Review

Future Medicinal Chemistry

Cancer cell metabolism as new targets for novel designed therapies

Metabolic processes are altered in cancer cells, which obtain advantages from this metabolic reprogramming in terms of energy production and synthesis of biomolecules that sustain their uncontrolled proliferation. Due to the conceptual progresses in the last decade, metabolic reprogramming was recently included as one of the new hallmarks of cancer. The advent of high-throughput technologies to amass an abundance of omic data, together with the development of new computational methods that allow the integration and analysis of omic data by using genome-scale reconstructions of human metabolism, have increased and accelerated the discovery and development of anticancer drugs and tumor-specific metabolic biomarkers. Here we review and discuss the latest advances in the context of metabolic reprogramming and the future in cancer research.‡Authors contributed equally

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Cancer is still one of the major causes of death worldwide and the statistics are devastating. According to the WHO the global burden of cancer has risen to 14.1 million new cases and 8.2 million cancer deaths in 2012 and the estimates predict that it could increase in its global incidence [1].

It was proposed 15 years ago by Hanahan and Weinberg that cancer development relies on the following basic biological capabilities, known as the 'hallmarks of cancer' that are acquired during the multistep process of tumor development: the capability to sustain proliferative signaling, resistance to cell death, evasion of growth suppression, ability of replicative immortality, tumor-promoting inflammation, genome instability and mutation, induction of angiogenesis and activation of invasion and metastasis. Owing to conceptual progress in the last decade, two new hallmarks, metabolic reprogramming and evasion of immune destruction, have been identified (Figure 1) [2].

Nowadays, it is widely recognized that metabolic reprogramming is essential to sustain tumor progression. Several metabolic adaptations described in cancer cells, such as the metabolization of glucose to lactate in the presence of oxygen (Warburg effect), are quite common among different cancer types. These changes are promoted by genetic and epigenetic alterations producing mutations or alterations in the expression of key metabolic enzymes that modify flux distributions in metabolic networks, providing advantages to cancer cells in terms of energy production and synthesis of biomolecules [3,4].

Understanding the mechanisms that trigger metabolic reprogramming in cancer cells and its role in tumoral progression is crucial, not only from a biological but also from a clinical stance, since this can be the basis towards improving existing cancer therapies or developing new ones.

In this review, we discuss the role of: the crosstalk between oncogenic signaling pathways and metabolism; the influence of nongenetic factors, such as tumor microenvironment, on metabolic reprogramming of cancer and stromal cells; the changes in isoenzymes patterns as potential therapeutic targets; and the new computational tools used by a systems biology approach in drug-target and biomarker discovery based on genome-scale metabolic models (GSMMs). Finally, we also discuss the future

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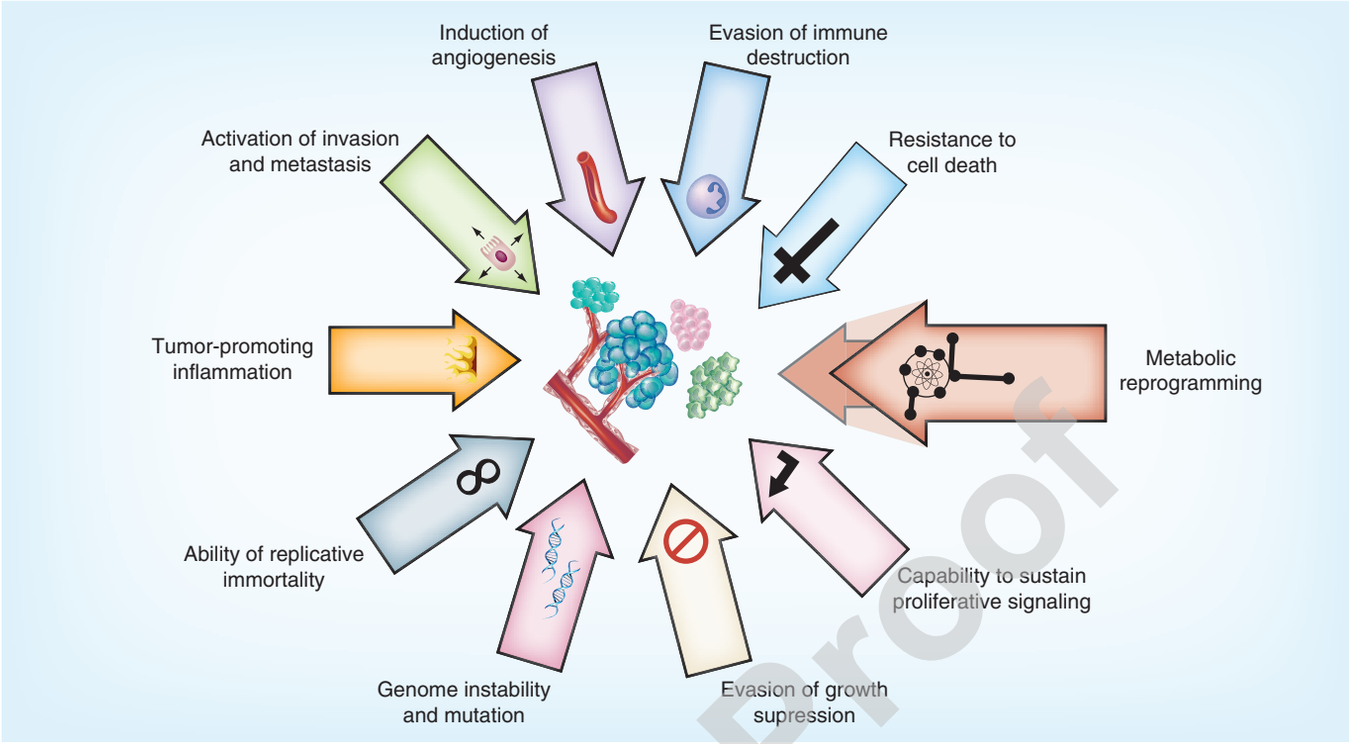

Figure 1. Hallmarks of cancer. The hallmarks of cancer comprise ten capabilities required during a multistep tumor pathogenesis to enable cancer cells to become tumorigenic and ultimately malignant. Metabolic reprogramming has been identified as an emerging hallmark and as a promising target for the treatment of cancer as there is a deregulation of bioenergetic controls and an abnormal use of metabolic pathways to sustain their biosynthetic and energetic needs.
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challenges in developing new strategies and methods to drug and biomarker discovery, exploiting the reprogramming of metabolism that sustains cancer progression.

Crosstalk between oncogenic signaling events & cancer cell metabolism

Through a better understanding of the complex networks of oncogenic signaling pathways, altered cellular metabolism emerges as one of the major routes through which oncogenes promote tumor formation and progression. Many key oncogenic signaling pathways converge to adapt tumor cell metabolism in order to support their growth and survival. The identification of new metabolic coordination mechanisms between altered metabolism and regulators of cell signaling networks, controlling both proliferation and survival, triggers the interest for new metabolism-based anticancer therapies. Several oncogenes, tumor suppressor genes and cell cycle regulators controlling cell proliferation and survival are intimately involved in modulating glycolysis, mitochondrial oxidative phosphorylation (OXPHOS), lipid metabolism, glutaminolysis and many other metabolic pathways (Figure 2). The accumulation of genetic abnormalities required for oncogenesis leads to changes in energetic and biosynthetic requirements that in turn affects the metabolic signature of cancer cells through interactions between enzymes, metabolites, transporters and regulators. High-throughput sequencing data reveals that the mutational events causing tumorigenesis are much more complex than previously thought and that the mutational range can vary even among tumors with identical histopathological features [5]. Some of the metabolic adaptations driven by oncogenic signaling events have been described as common to different tumors, but metabolic profiles can be significantly tissue/cell specific [6]. Here, we will highlight some of the most prevalent examples of crosstalks between oncogenic signaling events and pivotal

Key terms

Metabolic reprogramming: Process in which the cellular metabolism evolves in order to adapt to new environmental conditions and perturbations. In the case of tumor, the energy metabolism is reprogrammed in order to sustain the high proliferative rate of cancer cells.

