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Anticancer drug discovery through genome-scale metabolic modeling

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Abstract
Altered metabolism has long been recognized as a defining property of cancer physiology, but is experiencing renewed interest as the importance of such alterations are becoming fully realized. Once regarded merely as a side effect of a damaging mutation or a general increase in proliferation rate, metabolic network rewiring is now viewed as an intentional process to optimize tumor growth and maintenance, and can even drive cancer transformation. This has motivated the search for anticancer targets among enzymes in the metabolic network of cancer cells. Genome-scale metabolic models (GEMs) provide the necessary framework to systematically interrogate this network, and many recent studies have successfully employed GEMs to predict anticancer drug targets in the metabolic networks of various cancer types.

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Introduction

Cancer remains a leading cause of death worldwide—approximately one-third of individuals will develop some form of the disease within their lifetime [1]. Although there have been substantial advancements in the detection, diagnosis, and treatment of many cancer types, the highly complex and heterogeneous nature of the disease continues to impede further progress. There are many contributors to the initiation and progression of cancer, and are generally grouped into distinct categories termed the “hallmarks” of cancer [2]. In addition to characteristics such as resisting cell death and evading growth suppressors, a recent addition to the hallmarks was the reprogramming of energy metabolism [2,3].

Perturbed metabolic activity in cancer cells is not a new concept. Indeed, one of the most notable metabolic alterations in cancer cells, the Warburg effect, was identified in the 1920s [4,5]. However, a metabolism-centric approach to understanding and treating cancer has experienced a revived interest in recent years, due to advancements in high-throughput biological profiling techniques (e.g., transcriptomics, proteomics, metabolomics), which now enable a systematic and mechanistic mapping of cancer-specific remodeling of metabolism. Furthermore, the strong link between metabolic behavior and cancer outcomes, as well as the identification of many cancer-driving “oncometabolites”, has highlighted the metabolic network as a promising source of novel anticancer drug targets [6,7].

The complexity of the metabolic network, which is further obscured by the substantial heterogeneity of cancer, prevents tracing specific properties or outcomes back to an individual metabolic feature or subsystem. In order to investigate such a broad and interconnected system, a computational approach is required [8]. One such class of approaches employs the use of genome-scale metabolic models (GEMs), which are mathematical representations of the network of reactions comprising the metabolic functionality of the cell [9]. A number of recent approaches have demonstrated that GEMs can with success be used to gain a more mechanistic understanding of tumor physiology, as well as to identify novel anticancer drug targets in the cancer metabolic network.

We review here the recent use of GEMs in the investigation of cancer metabolism, focusing specifically on their application for predicting targets or therapies for cancer treatment. We further discuss the limitations of current GEM-based approaches, as well as perspectives on future developments that seek to improve their accuracy and versatility. The recognition of the critical role metabolism plays in cancer, in addition to the demonstrated success of employing GEMs for anticancer target discovery, highlights an upward trend in the importance of GEMs to the ongoing battle against cancer.

Metabolism as a target of anti-cancer therapies

The importance of metabolism in the context of cancer was highlighted nearly a century ago in the work of Otto Warburg, where his discovery of increased glucose consumption by cancer cells compared with normal tissue is still exploited in modern clinical applications, such as tumor imaging with ^{18}F-deoxyglucose positron emission tomography (FDG-PET) [5,10]. This altered metabolic behavior, termed the "Warburg effect", includes a fermentation-like shift in glucose usage away from the TCA cycle and oxidative phosphorylation toward lactate production, despite the presence of sufficient oxygen to operate the seemingly more optimal aerobic respiratory pathway [4]. Extensive work since the discovery of this behavior has shed new light on the underlying cause, suggesting an intentional rewiring of metabolism to support the increased demands of precursor metabolites, in particular those that are part of glycolysis, for the synthesis of building blocks and further to macromolecules, rather than the initially proposed byproduct of "injured" mitochondria [3,11]. However, a definitive mechanism is still unclear, and the emerging picture is one of increasing complexity—not only is the Warburg effect absent in some cancers, there are a growing diversity of metabolic patterns exhibited among different cancer types, and even among cells comprising the same tumor [12].

Rewiring metabolism can confer a number of benefits to tumors ranging from rapid proliferation to improved oxidative stress tolerance, but often come with penalties such as increased nutrient demand or enhanced sensitivity to other forms of stress [13]. These new vulnerabilities and any other metabolic features that differentiate cancer from normal healthy cells constitute an attractive pool of metabolic targets for anti-cancer therapy development [6,14].

