1 New paradigms for metabolic modeling of human cells

2 **Short title: Metabolic modeling of human cells**

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26 **Abstract**

27 Abnormalities in cellular functions are associated with the progression of human diseases,
28 often resulting in metabolic reprogramming. GENome-scale metabolic Models (GEMs) have
29 enabled studying global metabolic reprogramming in connection with disease development in
30 a systematic manner. Here we review recent work on reconstruction of GEMs for human

1 cell/tissue types and cancer, and the use of GEMs for identification of metabolic changes
2 occurring in response to disease development. We further discuss how GEMs can be used for
3 the development of efficient therapeutic strategies. Finally, challenges in integration of
4 cell/tissue models for simulation of whole body functions as well as integration of GEMs with
5 other biological networks for generating complete cell/tissue models are presented.

6 **Keywords:** genome-scale metabolic models; cell-type models; cancer models; personalized
7 medicine

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2 **Introduction**

3 Type 2 diabetes (T2D), cardiovascular disease (CVD), non-alcoholic fatty liver disease
4 (NAFLD) and cancer are caused by the abnormalities in cell/tissue functions and have
5 become prevalent worldwide [1,2]. These chronic diseases are strongly linked to obesity that
6 has reached epidemic proportions in almost all developed countries [3]. Currently used
7 methods for development of drugs to treat these disorders are costly and inefficient, and
8 unfortunately result in more failures than successes. For cancer, drug development is even
9 more challenging due to the heterogeneous molecular mechanisms underlying human tumor
10 progression [4]. Therefore, the development of innovative approaches is vital for accelerating
11 the discovery of potentially effective products for the treatment of these disorders [5]. In this
12 context, generation of computer models for cells/tissues in health and disease states may
13 provide further insights for revealing the adaptations in cell/tissue functions in response to a
14 chronic disease through the use of systems biology based approaches.

15 The specific application of systems biology for studying complex diseases is generally
16 referred to as systems medicine. One of the key objectives of systems medicine is to
17 understand biological processes of cells/tissues in health and disease states and gain new
18 insights into what drives the appearance of the disease [6]. A principal tool of systems
19 medicine is GEnome-scale metabolic Models (GEMs) which can aid in understanding the
20 mechanistic relationship between genotype and phenotype and in revealing the underlying
21 molecular mechanisms of complex diseases [7]. GEMs are the collection of the annotated
22 stoichiometric chemical reactions as well as enzymes associated to those reactions in a
23 particular cell/tissue (Figure 1A). This network information can be converted into a
24 computational model and analyzed using various algorithms. GEMs are powerful tools for
25 dealing with the rising torrent of biological information and allow for sifting through huge
26 datasets to look for emergent properties of enzymes and other metabolic functions as they
27 interact in a cell/tissue. The harvested knowledge through the use of GEMs may be used for
28 designing diets for disease prevention, discovery of novel biomarkers for stratification,
29 identification of drug targets for designing effective treatment strategies, and prediction of the
30 toxicity caused by the drugs, i.e. personalized treatment [8-11] (Figure 1A).

31 **Global reconstructions of the human metabolism**

32 Alterations in metabolism may be the cause or consequence of a disorder, and changes in
33 metabolite concentrations can be used as biomarkers for diagnosis and monitoring of the
34 diseases whereas enzymes can be targeted for disease treatment. GEMs allow for studying the
35 interactions between metabolites and enzymes in a holistic manner rather than by more
36 traditional reductionist, "one metabolite and one enzyme", approaches. Two global
37 reconstructions of the human metabolic network, Recon1 [12] and EHMN [13] were manually
38 reconstructed through evaluation of bibliomic data and these networks revealed the existing
39 gaps in understanding of human metabolism for further experimental investigations. These
40 networks, which focus on different parts of the metabolism were merged with the knowledge
41 databases for biochemical reactions resulting in Human Metabolic Reaction database (HMR)
42 [14] (Figure 1B). Recon1 and EHMN also formed the basis for a community-driven effort

1 resulting in Recon2 [15] that also incorporated information from metabolic reconstruction of
2 the human hepatocyte (HepatoNet) [16]. Recently, a functional GEM for adipocytes,
3 *iAdipocytes1809*, with a strong focus on lipid metabolism was reconstructed [17] and
4 integrated to HMR together with HepatoNet for the construction of HMR2 [18]. The number
5 of the reactions, associated genes and the unique metabolites incorporated in the generic
6 human GEMs as well as in cell-type GEMs which are used in their improvement are
7 presented in Figure 1C. From this, it is seen that HMR2 is currently the most comprehensive
8 global reconstruction of the human metabolism.