Genome-scale metabolic models: Those models that summarize and codify the information known about the metabolism of an organism based on the literature and databases. These models represent the metabolic reaction encoded by an organism's genome and can be transformed into a mathematical formulation in order to study the metabolic cell behavior.

metabolic pathways. *HIF-1* is a key regulator that initiates a coordinated transcriptional program activated by hypoxic stress (in response to low-oxygen conditions), to promote the metabolic shift from mitochondrial OXPHOS to glycolysis (Figure 2) through the induction of several genes, including glucose transporters and glycolytic enzymes, leading to an increased flux of glucose to lactate [7]. Additionally, *HIF-1* actively downregulates the OXPHOS flux by activation of PDK1, which inhibits the conversion of pyruvate to acetyl-CoA catalyzed by the tricarboxylic acid (TCA) cycle enzyme PDH.

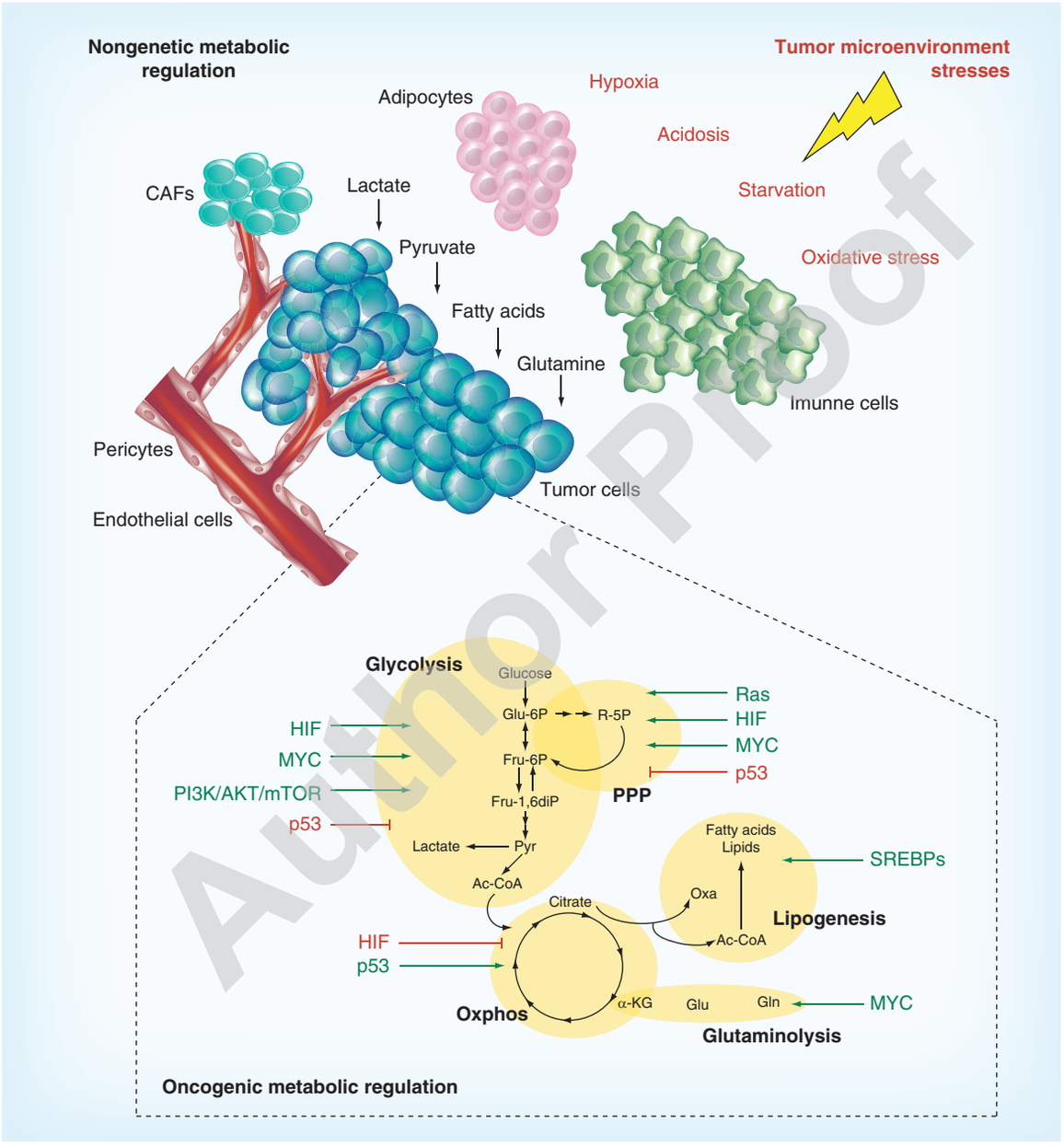

Figure 2. Nongenetic and oncogenic influences on tumor metabolic reprogramming. The nongenetic component (the tumor microenvironment) influences metabolic changes in tumor cells as a result of gradients of oxygenation and pH, nutrient availability, oxidative stress and the intercellular communication with stromal cells by means of metabolites such as lactate, pyruvate, fatty acids and glutamine. Combined with tumor microenvironment, the genetic component (oncogenes and tumor suppressors) plays a key role in metabolic reprogramming to ensure metabolites are shunted into pathways that support the energetic requirements and the biosynthesis of structural components, achieved by maintaining high rates of glycolysis and/or glutaminolysis, promoting the pentose phosphate pathway, slowing mitochondrial metabolism (oxidative phosphorylation) and utilizing tricarboxylic acid intermediates for biosynthetic precursors (e.g., fatty acids and lipids).

Similar to *HIF-1*, oncogenic activation of *Myc* also triggers a transcriptional program that enhances glycolysis by directly inducing glucose transporters and glycolytic enzymes. Indeed, there is a crosstalk between *HIF-1* and *Myc*, whereby they cooperate to confer metabolic advantages to tumor cells by oxygen-dependent mechanisms, with a difference that, contrary to *HIF-1*, *Myc* upregulation has more significant consequences for many cells as it alters not only glycolysis but also glutaminolysis (Figure 2) and many other biosynthetic pathways [8]. The *Myc* oncogene stimulates glutamine uptake and glutaminolysis by inducing glutamine transporters directly and GLS, the enzyme that converts glutamine to glutamate, indirectly [9]. Besides glycolysis, glutaminolysis is another important metabolic pathway in cancer cells, which contributes not only as a source to replenish the TCA cycle, but also to control the redox potentials through generation of reductive equivalents, such as NADPH. In addition to glucose, a vast amount of glutamine is consumed by cancer cells. Glutamine is converted to glutamate and then to α-ketoglutarate (α-KG), which feeds the TCA cycle. Some tumors that show an upregulation of glutamine metabolism have been reported to exhibit 'glutamine addiction', that is, glutamine becomes essential during rapid growth. However, glutamine consumption and addiction are dependent on the metabolic profile of the cancer cells and in particular on the oncogene/tumor suppressor involved in tumor progression [10].