Although proliferating cancer cells still utilize oxidative phosphorylation for a significant fraction of their ATP production, their metabolic network is generally reprogrammed to optimize production or import of metabolites required for rapid cell proliferation, such as NADPH and glutamine [3]. Glutamine serves as an excellent source of reduced nitrogen to generate purine and pyrimidine bases for nucleotide biosynthesis, as well as for the production of nonessential amino acids [13]. Some cancers even exhibit "glutamine addiction," where high uptake rates of the amino acid are required to support additional functions such as NADPH production for macromolecular biosynthesis and redox balancing, generation of oxaloacetate to replenish TCA cycle intermediates (anaplerosis), and driving exchange reactions to import additional extracellular amino acids [15,16]. This glutamine requirement has been targeted in approaches such as using glutamine analogs to inhibit its utilization or enzymatic depletion of glutamine levels in the blood; however, many of these treatments exhibit high host toxicity, and thus require further development [15].

Another non-essential amino acid that many cancers import or synthesize at an increased rate is serine, which is used to produce phospholipids and other amino acids, in addition to providing one-carbon units for folate metabolism [17]. The increased serine demand in tumors represents a promising metabolic target, and development of inhibitors for the serine biosynthetic pathway are currently ongoing [18]. The folate cycle is often upregulated in certain cancers [13,19], and generates precursors for purine biosynthesis and methylation, and can contribute to nearly half of the total cellular NADPH supply [20]. As such, folate metabolism represents yet another attractive target for anticancer therapy development. Interestingly, one of the first-developed chemotherapy treatments (methotrexate) functioned by interfering with folic acid utilization [21], and is still in use today [14,17].

Targeting the unique metabolic behavior of tumor cells has been demonstrated to be an effective anticancer approach, but the frequent host toxicity of many treatment strategies highlights the difficulty of working in such a narrow therapeutic window. Future efforts to target cancer metabolism therefore require approaches that account for the tightly connected and interactive nature of the metabolic network, to minimize potential collateral damage. One such promising approach employs the use of GEMs to help analyze and predict potential anticancer therapeutics in the metabolic network.

Construction and application of genome-scale metabolic models

GEMs are a mathematical representation of the network of reactions comprising all known metabolic functions of the biological system under study [22]. The stoichiometry of all the reactions are collected in a matrix, which specifies the involvements and molar ratios of reactants and products participating in each reaction. Another feature of GEMs is that for each reaction the corresponding enzyme(s) and its associated gene(s) are specified, and the models hereby also provide gene—protein—reaction—metabolite associations [23]. The relationship between each of these GEM components enables translation between gene, protein, reaction, and metabolite information, thus facilitating the use and integration of many different types of high-throughput omics data [24].

GEMs have been constructed for a wide spectrum of species and biological systems, including those of plants, bacteria, and fungi, and have been applied for purposes ranging from metabolic engineering of yeast

for improved biofuel production [25], to the identification of virulence factors in pathogens [26]. Human GEMs have been reconstructed and undergone extensive development over the past decade, the most recent and often-used of which are Recon2 [27] and HMR2 [28] (updated from Recon1 [29] and HMR [30], respectively). These models represent the metabolic functionality of all human tissues and organs, and therefore are often "contextualized" with data from a specific tissue and/or patient to enable more accurate simulations or predictions of that particular domain (Figure 1). This approach is critical to driving the development of personalized medicine, enabling treatments that are tailored to a specific individual or disease variant [31]. Furthermore, recent developments in single-cell sequencing and microdissection technologies have opened the possibility of contextualizing GEMs at the level of individual cells comprising a tissue or tumor, thus more accurately representing their heterogeneity [32]. A number of different algorithms have been developed to generate context-specific GEMs from various data types, but they generally function by eliminating or partially constraining reactions that the data suggest are absent or in relatively low abundance [33].