9 **Cell/tissue-type and cancer specific GEMs**

10 Metabolism differs in each cell/tissue type and it is therefore necessary to reconstruct
11 cell/tissue specific GEMs for simulation of their physiological and patho-physiological states.
12 Hence, a number of algorithms which use gene expression and proteomics data as input, have
13 been developed [8] and systematically evaluated [19]. These algorithms relate the expression
14 of transcripts or abundance of proteins in a cell/tissue to the likelihood that an enzyme present
15 in the generic GEMs is part of the metabolism in a specific cell/tissue type. However, the
16 functional capacity of these automatically generated networks often fails to recapitulate the
17 distinctive metabolic objectives in particular cell/tissue types. A task-driven model
18 reconstruction (tINIT) algorithm [20] was therefore developed for the generation of functional
19 personalized and cell-type specific GEMs based on the protein evidence in the Human Protein
20 Atlas [21] and HMR2 [22]. The tINIT algorithm tailors generic human GEMs to simulation-
21 ready context-specific GEMs through the use of cell type specific proteomics and
22 transcriptomics data as well as the defined metabolic tasks that the generated model should be
23 able to perform. The metabolic tasks include the aerobic rephosphorylation of ATP from
24 glucose or *de novo* synthesis of cholesterol from glucose.

25 Even though the tINIT algorithm is able to reconstruct functional models, manual curation is
26 still essential for generating high quality models. One of the limitations of the tINIT algorithm
27 lies in defining metabolic tasks that should occur in a particular cell/tissue type. This requires
28 an extensive literature search and further experimental investigations. However, in the era of
29 Big Data, vast amounts of biological information including proteomics data in major human
30 cell types [21] and tissues [23,24] as well as transcriptomics data in major human tissues [25-
31 29] are available and these data can be complemented with the tINIT algorithm for identifying
32 the metabolic functions occurring in particular cells/tissue types. Recently, tissue-specific
33 GEMs for 32 human tissues were reconstructed and the demonstration of how the high-quality
34 transcriptomics data can be used to drive further analysis was presented [29]. The tissue-
35 specific reconstructions were used to perform comparative analysis of the metabolism
36 between different tissues at genome-wide level.

37 A consensus functional GEM for hepatocytes, *iHepatocytes2322* was reconstructed manually
38 by integrating the contents of previously published liver tissue GEMs [18]. The network
39 centric view of *iHepatocytes2322* also enabled re-evaluation and improved annotation of the
40 proteomics data used in the reconstruction process. The resulting GEM was used to analyze
41 gene expression data obtained from NAFLD patients in order to identify potential biomarkers
42 and therapeutic targets. It was found that the expression of the genes involved in the

1 biosynthesis of chondroitin sulphates (CS) were up regulated and the biosynthesis of heparan
2 sulphates (HS) were down regulated. The study proposed the blood levels of CS and HS as
3 potential biomarkers for staging NAFLD. It was also shown that enzymes involved in the
4 biosynthesis of the serine were down regulated and branched chain amino-acid transaminase 1
5 (BCAT1) that converts AKG and valine to glutamate and branched chain keto acids in the
6 cytosol was up regulated in the liver tissue of NAFLD patients. Hence, it was suggested that
7 the inhibition of BCAT1 can potentially be used for the development of effective treatment
8 strategies for NAFLD patients.

9 Moreover, cancer-specific GEMs have been used for providing insights about tumor
10 progression, elucidating the differences between metabolic signatures of different tumors and
11 discovering putative anti-cancer targets [14,20,30,31]. The tINIT algorithm as well as other
12 algorithms can be used for the generation of the high-quality cancer and cell-line specific
13 GEMs through the use of proteomics [32,33], transcriptomics [34,35] and fluxomics [36] data
14 and these models may allow for increased understanding about tumor progression. During the
15 reconstruction of cancer GEMs through the use of tINIT algorithm, formation of biomass
16 were added as an additional metabolic task to ensure growth.

17 **Integration of omics data through the use of GEMs**

18 Besides the use of high throughput datasets in the reconstruction process of high quality
19 GEMs, these datasets can also be mapped onto reconstructed GEMs for analysis of metabolic
20 differences between two different conditions.

21 Even though there is much progress on metabolomics for profiling tissues and biofluids, e.g.,
22 blood and urine [37,38], these studies provide information only about the current metabolic
23 status rather than providing a mechanistic explanation for the appearance of the phenotype.
24 For instance, Newgard et al. [39] performed the metabolic profiling of lean and obese subjects
25 and increased level of branched-chain amino acids (BCAAs), isoleucine, leucine and valine
26 were reported in the plasma of the obese subjects. Moreover, Wang et al. [40] followed more
27 than 2,000 normoglycemic individuals for 12 years and profiled 61 metabolites in T2D
28 developing subjects, and a combination of BCAAs and aromatic (tyrosine and phenylalanine)
29 amino acid levels were proposed for predicting the appearance of T2D. Even though both
30 studies associated increased levels of BCAAs with obesity and insulin secretion, further
31 mechanistic explanations are necessary for explaining what causes the elevated level of
32 BCAAs and for translating it into medicine. GEMs can aid in interpretation of the differences
33 between the metabolomics data performed for two different clinical conditions and provide
34 detailed information about the contribution of involving tissues through the use of
35 transcriptomics and/or proteomics data (Figure 2A). Recently, the content of *iAdipocytes1809*
36 was validated through the use RNA-Seq data and the model was used for investigating the
37 contribution of adipose tissue to the differences in the metabolite levels of plasma collected
38 from lean and obese subjects [41]. From this analysis, it was hypothesized that plasma
39 BCAAs levels increase in the plasma of obese subjects due to decreased catabolism of
40 BCAAs in the adipose tissue.