Activated PI3K/AKT/mTOR pathway is one of the most common signaling cascades altered in tumor cells and this pathway is one of the most heavily targeted to develop anticancer therapies. Many cancers are driven by aberrations in the PI3K/AKT/mTOR pathway promoting metabolic transformation through multiple metabolic pathways, including an increase in glucose and amino acid uptake (Figure 2), upregulation of glycolysis and lipogenesis and enhanced protein translation through Akt-dependent mTOR activation [11].

In cancer cells, the increased rate of *de novo* lipid biosynthesis is an important aspect of the metabolic reprogramming during oncogenesis. Lipid metabolism is regulated via activation of the sterol regulatory element binding proteins (SREBPs) (Figure 2), which are important regulators of the Akt/mTOR signaling pathway [12]. Indeed, various genes coding for enzymes involved in fatty acid and cholesterol biogenesis are targets of SREBPs, including ATP-citrate lyase, acetyl-CoA carboxylase and fatty acid synthase [13]. Lipogenesis is also controlled by the *RAS* oncogene through the action of *HIF-1*, which has been reported to induce the expression of fatty acid synthase in human breast cancer cell lines [14]. However, the *RAS* oncogene also modulates mitochondrial metabolism roughly increasing the activity of *Myc* and *HIF-1* [4], glycolysis and the pentose phosphate pathway (PPP) [15]. Proliferating cells, such as tumors, require high amounts of pentose phosphates for biosynthesis of macromolecules and NADPH for redox homeostasis maintenance [16]. Therefore, PPP plays a fundamental role in defining the metabolic phenotype of tumor cells. Hence, there are also examples of coordinated crosstalk between the main enzymes that control the PPP during oncogenesis and oncogenic signaling pathways. K-RAS and PI3K signaling have been shown to positively regulate G6PD, whereas p53, which is a transcription factor and regulator of the cell cycle and apoptosis, physically interacts with G6PD to negatively modulate its activity [17], and thereby downregulates PPP. On the other hand, active *HIF-1* signaling has been linked to both TKT and TKTL1, the enzymes catalyzing the rate-limiting step of the non-oxidative branch of the PPP [18].

In addition, alterations in *p53* are frequent events in tumorigenesis. The loss or inactivation of *p53* downregulates OXPHOS by inducing aerobic glycolysis through inhibiting glucose transporters and the glycolytic enzyme PGM and inducing TP53-induced glycolysis and apoptosis regulator, a negative regulator of glycolysis [19]. On the other hand PHF20 stabilizes and upregulates p53 resulting in a gain of functionality that drives the reprogramming of the metabolism of certain cancers cell lines, such as U87 (glioblastoma) or MCF7 (breast cancer) [20].

Other examples of oncogene-mediated metabolic reprogramming include mutations in genes encoding FH and succinate dehydrogenase, which are loss-of-function mutations and behave as tumor suppressor genes [21]. On the other hand, mutations in IDH-1 and IDH-2, do not result in inactivation of normal IDH enzymatic function but generation of novel gain-of-function mutation that enables the conversion of α-KG to D2-HG, which may act as an 'oncometabolite' by inhibiting multiple α-KG–dependent dioxygenases involved in epigenetic regulation [22].

Tumorigenesis occurs as a consequence, not only of the dysregulation of numerous oncogenic pathways, but also due to many nongenetic factors, including tumor microenvironment stresses, such as hypoxia, lactic acidosis and nutrient deprivation. The integration of these nongenetic factors within the genetic

Key term

Tumor heterogeneity: Variability among different tumors in the same organ (intertumoral heterogeneity) or the variability among cells in a tumor (intratumoral heterogeneity).

framework of cancer is the next logical step in understanding tumor heterogeneity. Research over the years has elucidated the cellular and molecular interactions (including metabolic reprogramming) occurring in the tumor microenvironment and are closely linked to the processes of angiogenesis and metastasis.

Tumor microenvironment

Since the discovery of immune cells in tumor samples by Rudolf Virchow in 1863, various studies have shown the linkage of cancer to inflammation, vascularization and other conditions, which suggest that tumors do not act alone. Without its 'neighborhood' the survival of tumor cells could be a big question mark. The cellular heterogeneity in this microenvironment is complex and comprises of extracellular matrix, tumor cells and non-transformed normal cell types that co-evolve with the tumor cells (e.g., cancer-associated fibroblastic cells [CAFs], infiltrating immune cells and endothelial cells that constitute the tumor-associated vasculature) that are embedded within this matrix and nourished by the vascular network. In addition, there are many signaling molecules and chemicals, such as oxygen and protons, all of which can influence tumor cell proliferation, survival, invasion, metastasis and energy metabolism reprogramming. CAFs, one of the most abundant stromal cell types in different carcinomas, are activated fibroblasts that share similarities with fibroblasts, stimulated by inflammatory conditions or activated during wound healing. But, instead of suppressing tumor formation, CAFs can significantly promote tumorigenesis, invasion and *de novo* cancer initiation by some unique growth factors and cytokines secretion (e.g., EFG, FGF, IL6, IL8, VEGF etc), extensive tissue remodeling mediated by augmented expression of proteolytic enzymes (e.g., matrix metalloproteinases), deposition of extracellular matrix and pathogenic angiogenesis by liberating pro-angiogenic factors within the matrix [23]. Significant cell plasticity exists within this cell population, as both mesenchymal-to-epithelial and epithelial-to-mesenchymal transitions are known to occur, further enhancing stromal heterogeneity. Moreover, CAFs can enhance proliferation and invasion by inducing the epithelial-to-mesenchymal transitions on tumor cells [24,25]. Immune cell recruitment and localization in the tumor milieu vary widely in the lesions. Heterogeneity of tumor immune contexture is influenced by various factors, including those secreted by CAFs, the extension and permeability of the vasculature, and the tumor cells themselves. Importantly, macrophages comprise the most abundant immune population in the tumor microenvironment and are responsible for the production of cytokines, chemokines, growth factors, proteases and toxic intermediates, such as nitric oxide and reactive oxygen species [26]. Their contribution to tumor initiation, progression and metastasis can be attenuated by antioxidant treatments, such as butylated hydroxyanisole, as reactive oxygen species levels have been reported to regulate the differentiation and polarization state of macrophages. Endothelial cells that are 'hijacked' by the tumors play an important part in forming a transport system, although ineffective, but essential for its survival and growth. In addition, blood vessel formation needs a protein matrix for the endothelial cells to be attached to and also it needs pericytic cells to strengthen these vessels. But, since the pericytes are not known to function very well in tumor vessel formation, the vessels are always malformed and leaky [27].