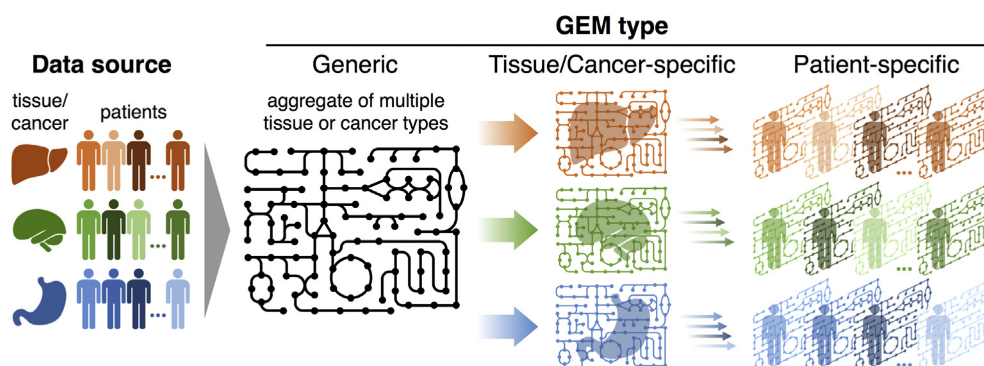
Additional information, such as metabolite exchange rates, can be incorporated into GEMs to enable simulation of flux through the different branches of the metabolic network. One of the most common approaches is constraint-based modeling, where biologically-based constraints are enforced on the flux that can pass through a particular reaction [34]. These constraints further enable an approach known as flux balance analysis (FBA), where the flux through the network is estimated based on a number of assumptions, the foremost of which is the quasi-steady state assumption that the intracellular metabolite pools are being replenished at the same rate as their depletion [35]. Since the problem is under-defined (e.g., there exists an infinite set of solutions that can satisfy the given constraints), it is often posed as an optimization problem, where the cell is assumed to be optimizing an objective such as production of biomass or ATP generation.

Application of GEMs in predicting anticancer drug targets

Many recent studies have employed GEMs to elucidate cancer-specific metabolic sub-networks, and to predict targets that impair tumor growth or viability (Figure 2). There are several recent reviews on this topic [8,9,36–40], and we will therefore only focus here on a few recent studies that illustrate the versatility in the application of GEMs for drug target discovery.

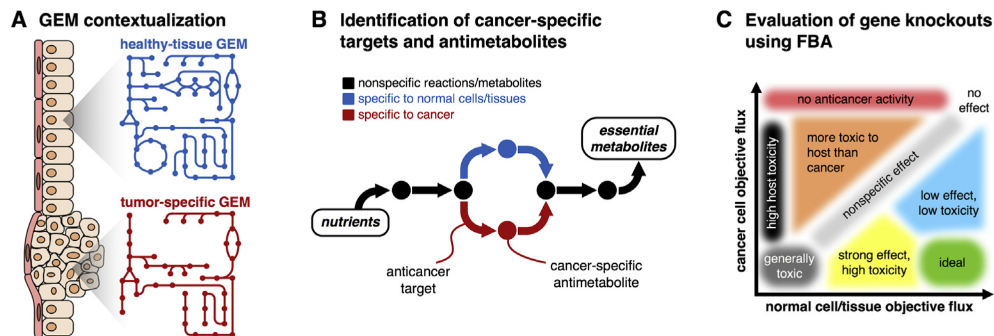
A generic cancer GEM was reconstructed by Folger and colleagues by integrating Recon1 with gene expression data from cancer cell lines in the NCI-60 collection [41]. Only reactions catalyzed by a "core" set enzymes that exhibited relatively high gene expression in over 90% of the NCI-60 measurements were included in the cancer GEM, in addition to a minimal set of reactions necessary to enable balanced flux through each of the core pathways and a biomass reaction. FBA was used with the cancer GEM to identify single gene knockouts that decreased proliferation rate (i.e., biomass reaction flux), after which FBA was performed with the full human model (Recon1) to eliminate genes that impaired growth or ATP production in normal cells. The resulting set of 52 genes represented potential anticancer targets; 21 were previously known targets of FDA-approved anticancer drugs, or undergoing testing for use in cancer therapy, whereas the remaining 31 targets served as candidates for novel anticancer therapies. More recently, Frezza and colleagues investigated

Figure 1



Human GEMs can be generated and utilized with varying degrees of specificity. Biological data is collected from one or more tissue or cancer types, ideally with many patients per type. A generic GEM is a whole-body human reconstruction (e.g., HMR or Recon), or is generated upon integrating such models with data spanning multiple types of cancers or tissues. A tissue- or cancer-specific GEM is generated by integrating a generic human GEM with data specific to a single type of cancer or tissue collected from many individuals, whereas a patient-specific GEM is contextualized only with data obtained from a single individual.