GEMs have also been used for simulation of internal fluxes by incorporating experimental determinations of external fluxes and gene expression data [19]. Using *iAdipocytes1809*, the *in vivo* uptake and secretion rates for glucose and fatty acids in the adipose tissue of lean and obese subjects were used for predicting the internal flux differences between the adipose tissue metabolism of lean and obese subjects using a random sampling algorithm (43). Through the integration of the simulated fluxes with gene expression data, it was possible to identify transcriptionally regulated reactions [17].

Integration of models for simulations of whole body functions

In order to describe overall human metabolism, cell/tissue models should be merged for simulations of whole body functions and for predicting the contributions of each tissues to the altered phenotype in disease states. In this context, suitable cell/tissue type-specific objective functions should be generated for simulation of the cell/tissue functions. For instance, in order to simulate the metabolic differences in the adipocytes of lean and obese subjects, formation of lipid droplets in post-prandial state was used as an objective function [17]. Similarly other objective functions need to be defined for different cell types, and these objective functions need to be evaluated based on the biological question. Furthermore, in order to link GEMs for different tissues it will be necessary with a model describing blood circulation in the body [42,43], and how there is exchange between the different tissues and blood. This kind of model will be dynamic in nature and it will be challenging to integrate GEMs with this kind of circulation model.

In connection with considering whole body metabolism it is important to also consider the high metabolic activity of the gut microbiome. GEMs for gut microbiota have been generated based on metagenomics data and used for simulation of their interactions [44,45], and different algorithms have been developed for analyzing the interactions between the microbial community [46,47]. Gut microbiota models should also be integrated with host models for studying the contributions of gut microbiota on the overall body metabolism. This will allow for generation of hypotheses such as how different species contribute to human body functions and hereby provide a link between microbiota, food intake and nutrient absorption by the human body in health and disease states.

Perspectives

Clearly, more work is required to understand heterogeneous molecular mechanisms of chronic diseases and cancer, discover biomarkers and identify drug targets. Biologists have been studying the functions of individual proteins and molecular pathways, and their contribution in the progression of the disease for many years. In fact, understanding the role of individual components is an important step but, to develop preventive, diagnostic and therapeutic strategies, an integrated approach must be taken. Systems medicine based approaches may provide potential solutions and rational approaches for discovering emergent properties of biological systems and for describing their dynamic and complex behaviors in health and disease states.

Reconstructed GEMs can be used as scaffolds for context-dependent analysis of high-throughput data, but even though there has been much recent progress it is still necessary to

1 develop novel algorithms to obtain further insights. Specifically, novel methods for the
2 simulation of the metabolic interactions between different cell types, tissues and human oral
3 and gut microbiota should be developed. This will involve identification of common
4 metabolic operations between host and gut microbiota, e.g., bile acid metabolism, and such
5 simulations may provide essential input to the simulation of whole body functions.

6 The contents of human GEMs should continue to be enhanced together with the
7 improvements in biochemical knowledge, and these models will hereby provide for an
8 accumulative knowledgebase of human metabolism. Hereby they provide a platform for better
9 interpretation of cell/tissue biology. Integration of GEMs with other biological networks, e.g.,
10 regulatory [48,49], signaling [50,51] and protein-protein interaction networks [52-54] (Figure
11 2B), will allow for further integration of biological knowledge into these models and may in
12 the long run enable dynamic simulation of human metabolism.

13 **Figure Legends**

14 **Figure 1** A) GEMs contain the biochemical reactions as well as the associated genes in
15 particular cells/tissues. This network information can be converted to computational models
16 through the use of a stoichiometric matrix (**S**) and these models can be used in various
17 translational medicine applications including: omics data integration, comparative and
18 network dependent analysis, and simulations. B) The timeline of generation and evaluation of
19 global reconstructions of human metabolism. C) Bar plot showing the number of the
20 reactions, unique metabolites and genes incorporated to the generic human GEMs and cell-
21 type specific GEMs used in their improvement.

22 **Figure 2** A) GEMs can be used for predicting the metabolic alterations in an individual tissue
23 through the mapping of transcriptomics and proteomics data to the network structure.
24 Individual response of the tissues can be integrated for predicting the changes in the
25 metabolite level of biofluid e.g., blood. Red and blue colors indicate up and down regulated
26 reactions, respectively, and black indicates no change. B) Integration of the GEMs with other
27 biological networks including regulatory, signaling and protein-protein interaction networks
28 for better prediction of the cell phenotype. Reactions (R), metabolites (m), proteins (p),
29 transcripts (t), genes (g) and transcription factor (TF) are shown in the integrated toy model.

30 **Competing interests**

31 The authors have declared that no competing interests exist.

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18 network can be integrated to other biological networks.

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***Graphical abstract (for review)**
Integration of genetic and biochemical knowledge

Global human GEMs
Recon2, HMR2

Omics data

Algorithms

**Manual
evaluation**

Context-specific GEMs
Cell/tissue & cancer GEMs

A. Figure **B.**