In the last few years the concept of cancer stem cells (CSC), a small minority of cells in the tumor, has evolved to be a possible cause and source of tumor heterogeneity. Currently there are two models that describe tumor cell heterogeneity: the hierarchical CSC model, where self-renewing CSCs sustain the stem cell population while giving rise to progenitor cells that are not capable of self-renewal and can give rise to differentiating clones that contribute to overall tumor heterogeneity, and the stochastic (tumor microenvironment-driven). model in which cancer cells are clonally evolved, and virtually every single cell can self-renew and propagate tumors. In this model, the self-renewal capability of each cell is determined by distinct signals from the tumor microenvironment. Recent studies have suggested that tumor heterogeneity may exist in a model coordinating with both the CSC and the stochastic concepts [28].

Metabolic reprogramming associated with cancer & stromal cell interaction

Recently, the relationship between tumor microenvironment and metabolic reprogramming has been highlighted and there has been extensive research about metabolic symbiosis between cancer and stromal cells. Among these interactions, it was shown that epithelial tumor cells induce oxidative stress in the normal stroma, inducing aerobic glycolysis in CAFs, as well as changes in inflammation, autophagy and mitophagy (Figure 2). As a consequence of this rewiring in CAFs metabolism, energy-rich metabolites (such as lactate, pyruvate and ketones) are secreted, feeding adjacent cancer cells. This tumor–stroma metabolic relationship is referred to as the 'reverse Warburg effect'. CSCs that are present within the tumor also rely more heavily on glycolysis, even in the presence of oxygen (Warburg effect), and decrease their mitochondrial activity in order to limit reactive oxygen species production. As these glycolytic and mitochondrial signatures help

to maintain the CSC phenotype, recent studies have focused their attention to these metabolic weaknesses to be combined with traditional chemotherapy that, alone, usually fails to target CSCs [29,30]. In addition, other stromal cells, such as adipocytes, are able to act as energy sources, transferring fatty acids that come from lipolysis to ovarian tumor cells for β-oxidation [31]. Deregulated lipogenesis has been shown to play an important role in the interactions between cancer cells and the surrounding stromal cells. Studies suggest that it affects the epithelial cell polarity during the early stages of cancer development [32], inducing cancer cell migration [33] and activation of angiogenesis involving signaling lipids (e.g., diacyl glycerides, lysophosphatidic acid and prostaglandins), fatty acid synthesis enzymes and overof the monoglyceride-lipase [34–36].

Loss of stromal caveolin-1 in CAFs has been associated with tumor progression and metastasis [37] and causes oxidative stress and induction of autophagy, which results in increased levels of glutamine and ammonia in the stromal microenvironment. This glutamine could be consumed by cancer cells for energy and anaplerotic reactions and ammonia acts as a potent inducer of autophagy, creating a vicious cycle [37]. The migration stimulating factor, a truncated isoform of fibronectin identified to be overexpressed by CAFs and other 'activated' fibroblasts, has been shown to increase lactate production in the stromal environment and decrease mitochondrial activity, suggesting a shift towards glycolysis during hypoxia in addition to promoting tumor growth without affecting tumor angiogenesis [38].

Angiogenesis has been long known to play a major role in supporting cancer cell growth in the tumor microenvironment. But since the newly formed blood vessels are mostly defective there is always a nutrition deficiency and acidosis in these areas (Figure 2). A biomarker study in the gastric cancer environment where a quantitative analysis of the organic acids that are the end products of metabolism, using GC–MS, showed an increase in glycolytic end-products, such as pyruvic and lactic acids, with respect to normal tissues [39]. The pattern of high acidification in the tumor microenvironment due to the accumulation of glycolytic end-products results in a nutrient-deficient environment. In addition, metabolic reprogramming of tumor-associated endothelial cells has been showing up wide interests. Upon tumor angiogenic activation, endothelial cells are pushed to a state of metabolic stress for increasing their proliferation rate to form new blood vessels, although the resulting network is abnormal and inefficient. These normal cells show higher glycolytic enzyme activities and lactate production, even in the presence of oxygen [40], and they continue proliferating even in the presence of hostile conditions and high nutrient deficiency [41]. Also it has been shown that endothelial cells, similar to tumor cells, have a high expression of monocarboxylate transporter 1 required for the lactate influx, revealing that these cells seek alternative metabolites in a nutrition-deficient environment [42]. Moreover, the inhibition of glycogenolysis in human umbilical vein endothelial cells has been shown to decrease cell viability and migration, elucidating the importance of glycogen for the survival of these cells [43]. The role of the PPP in cell viability has also been demonstrated, in that, the direct inhibition of G6PDH has been shown to decrease endothelial cell survival [43]. When tumor cells choose the less energy-efficient metabolic pathways, such as glycolysis and glutaminolysis, both leading to the production of lactic acid, the pH of the tumor microenvironment decreases. It has been shown that endothelial cells behave in a similar fashion while forming new tumor blood vessels. While this phenomenon is known, it has also been found that the decrease in pH in the surrounding microenvironment actually increases cancer survival by immune suppression. Loss of T-cell function has been reported under low pH environment, while restoring the pH to normal conditions has been found to restore T-cell function [44]. Similarly, the lactic acid generated has shown to increase the proliferation of endothelial cells by increased interleukin8/CXCL8 production [41,45]. From a therapeutic point of view, targeting the altered metabolic pathways leading to lactic acid accumulation in tumor microenvironment could inhibit tumor growth as this mechanism would restore the impaired immune response and also a combinatorial therapy with antiangiogenesis drugs could reduce the proliferation of endothelial cells and formation of new blood vessels [46].

An important event that occurs during the changes in tumor microenvironment, as the cancer progresses, is the metastasis of some selected cancer cells to distant sites. A receptive microenvironment is required for tumor cells to engraft distant tissues and metastasize. Although several studies have indicated the formation of a premetastatic niche in the secondary sites before the primary tumor metastasizes [47], we have to consider how metastatic cells are able to adapt to their new metabolic environment, which can differ to a greater or lesser extent with respect to its nutrient and oxygen availability. Metastatic cells should exhibit a remarkable and dynamic flexibility that enables them to rapidly switch between metabolic states [48]. In addition, the homeostasis of the sites for metastasis can be disrupted as consequence of the metabolic activity of metastatic cells. This has been observed in bone, where metastatic prostate cancer cells secrete glutamate into

their extracellular environment as a side effect of cellular oxidative stress protection, promoting the development of pathological changes in bone turnover [49]. Further studies are required to analyze these metabolic interplays between metastatic cells and tumor microenvironment in order to obtain more specific treatments and therapies.

Isoenzymes: therapeutic targets in cancer

The technological advances that have occurred over the past decade and the increasing number of evidences that have emerged from previous studies show a wide array of metabolic rewiring in cancer cells. Many metabolic enzymes that are specific to important metabolic pathways and those altered in cancer cells have been identified. These enzymes have a key role in mediating the aberrant metabolism of cancer cells and could serve as a promising source of novel drug targets. Isoforms of many of these metabolic enzymes are found to be specifically expressed in tumor cells affecting important pathways of the energetic metabolism. The current research is being refocused on specifically targeting these isoforms that has shown to be a promising strategy to develop new anticancer treatments. In this part, we will highlight some of the most important, altered pathways and the specific isoenzymes, that could be used for drug targeting, in cancer disease.