Figure 2



Application of cancer-specific GEMs for anticancer drug discovery. (**A**) Molecular or phenotypic data from a tumor or normal healthy tissue is used to generate a cancer-specific or healthy-tissue GEM, respectively. (**B**) The structure of a cancer-specific GEM can be compared with that of a healthy tissue to identify differences in pathways required to convert nutrients into essential metabolites (e.g., precursors for biomass or energy generation). Enzymes required in the cancer GEM but not the healthy tissue GEM represent potential anticancer targets, whereas metabolites used by such enzymes represent potential antimetabolites for cancer treatment. (**C**) FBA can be employed in a gene deletion analysis to evaluate the impact of inhibiting the encoded protein in cancer vs. normal tissue on the flux through an objective reaction(s); e.g., biomass production or ATP hydrolysis. Deletions that reduce the objective flux in the cancer GEM more than the healthy network (lying below the 45° line) are more desirable, where the ideal situation is complete inhibition of the cancer objective without affecting the normal cell.

the metabolism of fumarate hydratase (Fh1)-deficient kidney cells (from knockout mice), as this gene was known to be mutated in hereditary leiomyomatosis and renal-cell cancer (HLRCC) patients, which can lead to fumarate accumulation and activation of hypoxia-inducible factors even without oxygen limitation [42]. The authors constructed context-specific GEMs from Recon1 for cell lines with and without Fh1 based on corresponding gene expression data, with the addition of metabolic genes expressed highly across many cancer types. FBA was used to predict gene knockouts that were synthetically lethal with Fh1 (i.e., they were lethal in the GEM lacking Fh1), but would not affect growth of the normal Fh1-containing cell line. This identified 24 reactions that were lethal with the Fh1 deletion, the majority of which were involved in the heme metabolism pathway. The authors concluded that targeting the heme biosynthesis/degradation pathway constitutes a promising potential treatment approach for HLRCC patients.

Recognizing the need for a systematic method to generate cell-specific GEMs, Agren et al. developed the integrative network inference for tissues (INIT) algorithm [43], which was later expanded to incorporate a range of required metabolic tasks (tINIT) to ensure a biologically functional GEM [44]. Using tINIT, Agren and colleagues reconstructed personalized hepatocellular carcinoma (HCC) GEMs for six patients from the HMR2 model using proteomic data from each of the patients [44]. An analogous procedure was used to reconstruct 83 healthy cell type specific GEMs using HMR2 and proteomics data from the human protein atlas (HPA) [45]. The HCC GEMs were then used to predict "antimetabolites", which are critical metabolites whose replacement with an analog would impair cell viability (Figure 2B). Upon constraining the flux of all reactions utilizing a particular metabolite to zero, it was classified as an antimetabolite if the resulting network was unable to satisfy the given set of metabolic tasks. Predicted antimetabolites that also impaired the 83 healthy GEMs were discarded, to eliminate candidates that would potentially exhibit host toxicity. Hereby 101 metabolite analog drug candidates were predicted to impair HCC without affecting normal cell growth. One of the candidate antimetabolites, L-carnitine, was evaluated experimentally through the use of perhexiline, which mimicked the effects of an L-carnitine analogue by inhibiting the palmitoyltranferase enzymes that utilize L-carnitine as a substrate. Treatment of HepG2 cells with perhexiline exhibited similar inhibitory activity as sorafenib, which is an approved treatment of HCC.

A different algorithm to contextualize GEMs with molecular and phenotypic data was developed by Yizhak et al., termed PRIME (personalized reconstruction of metabolic models) [46]. The authors applied PRIME to Recon1 using data from 224 lymphoblast cell lines and the NCI-60 cell lines to reconstruct individual models for each cell line, and demonstrated that the models could accurately predict proliferation rates and responses to a collection of metabolic drugs. The cell-specific GEMs were further used to predict gene knock-downs that impaired cancer cell proliferation, without affecting normal cell growth (Figure 2C). Their predicted cancer-specific drug targets were enriched with enzymes targeted by newly developed drugs, and a top candidate, malonyl-CoA decarboxylase (MLYCD), was evaluated experimentally. An siRNA-mediated silencing of MLYCD impaired proliferation of leukemia cell lines while having no effect on normal lymphoblast cells, validating the activity and cancer

specificity of the predicted target. Yizhak et al. also used their NCI-60 cancer-specific GEMs to investigate metabolic activity relating to the Warburg effect, and targeted this phenomena to predict novel anti-migratory targets in cancer [47]. The extent to which the Warburg effect was active in the cancer specific GEMs was quantified using the glycolytic to oxidative ATP flux ratio (AFR). After discovering that AFR exhibited significant positive correlation with cancer cell migration rates, the authors conducted a single gene knockout screen to predict targets that would reduce the AFR. The top candidates were evaluated experimentally in breast and lung cancer cell lines, where up to 13 out of 17 were found to significantly attenuate migration.

