Commentary

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Applications of genome-scale metabolic network models in the biopharmaceutical industry

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Biotechnology is currently evolving through the era of big data, thanks to advances in the high-throughput technologies for rapid and inexpensive genome sequencing and other genome-wide studies [1]. With the daunting amount of data, it has been possible to put them together into a coherently organized biological network that provides counterintuitive insights on biological systems [2]. Among such biological networks, a genome-scale metabolic network model is expected to play an increasingly important role in the biopharmaceutical industry [3]. Before enumerating their specific strengths, it is important to note that principles underlying genome-scale metabolic network models are consistent with the holistic perspective of systems biology, the aim of which is to unveil hidden factors causing diseases and to find relevant treatment strategies [4]. Despite the importance of metabolism in a biological system, studies on diseases in relation to metabolism were far fewer in number than those performed on signaling and transcriptional regulatory networks [5]. However, metabolism, highly linked with observable phenotypes, is a biological network that is more comprehensively characterized when compared with the other two types of networks [6]. Metabolism is, therefore, amenable to large-scale mathematical modeling and simulation. It is with this motivation that the genome-scale metabolic simulation deserves more attention in drug discovery campaigns and optimization of a host strain for the production of biopharmaceuticals.

Reconstruction and application of genome-scale metabolic network models have been forged as a major research strategy of systems biology. Over the last decade, genome-scale metabolic models have been built for almost all biologically important organisms across the domains of archea, bacteria and eukaryotes [3]. They range from simple microorganisms such as *Escherichia coli* [7] and *Saccharomyces cerevisiae* [8] to higher organisms including Chinese hamster ovary (CHO) cells [9,10] and a generic human cell [11,12]. It should be noted that all these organisms that have been subjected to metabolic modeling are important cellular hosts for biopharmaceutical production or medically meaningful organisms that need to be cured (e.g., specific cancer cells) or destroyed (e.g., pathogens). A recent notable development of importance in the genome-scale metabolic modeling would be the newly updated human metabolic network Recon 2 [12]. Recon 2 is a result of efforts from a group of researchers, going over a vast amount of literature and biochemical data and reconciling conflicting information. Scope of the hitherto reconstructed genome-scale metabolic models manifest high expectations for their potential contributions to biopharmaceutical industry.

Genome-scale metabolic network models are not just a simple pileup of biochemical reactions, but allow mathematical simulation under precisely defined conditions of constraints [13]. Once the experimentally

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or theoretically validated biochemical reactions are incorporated into the metabolic network, the network model is mathematically expressed in a matrix using stoichiometric coefficients of associated metabolites. The complete metabolic network model is simulated using numerical optimization, often called constraint-based flux analysis [14]. The constraint-based flux analysis has proved to be extremely useful in predicting metabolic fluxes for individual reactions under specific genetic and environmental conditions, without requiring kinetic parameters. This approach allows prediction of the effects of consuming specific nutrients or inactivating genes on the metabolic status at genome-scale. Another strength of constraint-based simulations of genome-scale metabolic network models is that it can be easily combined with experimental data [15], mainly omics data, or different modeling approaches (e.g., dynamic reactions) for more precise description of the target organisms [16]. These features are especially useful in designing environmental conditions, including nutritional composition, for optimal growth of the production host, and predicting drug targets that effectively inhibit the proliferation of cancer cells or pathogens. We herein introduce recent contributions of genome-scale metabolic network models in various aspects of the biopharmaceutical industry.

CHO cells can be considered one of the most important production hosts for recombinant therapeutic proteins. In bioprocess engineering, increasing the production titer and yield is always a great challenge, and the CHO cell is not an exception. For this reason, systems-level metabolic analysis is critical, and this approach was reinforced through the use of the genome-scale metabolic model [9,10] in addition to various omics technologies. For instance, the metabolic model was used to characterize the CHO cell's metabolism at growth phases at which lactate was produced or consumed [9]. As the CHO cell consumes glucose, it secretes lactate, and once the glucose is depleted, it then starts to consume lactate. A detailed analysis employing the metabolic model revealed that the lactate-consuming cells had greater efficiency of energy production than the glucose-consuming (or lactate-producing) cells. Likewise, CHO cells were subjected to systematic metabolic analysis using their metabolic model and analytical metabolomics for the three different growth phases during a fed-batch cultivation: early and late exponential and nongrowth phases [10]. This integrative approach identified changes in metabolism associated with energy, glutathione and glycerophospholipid as potential causes for the growth limitation towards the end of the fed-batch cultivation. Although these studies did not provide specific strategies as to what should be done to overcome the confronted limitations for cultivating the CHO cells at high efficiency, they nonetheless provided best possible scenarios on what is really happening inside the cell throughout the course of cultivation. Such insights would no doubt be reflected accordingly in the design of more efficient CHO cell-based bioprocesses.

Drug targeting for various diseases is also an important aspect of fundamental research in biopharmaceutical industry. Genome-scale metabolic network models have made significant contributions to predicting the effects of genetic and environmental perturbations *in silico*. A relevant implication is the prediction of selective gene targets for cancers [17]. A genome-scale generic cancer metabolic model was created using a generic human metabolic network model Recon 1 [11], a previous version of Recon 2, encompassing cancerous features that are generally observed across specific cancer types. After validation with experimental shRNA gene silencing data, the generic cancer metabolic model was subjected to constraint-based flux analysis that looked for synthetic lethal genes that could selectively target cancer cells against healthy cells. The rationale for this selective targeting is that it is possible to damage cancer cells without harming healthy cells if a drug interferes with one of the metabolic genes in a synthetic lethal pair while the pair gene is inactive or very lowly expressed only in cancer cells. Under this circumstance, cancer cells die because they cannot produce the necessary metabolite, whereas healthy cells can still produce the next metabolite using an alternative reaction through the synthetically lethal pair gene.

Drug targeting to treat microbial pathogens can also be predicted in a similar manner, but because of their relatively simple biological system, the predicted information can be more directly validated using experimental drug screening [18,19]. A relevant example is the drug targeting of a human opportunistic pathogen *Vibrio vulnificus* that causes septicemia, necrosis and gastroenteritis for patients with damaged liver or a compromised immune system [18]. With its manually reconstructed genome-scale metabolic network model, VvuMBEL943, it was used to predict so-called essential metabolites, the removal of which stops the cellular growth. The predicted final five essential metabolites were then used as templates to find their chemical analogs out of the large-sized chemical compound library. This approach was based on a rationale that commercially available effective drugs appear to have structural similarity with metabolites [20]. Subsequently, the selected structural analogs were subjected to whole-cell screening for the

identification of hit compounds. This study demonstrated how systematically predicted information can usefully be combined with other scientifically proven conventional experimental tools.

The aforementioned studies using the metabolic models are just the tip of the iceberg, and may more groundbreaking achievements are expected to come. This trend can be anticipated based on the fact that medically important organisms' models are continuously upgraded, as in the case of Recon 2, and biologically more reasonable algorithms (e.g., numerical constraints or objective functions) are actively being developed [15]. It is this reason that genome-scale metabolic network models will play more powerful roles in biopharmaceutical industry. However, it should also be remembered at the same time that this computational tool is just an option among many other available tools, and certainly has its own limitations. Therefore, the most ideal scenario is to use this metabolic modeling and simulation approach with other computational and experimental tools in a complementary way. In fact, this metabolic modeling and simulation approach is one of the best tools that can easily be integrated with other platform technologies, including kinetics equations or omics data, as mentioned above. The genome-scale metabolic network models await more challenges in biopharmaceutical industry for their practical applications.

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