Glycolytic isoenzymes

Glycolytic pathway serves as the principal energetic source for a cell. The higher dependency of cancer cells upon glycolytic metabolism for the production of ATP provides a greater motive to target glycolytic enzymes (Figure 2). Many isoforms of these enzymes have been found to be specifically expressed in tumor cells and are being exploited as potential candidates to be used as drug targets. The transport of glucose across the plasma membrane is regulated by various isoforms of glucose transporters (GLUT1–14 or SLC2A1–14). GLUT1, -3 and -4 are found to be expressed at higher levels in cancer [50]. GLUT3 and other transporters could be targeted by the use of specific antibodies or drugs, such as phloretin or ritonavir, causing the cells to starve by blocking their nutrient uptake through these transporters.

Another important metabolic enzyme of the glycolytic pathway is HK, which regulates the first rate-limiting step of glucose metabolism. Cancer cells are heavily dependent on HK isoforms, such as HK2 [51]. The specific expression of HK2 in adipose tissue and skeletal muscles provides an opportunity to target this enzyme without having the risk of affecting other tissues. Compounds such as methyl jasmonate isolated from plants have been shown to disrupt the association between mitochondria and HKs (HK1 and -2). involved in regulating apoptosis [52] and have shown to be lethal to cancer cells *in vitro* [53].

Recent publications suggest a key role of PK isoenzyme – PKM2 – in mediating the Warburg effect in cancer cells [54], proving its prospective as an enzymatic anticancer drug target. The enzyme activity of PKM2 is inhibited downstream of cellular growth signals [55]. Cell proliferation and aerobic glycolysis in tumors are greatly dependent on this ability to inhibit the activity of the PKM2 enzyme. Many approaches using small-molecule inhibitors and small-hairpin RNA-based inhibition of *PKM2* have been shown to cause cell death and slow down cell proliferation *in vitro* [54,56]. The PFKFB3 isoform is shown to be important in RAS-mediated tumors and inhibition of PFKFB3 by small-molecule inhibitors has been shown to have cytostatic effect on the growth of cancer [57]. Inhibition of LDHA using FX11 or oxamate has been shown to induce oxidative stress and cause cell death in cancer cells [58,59]. Targeting LDHA combined with NAMPT inhibitors has been shown to slow down tumor regression and thus making it a potential candidate for drug targets [59].

TCA isoenzymes/mitochondrial complex

PDK phosphorylates PDH and inhibits the conversion of pyruvate to acetyl-CoA, a key metabolite in the TCA cycle (Figure 2). Isoenzyme PDK3 is induced by upregulation of HIF-1α under hypoxic conditions and results in cells undergoing glycolysis instead of TCA for energy production. Inhibition of PDK3 increases the susceptibility of tumor cells towards anticancer drugs and causes inhibition of hypoxia-induced glycolysis [60]. Thus PDK3 could be used as a drug target to overcome drug resistance and improve chemotherapy.

Isoforms of IDH1 and -2 are found to be mutated in glioma and acute myeloid leukemia [61,62]. Mutations in IDH1 and -2 result in the overexpression of both of these enzymes and the production of 2-HG, which inhibits α-KG-dependent dioxygenase enzymes. Association between high levels of 2-HG and tumorigenicity is yet to be established, but interestingly the levels of several TCA metabolites remain unaltered, suggesting an alternate pathway that could be acting in normalizing the metabolite levels in cells with IDH1 mutations.

Isoenzymes of the PPP

Cancer cells are in a constant demand for greater amounts of purines and pyrimidines to maintain their high proliferative nature (Figure 2). The key enzyme for the oxidative PPP, the G6PDH enzyme, is overexpressed in certain types of cancers and it has been

shown to transform fibroblasts and help in tumor cell proliferation [63]. On the other hand, the overexpression of TKTL1 in many forms of cancer could increase the concentration of glyceraldehyde-3-phosphate and help in mediating the Warburg effect in cancer cells [64]. Combinatorial approach of targeting G6PDH and TKTL1 can help overcome drug resistance and may cause cell death [65].

Targeting isoenzymes of glutamine metabolism

Recent findings that point to the use of glutamine as a carbon source for the TCA cycle [66] in cancer cells encouraged researchers to consider enzymes of glutamine metabolism as potential therapeutic targets. 6-diazo-5-oxo-L-norleucine- or bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulphide-mediated inhibition of GLS or siRNA-induced silencing of GLS and GDH have been shown to inhibit the activation of mTORC1 [67]. Thus, combinatorial targeting of GLS and GDH along with chemotherapy may prove to be more effective in cancer treatment. The differential expression of these cancer-associated isoenzymes can be used as potential biomarkers for early cancer prognosis or as enzymatic drug targets. However, the role and importance of these mutations in the reprogramming of the energetic metabolism observed in cancer cells is not always obvious. This makes it extremely difficult to evaluate the effects of these mutations in the cancer metabolism qualitatively or quantitatively. Additionally, the effects of these isoenzymes on metabolism can be attenuated or enhanced by compensatory and regulatory mechanisms. Taking into account these rationales, the need for a tool that permits a holistic analysis of the metabolic system is essential, in order to qualitatively evaluate the effects of a single or combination of different mutations within the whole metabolic network system. In the last few years, genome-scale metabolic network models have demonstrated their suitability for the integrated analysis of large and complex metabolic networks providing new clues for identifying drug targets.

GSMMs as new tools emerging from systems biology approach to drug discovery

In the previous sections, we have presented evidences that support cancer onset and that the progression relies on metabolic abnormalities to balance energy demand and biomolecular synthesis (metabolic reprogramming) [68]. GSMMs are emerging as a potential solution to decipher the molecular mechanisms underlying cancer in the context of systems biology [69]. GSMMs represent the metabolic reaction complement encoded by an organism's genome. These models are built based on the literature and databases and enable one to summarize and codify information known about the metabolism of an organism.

Over 100 GSMMs have been built for different species, ranging from archea to mammals [70–84]. Reconstructions of human metabolism, such as Recon1 [81], Edinburgh Human Metabolic Network [82] or the most recent reconstructions of human metabolism, Recon2 [83], are widely used to study the mechanism of diseases with a strong metabolic component, such as cancer or diabetes [85–88].

This systems biology tool enables the mathematical representation of biotransformations and metabolic processes occurring within the organism and offers an appropriate framework to integrate the increasing amount of 'omic' data generated by the different high-throughput technologies.

The transformation into a mathematical formulation is mostly driven by constraint-based modeling (CBM) [89] and allows the systematic simulation of different phenotypes, environmental conditions, gene deletion and so on. This approach allows for modeling the complexity of cancer metabolism and tackling more problematic biological questions, such as the role of metabolism in cancer disease [90].

Genome-scale constraint-based metabolic models have been used for a variety of applications, involving studies on evolution [91], metabolic engineering [92–94], genome annotation [95] or drug discovery [96], with a high relevance in cancer research.

Indeed, GSMMs can efficiently capture the complexity of cancer metabolism in a holistic manner and permit to improve existing therapies or develop new ones [97].

In this chapter we discuss methods for building GSMMs and computational approaches to analyze and integrate 'omic' data into these large-scale metabolic network models. Finally, we introduce some of the most relevant softwares and algorithms developed for drug-target discovery that can be used in cancer research.