In an effort to streamline the gene-reaction association data encoded in GEMs, Zhang and colleagues developed a framework termed logic transformation of model (LTM), which incorporates pseudo reactions and metabolites into GEMs to establish one-to-one gene-reaction associations without changing the underlying network logic [48]. The transformation improved the efficiency and accuracy of gene-based analyses or optimization algorithms using GEMs by translating protein complex and isozyme information into an explicit gene-reaction association matrix. To demonstrate the efficacy of their approach, the authors applied LTM to the HCC GEM constructed by Agren et al. [44], and used it to predict individual anticancer gene targets rather than antimetabolites. Furthermore, their algorithm enabled an exhaustive search for double and triple synthetic lethal gene sets, which were targets predicted to impair HCC growth if perturbed in combination. Among the predicted combination gene targets were those involved in glutaminolysis, production of cytosolic acetyl-CoA from pyruvate, and the pentose phosphate pathway.

Recently, Gatto and colleagues [49] reconstructed a clear cell renal cell carcinoma (ccRCC) GEM by constraining the HMR model with proteomic data using the INIT algorithm [43]. The ccRCC-specific GEM was used to critically evaluate the accuracy of FBA in predicting anticancer drug targets in the metabolic network [50]. By conducting a single gene deletion analysis, targets were predicted as genes whose deletion greatly reduced or completely eliminated the ability to carry flux through the biomass reaction. These predictions were then compared to *in vitro* results, where 7 ccRCC cell lines were transfected with a library of siRNAs targeting 230 metabolic enzymes. The FBA predictions exhibited significant accuracy; however, using the same approach with a different cancer type, prostate adenocarcinoma (PA), resulted in poor predictive performance. This revealed that the accuracy of an FBA-based approach for predicting anticancer targets can be cancer-type specific, and highlights an area where further improvement is required. Ghaffari and colleagues expanded this approach to include eleven different cancer types, for which specific GEMs were reconstructed from HMR2 using RNA-Seq data measured in the corresponding cell lines [51]. These cancer-type-specific GEMs were utilized to predict antimetabolites across the different cancers. Out of the 138 identified antimetabolites, 106 were predicted to eliminate growth across all cancer types, whereas the remaining 32 affected at least one of the cell lines, highlighting the advantage gained by utilizing individual cell-line-specific GEMs. In an effort to avoid treatments that would exhibit toxicity in normal cells, the same analysis was conducted using 83 healthy tissue-specific GEMs developed in a previous study [44]. Ultimately, 85 antimetabolites were predicted to inhibit growth in at least one cancer cell line without affecting normal cellular growth.

Björnson and colleagues reconstructed a population-based GEM for hepatocellular carcinoma (HCC) from HMR2 using transcriptomic and proteomic data for 361 individuals from The Cancer Genome Atlas (TCGA), termed iHCC2578 [52]. The GEM was further developed using the tINIT algorithm, maintaining reactions as necessary to ensure that vital biological functions, including biomass generation, were possible. The authors employed iHCC2578 to investigate the degree of deregulation in various metabolic subsystems among HCC patients using differential expression analysis and differential rank conservation (DIRAC) [53] analysis. Their investigation revealed tight regulation of fatty acid biosynthesis (FAB) among HCC patients and pointed toward mitochondrial acetate as a key substrate for FAB, which was further supported by the increased expression of mitochondrial acetyl-CoA synthetase (ACSS1) in HCC compared to noncancerous liver. Stratification of HCC patients based on low or high ACCS1 expression revealed significant transcriptomic differences between the groups, and highlighted the protein as a potential anticancer drug target.

Further applications of GEMs in cancer

Additional recent studies, such as those by Gatto et al. [54] and Zielinski et al. [55], have successfully employed GEMs to study the metabolic alterations specific to cancer cells. By integrating additional layers of molecular and phenotypic data with the GEMs, these studies were able to identify convergent metabolic subregions that are critical to the transformation and maintenance of many different cancer types, namely arachidonic acid and xenobiotic metabolism [54], as well as elucidate key drivers to known metabolic signatures of cancer, such as glutamine addiction and the Warburg effect [55].

