial endotoxin as an immunostimulator [11]. The known genome-wide interaction network, retrieved from Ingenuity Pathway Analysis (www.ingenuity.com), was explored to identify significant functional groups in response to an inflammatory stimulus with bacterial endotoxin. This analysis revealed that response of peripheral blood leukocytes to inflammatory stimulus with bacterial endotoxin was mainly dysregulation of distribution in energy flow and modulation of translational mechanisms. However, understanding underlying mechanisms of such a biological phenomenon through transcriptomics studies is challenging due to inter- and intra-patient variability and variability of transcriptomics studies [42,49]. Reproducibility of transcriptomics studies can be monitored by measuring expression levels of the genes of interest by quantitative real-time PCR [23]. Inter- and intra-patient variability in omics studies is reduced with stringent statistical methods [50,32].

mAbs may exhibit complex, non-linear pharmacokinetics, with substantial inter- and intra-patient variability mostly due to changes in expression of the target [15]. Such variability is incorporated in PK/PD modeling if the covariates driving variability are identified. Typical exploratory covariates for PK analysis in clinical studies are weight, age, gender, and race of subjects. In addition to these typical covariates, disease-specific characteristics are also included in covariate analysis. For psoriasis, disease-specific covariates include duration of the disease, PASI score, diabetes, hypertension, and prior treatment with immunosuppressive or protein-based drugs [54]. (Psoriasis Area and Severity Index or PASI score is measured on selected skin regions where intensity of redness, thickness, and scaling of skin lesions is assessed; hence, it is a composite index reflecting the severity of the disease [4].) With the increasing knowledge and developing technologies, it is clear that variation in drug exposure and drug responses may emerge from genomic changes. Thus, identifying variability and clinical covariates should be based on clinical data obtained from a large population of patients and appropriate demographics [21]. Understanding the relationship between changes in gene expression at the level of genome in blood and exposure to mAb early in clinical development could facilitate the late stage clinical studies assessing efficacy. For example, significant changes in gene expression at the level of genome, e.g., group of important genes, may serve as an identifier for variability in exposure to mAb in blood and facilitate selection of patients.

An example of potential utilization of omics data in clinical studies is integrating placebo effect observed in the treatment of psoriasis into selection of patients. Administration of placebo to some patients with psoriasis resulted in significant effect on PASI score in multiple clinical studies; thus, these patients are not candidates for treatment. The time-course of the placebo effect on PASI score was captured in an empirical function form with an indirect response model along with that observed following administration of ustekinumab and brodalumab [54,44]. Since placebo effect could only be described by an empirical function and not a mechanistic model, the predictions based on the final model were uncertain. On the other hand, correlation of omics data with placebo effect and the drug effect may enable reliable selection of patients who do not need treatment.

Another modeling approach using genomics data is construction of a disease-specific network [8]. Nair et al. developed a protein–protein