GSMM reconstruction

Genome-scale metabolic reconstructions are created in a bottom-up manner based on genomic and bibliomic data and, thus, represent a biochemical, genetic and genomic knowledge base for the target organism [81–83]. However, to date we are still not able to completely and automatically reconstruct high-quality metabolic networks (Figure 3A) [98]. Genome-scale reconstruc-

Key term

Enzymatic drug target: A component in a metabolic pathway to which some other entity, such as a drug, is directed and/or binds.

tion starts with the generation of a draft, automated reconstruction based on the genome annotation and biochemical databases of the target organism. This task can be achieved by using software tools, such as Pathways tool [99]. The genomic sequence of the targeted organism is coupled with the most recent annotations available from databases [100], such as GOLD or NCBI Entrez Gene databases [101,102].

Metabolic reactions can be associated with the annotated metabolic genes by using enzyme commission (E.C.), ID and biochemical reaction databases (e.g., KEGG [103] and BRENDA [104]). This process permits both linking metabolic genes with their corresponding encoded enzymes and determining the stoichiometric relationship of metabolic reactions with the metabolites and cofactors that they consume and/or produce.

The gene–protein-reaction association (GPR). is represented as Boolean relationships in which isoenzymes that catalyze the same reaction have an "OR" relation (only one of the genes that encode the different isoenzymes is required to have the reaction active) and the complexes that catalyze a reaction have an "AND" relation (all the genes that encode the different complex subunits are necessary to have the reaction active) [81]. GPR associations enable the mapping of transcriptomics or proteomics to the level of reactions.

Reactions can be located into different subcellular compartments based on protein location [81]. Reaction directionality can be determined from thermodynamic data. Additionally, artificial reactions, such as biomass reaction that define the ratio at which biomass constituents are produced (nucleic acids, lipid, proteins, etc) or exchange reactions that define the overall rate of nutrients consumption or production, are also defined in the reconstruction. These artificial reactions are necessary to predict or impose certain phenotypic conditions on the mathematical model.

Next, it is necessary to manually curate and refine the draft, automated reconstruction. The main objective of curation is to identify and correct incomplete or erroneous annotation, add reactions that occur spontaneously and remove gaps and metabolites that cannot be produced or consumed [81] through search on the literature and other databases.

Once the model is curated, it is evaluated and validated in an iterative fashion by using mathematical tools [105]. The aim of the validation process is to evaluate if the model is stoichiometrically balanced, find gaps in the network and search for candidate reactions for gap filling, quantitative evaluation of biomass precursor production and growth rate, compare predicted physiological properties with known properties and determine the metabolic capabilities of the model.

It is worth noting that once a GSMM has been constructed, it can be used in future reconstructions in order to expand and refine the model [81,83].

Constraint-based methods as tools for tumor metabolism characterization

As was previously mentioned, GSMMs include stoichiometric details for the set of known reactions in a given organism. These large scale metabolic models require computational methods to be qualitatively analyzed. Traditionally, approaches based on ordinary differential equation have been used for characterization of dynamic cell states. However, this full-scale dynamic modeling is frequently infeasible for large-scale networks because of a paucity of necessary parameter values.

Constraint-based methods (CBMs) permit the analysis of large-scale biochemical systems under conditions where kinetic parameters need not be defined (steady state). Genome-scale constraint-based metabolic models can be used to predict or describe cellular behaviors, such as growth rates, uptake/secretion rate or intracellular fluxes [89]. Flux balance analysis (FBA) is one of the most widely used CBMs for the study of biochemical networks. The variables used in FBA include the fluxes through transport and metabolic reactions and model parameters include reaction stoichiometry, biomass composition, ATP requirements and the upper and lower bounds for individual fluxes, which define the maximum and minimum allowable fluxes of the reactions.

The first step in FBA is the mathematical representation of the metabolic reactions in the form of a numerical matrix, with stoichiometric coefficients of each reaction (stoichiometric matrix), where the metabolites are represented in rows and reactions in columns. FBA employs mass actions formalism for the mathematical representation of the metabolic networks: $dC/Dt = S.v.$, where v and C are vectors of reaction fluxes and metabolite concentration respectively, t is time and S is the stoichiometric matrix (Figure 3A).

The next step is to impose constraints to the metabolic network. Constraints are fundamentally represented in two ways:

Steady-state mass-balance imposes constraints on stoichiometry and network topology on the metabolic fluxes through the network. Additionally, steady state assumption also imposes constraints that narrow the space of solutions. By definition, the change in the concentration of a certain metabolite over time at steady state is 0: $dC/Dt = 0$, thus: $S.v = 0$. These constraints ensure that for each metabolite in the network the net production rate equals the net consumption rate;

Inequalities that impose bounds on the system: every reaction can also be given upper and lower