Beyond identifying potential anticancer targets, GEMs can similarly be used to predict metabolic cancer

biomarkers. For example, Jerby and colleagues developed a metabolic phenotypic analysis (MPA) algorithm to integrate mRNA or protein abundance data with a GEM for the purpose of quantifying differences in metabolic process activity between different samples [56]. Using MPA with gene expression profiles from normal and cancer breast tissue, the authors quantified the metabolic differences between estrogen receptor negative (ER^-) and positive (ER^+) breast cancer. The resulting top candidates were choline-containing compounds, which were validated by the previously suggested use of choline as a marker for breast cancer PET imaging, and the fact that choline and phosphocholine were known breast cancer biomarkers [56].

More recently, Gatto et al. investigated metabolic reprogramming associated with ccRCC by integrating tumor and normal tissue transcriptomic data with metabolic network information from the KEGG database and HMR2 [57]. Their integrated analysis revealed significantly coordinated changes to the regulation of glycosaminoglycan (GAG) biosynthesis in ccRCC, motivating the authors to evaluate the potential of using GAG-associated metabolites (chondroitin and heparan sulfate) as biomarkers for the metastatic disease. GAG metabolite properties measured in the plasma and urine of metastatic ccRCC and healthy patients were found to accurately predict disease status, and were subsequently validated in an independent cohort, offering a promising non-invasive metabolic biomarker of metastatic ccRCC [57].

Conclusion

The cancer-associated metabolic network is receiving increased attention as the importance of metabolic remodeling in connection with disease development provides a potential for therapeutic intervention [6,7,14]. The complexity and breadth of the metabolic network, compounded by the immense diversity and heterogeneity across different patients and cancer lineages, requires a computational approach to systematically isolate biologically meaningful features from noise [8,9]. Many recent studies have already demonstrated that GEMs are capable of such a task, confirming a large number of currently approved anticancer drugs, as well as providing new potential targets. Despite their success, there exist a number of challenges and areas of improvement for future use of GEMs in predicting anticancer targets.

The accuracy of GEM-based predictions can be improved by integrating more context-specific information with model properties such as nutrient uptake constraints or objective function(s). The set of nutrients and substrates available to a system can largely dictate the importance of different enzymes to cellular function or viability, and should therefore accurately reflect the cellular environment *in vivo* [47,50]. Regarding the objective function, maximization of biomass production is often a good assumption for microbes, but is generally not appropriate for human cells, where even tumor cells can exhibit slow or no growth [39,58]. Therefore, human GEM objective functions should be tailored in a more context-specific manner, such as defining unique objectives for each tissue based on its physiological function (e.g., bile acid synthesis for hepatocytes [59]), or inferring the objective from experimental measurements on that system [60].

Although the accuracy of GEM-predicted enzyme targets or antimetabolites is generally improved upon integration with additional data, a remaining challenge is to balance the extent of specialization with versatility, so as to avoid overfitting the GEM to a specific dataset. By incorporating data for different cancer types, or even the same cancer type but from independent studies, predictions will become more robust to batch effects and noise [61]. Furthermore, a large proportion of studies have used *in vitro* data from cell lines to contextualize cancer GEMs and/or validate its predictions. While this is often necessary due to a lack in the availability or quality of *in vivo* data, advancements in biological profiling techniques are now making such measurements cheaper and more accessible, and should therefore be prioritized in future GEM-based approaches.

Another area of improvement for GEMs lies within their assumption of tissue or tumor homogeneity, which derives from the nature of the experimental data used for their contextualization. Since omics profiling requires relatively large, multicellular samples to accurately quantify an extensive set of molecular-level properties, the result is an average picture of all the cells comprising the sample, lacking information on intra-tissue heterogeneity [62]. The extent and importance of genomic and phenotypic heterogeneity among cells within a single tumor has been demonstrated by a number of studies [63,64], highlighting the need to account for this variation when generating metabolic models of such systems. The continued development of microdissection and single-cell sequencing technologies [32] will provide the omics profiles necessary to capture intratumor heterogeneity within a collection of GEMs, enabling a more accurate representation of the disease.

GEMs are essential to interpreting and predicting how metabolic changes will impact a highly connected network. Although the application of GEMs for anticancer drug discovery is still relatively young, the approach has already shown strong potential. It is expected that GEMs will continue to serve as an increasingly important tool in understanding and exploiting cancer-specific metabolic reprogramming.

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- •• of outstanding interest

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