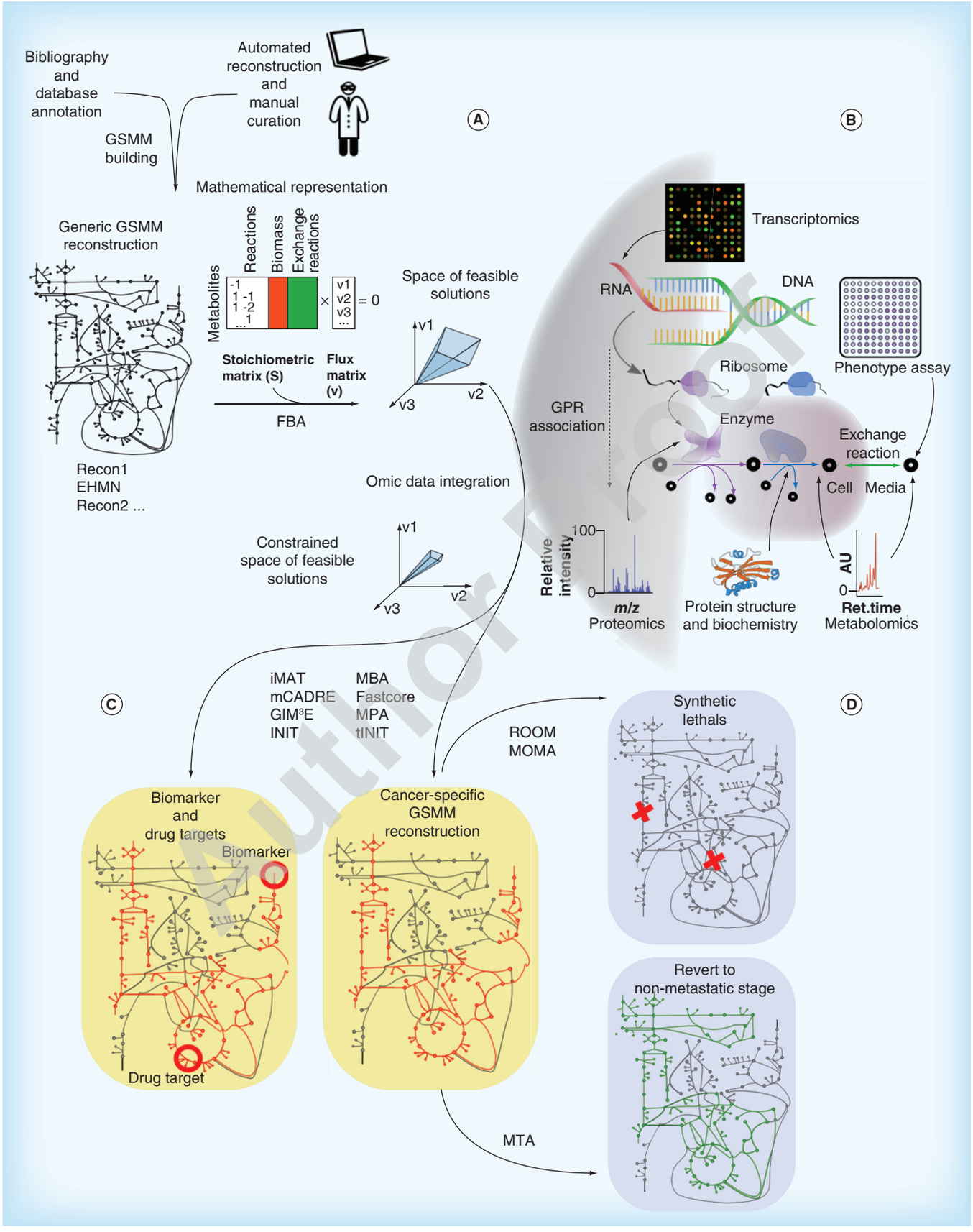

Figure 3. Genome-scale metabolic model building and analysis (facing page). (A) Genome-scale metabolic model (GSMM) reconstruction starts with a draft automated version based on literature and databases, finally this version is manually curated in order to refine the model. Typically, these models are analyzed by using flux balance analysis, assuming steady state. **(B)** GSMMs can be used as a platform to integrate and combine omic data from multiple layers. In these models, metabolomics data can be associated with metabolites, while genomics, transcriptomics and proteomics can be associated with metabolic reactions, these associations are established through gene–protein-reaction associations. The phenotypic assays can constrain properties of the network, such as growth rate under certain experimental conditions. **(C)** By integrating omic data into a GSMM we can determine either tumor-specific biomarkers or anticancer drug-targets and reconstruct cancer-specific GSMM. **(D)** Cancer-specific reconstructions can be used to determine synthetic lethals specific for each cancer type for which the non-tumor cells are insensitive (ROOM and MOMA methods), Additionally if we reconstruct an initial GSMM describing metastatic phenotype and a target GSMM describing non-metastatic phenotype we can determine the actors that would permit to revert the metastatic phenotype into a non-metastatic one (MTA method).
ret.: Retention.

bounds. These restrictions are based on measured rates (e.g., metabolite uptake/secretion rates) or reaction reversibility (e.g., irreversible fluxes have a zero lower bound) and are used to define the environmental conditions in a given simulation, such as nutrient or O_2 availability, which can be related with a specific tumor microenvironment or stages in tumor progression.

Finally it is necessary to define a phenotype in the form of a biological objective that is relevant to the problem being studied (objective function). Typically, objective functions are related to growth rate prediction. GSMMs define this phenotype by an artificial biomass production reaction, that is, the rate at which metabolic compounds are converted into biomass constituents (nucleic acids, lipid, proteins, etc). The biomass reaction is based on experimental measurements of biomass composition and is unique for each organism or cell type. Thus, an objective function could be the maximization of growth rate that can be accomplished by calculating the set of metabolic fluxes that result in the maximum flux through biomass production reaction. Since uncontrolled cell growth is the basis of tumor progression, this approach is widely used in the simulation of cancer cell metabolism. The objective function can be adapted to the specific cell type or organism; however, the objective that better defines our case of study is not always obvious, especially in multicellular organisms [106].

Taken together, the mathematical representation of the metabolic reactions and of the objective function, is defined as a system of linear equations that are solved by a number of algorithms and software developed for this purpose [105]. Predictions of values for these fluxes are obtained by optimizing for an objective function, while simultaneously satisfying constraint specifications.

Omic data integration

The advent of high-throughput technologies have transformed molecular biology into a data-rich discipline by providing quantitative data for thousands of cellular components across a wide variety of scales. However, extraction of 'knowledge' from this ocean of omic data has been challenging [107]. GSMMs have emerged as an advantageous platform for the integration of omic data (e.g., [108]; Figure 3B). In this framework cellular and molecular phenotypes are simulated allowing the development of biological hypotheses and discoveries [109]. Metabolic reconstruction of the human metabolism has been successfully used for a variety of analyses of omic data, including applications in data visualization [110], deducing regulatory rules [111], network medicine [112], constructing tissue-specific models [113] or multicellular modeling [114]. Thus, omic data can be used to further constrain the non-uniqueness of constraint-based solutions space and thereby enhance the precision and accuracy of model prediction (Figure 3A-C) [109]. To achieve this aim a number of FBA-driven algorithms that integrate omic data into GSMMs have been developed. Table 1 highlights some of the most relevant approaches recently developed to incorporate experimental omic data into GSMMs [86–87,113,115–117]

Drug-target & biomarker discovery

Cancer cells maintain their high proliferation rate by adapting their metabolism based on the environmental conditions, such as pH, O_2 availability, vascularization or nutrient availability [118]. The elucidation of diverse metabolic alterations for the identification of biomarkers and novel drug targets has, therefore, been increased in recent years. An increasing number of methods and algorithms have been recently developed to integrate tumor-specific omic data into GSMMs. It has enabled the gain of further biological and mechanistic understanding of how cancer benefits from metabolic modifications [90]. This model-driven approach allows the discovery of potential biomarkers and drug

Key term

Omic data integration: Computational process in which multi-omic data obtained from different high-throughput technologies, considering different aspects of the molecular biology, are integrated into genome-scale metabolic models in order to unveil emergent properties of the biological systems.

Table 1. Computation method for integrating omic data into global-scale metabolic models.

Name	Input	Description	Ref.
iMAT	Gene expression data	Seeks to maximize the similarity between the gene expression and the metabolic profiles.	[115]
mCADRE	Gene expression and metabolomic data	Uses tissue-specific data to identify a set of core reactions. Seeks to build a consistent network using all the core reactions and the minimum number of non-core reactions.	[86]
GIM^{3}E	Gene expression and metabolomic data	Builds a network that satisfies an objective function while penalizing the inclusion of reactions catalyzed by genes with expression below a certain threshold. It can be further constrained to produce certain metabolites based on experimental evidences.	[116]
INIT	Gene expression and metabolomic data	Seeks to build a model prioritizing the addition of reactions with strong evidence of their presence based on gene expression data. Can be forced to produce metabolites that have been detected experimentally.	[87]
MBA	Transcriptomic, proteomic, metabolomic, bibliomic data	Uses tissue-specific data to identify high and moderate probability core reactions. Seeks to build a network with all the high-probability core reactions, the maximum moderate probability core reactions and the non-core reaction required to prevent gaps.	[113]
Fastcore	Transcriptomic, proteomic, metabolomic, bibliomic data	Identify a set of core reactions based on tissue-specific data. Seeks to build a network that contains all reactions from the core set with the minimum set of additional reactions necessary.	[117]

targets [87,97,119]. The identification of new biomarkers is of major importance to biomedical research for early diagnosis and monitoring treatments efficiently. The identification of cancer biomarkers is possible due to aberrant metabolism of tumors that alters the profile of absorption and nutrients secretion.

Omic data of clinical samples (mainly transcriptomics data) can be used to infer the exchange rates of different metabolites for each individual sample via GSMM analysis (alterations in exchange reactions in the model). Thus, those metabolites that significantly differ between two clinical groups in their exchange rates are then considered as potential biomarkers. However, this task is especially challenging in the case of cancer owing to metabolic abnormalities resulting from complex and elaborate genetic and epigenetic alterations that modify the expression of a variety of cancer-associated isoenzymes. In order to determine potential biomarkers in cancer, several computational approaches has been developed. For example, the metabolic phenotypic analysis (MPA) method uses GPR association to integrate transcriptomic and proteomic data within a GSMM to infer metabolic phenotypes [88]. MPA was used to study breast cancer metabolism and predict potential biomarkers. These predictions, wich include amino acid and choline-containing metabolites, are supported by a number of experimental evidences [120]. Another recently developed algorithm is mCADRE, which has been used to systematically simulate the metabolic function of 26 cancer cell types (among other cell types) [86]. This algorithm has been able to identify several pathways, such as folate metabolism, eicosanoid metabolism, fatty acid activation and nucleotide metabolism, that are enriched in tumor tissue compared with their corresponding normal tissue. Many enzymes involved in these pathways are already used as chemotherapy targets. Other approaches, such as flux variability analysis [121] or sampling analysis [122], are also suitable to predict metabolic biomarker candidates by integrating omic data into a GSMM. The novel drug discovery is based on the abnormalities existing in various reactions/pathways of cancer metabolism. These differences can be used as drug targets to attack specific weaknesses of the tumor and hence compromising its viability, but not that of non-cancerous cells [123]. For example, the INIT method [87] was used to identify characteristic metabolic features of cancer cells by inferring the active metabolic network of 16 different cancer types and compare them with the healthy cell types where they come from. These metabolic differences may play an important role in proliferation of cancer cells and

could be potential drug targets. This method found significant differences in polyamine metabolism, the isoprenoid biosynthesis and the prostaglandins and leukotrienes pathways in cancer cells compared with healthy cells. Some of the reactions that were found that have different activity in cancer cells, are already used in the clinical practice as therapeutic targets [124,125]. Based on the rationale that the differences between normal and tumoral cells can be potential therapeutic targets, several approaches have been developed that consider different aspects of cancer metabolism for the discovery of new drug targets:

Antimetabolite

One of the most common anticancer drugs are antimetabolites. An antimetabolite is structurally similar to a certain metabolite but it cannot be used to produce any physiologically important molecule. Antimetabolite-based drugs act on key enzymes preventing the use of endogenous metabolites, resulting in the disruption of the robustness of cancer cells and reduction or suppression of cell growth. For example, antimetabolites, such as antifolates or antipurines, mimic folic acid and purines [126]. The GSMM approach can be used to systematically simulate the effect of potential antimetabolites in cancer research. To achieve this, methods such as the tINIT (Task-driven Integrative Network Inference for Tissues) algorithm have been developed [97]. This method has been used to reconstruct personalized GSMMs for six hepatocellular carcinoma patients based on proteomics data and the Human Metabolic Reaction database [87] and identify anticancer drugs that are structural analogs to targeted metabolites (antimetabolites). The tINIT algorithm was able to identify 101 antimetabolites, 22 of which are already used in cancer therapies and the remaining can be considered as new potential anticancer drugs.

Synthetic lethal

The genetic lesions occurring in cancer not only promote the oncogenic state but are also associated with dependencies that are specific to these lesions and absent in non-cancer cells. Two genes are considered 'synthetic lethal' if the isolated mutation on either of them is compatible with cell viability but the simultaneous mutation is lethal [127]. Analogously, two genes are considered to interact in a 'synthetic sick' fashion, if simultaneous mutation reduces cell fitness below a certain threshold without being lethal [127].

Enzymes encoded by genes that are in synthetic lethal or sick interactions with known, non-druggable cancer-driving mutations can be potential anticancer drug targets. This approach has two main advantages: first, we can indirectly target non-druggable cancer-promoting lesions by inhibiting druggable synthetic lethal interactors and secondly we can achieve a high selectivity by exploiting true synthetic lethal interactions for anticancer therapy. This is especially remarkable in the case of cancer-specific isoenzymes, which are emerging as one of the most promising anticancer drug targets. GSMMs provide an excellent tool for the systematic simulation of specific pairs of gene knockout (KO) to unveil those combinations that compromise the viability of cancer cells (synthetic lethal). By definition, gene KO is simulated by giving value zero to gene expression and the effect of gene deletion is transferred to the metabolic reaction level by GPR association. Thus, for instance, the flux through a reaction that is associated only to one knocked-out gene would be zero. If the reaction is catalyzed by isoenzymes or complexes, the effect of a gene deletion is more complex.

However, predicting the metabolic state of a cell after a gene KO is a challenging task, because after the gene KO the system evolves into a new steady-state that tends to be as close as possible to the original steady-state [128]. To overcome these difficulties several algorithms have been developed. For example, the MOMA algorithm minimizes the euclidean norm of flux differences between metabolic states of the KO compared with the wild type [129]. The ROOM method minimizes the total number of significant flux changes from the wild type flux distribution [129].

In other words, MOMA minimizes the changes in the overall flux distribution while ROOM minimizes the number of fluxes to be modified after the gene KO (Figure 3D). As an example of employing the concept of synthetic lethality in cancer, a GSMM approach has been used to develop a genome scale network model of cancer metabolism [119]. The model predicted 52 cytostatic drug targets (40% of which were known) and further predicted combinations of synthetic lethal drug targets, which were validated using NCI-60 cancer cell collection. In a remarkable example, synthetic lethality between heme oxygenase and fumarate hydratase was predicted by the GSMM approach and was also experimentally validated [130]. The number and the quality of these predictions prove the capabilities of this approach to identify synthetic lethal pairs of genes as potential novel drug target in cancer.

Future perspective

Metabolism represents the essence of how cells interact with their environment to provide themselves with energy and the essential building blocks for life. In this review, we highlighted the role of a wide range of factors that trigger the malignant transformation of cancer metabolism as well as experimental and computa-

tional approaches to develop new therapies. Despite the encouraging achievements and improvements in cancer research, there still exist limitations that need to be overcome in order to enhance the effectiveness of drug therapies in cancer disease.

One of the major challenges in targeting key metabolic pathways is the lack of clear understanding of how the cancer cell metabolic profile varies from a non-tumor proliferating cell and the potential toxicity risk associated with targeting metabolism. A better understanding of how the metabolism differs in a specific type of cancer or within the same type may help us predict and identify targets without affecting non-tumor cells. In this context, combination of metabolic and signaling pathway inhibitors has been proposed as one of the rational approaches [131]. Using computational approaches permits the systematic simulation of gene perturbations, either metabolic and/or non-metabolic, that could contribute to unveil novel key signaling nodes resulting in potential anticancer drug targets. Recently developed algorithms, such as PROM [111], allow the integration of transcriptomic data into GSMMs while considering the gene regulatory network structure of a given organism. This approach has been developed for predicting metabolic changes that result from genetic or environmental perturbation in *Escherichia coli*. However, it is obvious that algorithms accounting for both gene regulatory and metabolic networks could be used to analyze more precisely the effect of perturbations on oncogenes in cancer metabolism.

Tumor heterogeneity represents a hurdle that must be overcome in order to develop new and more efficient anticancer therapies. One of the factors triggering intratumoral heterogeneity is the tumor microenvironment, which interferes with the ability of drugs to penetrate tumor tissue and reach the entire tumor cells in a potentially lethal concentration. In addition, heterogeneity within the tumor microenvironment leads to marked gradients in the rate of cell proliferation and to regions of hypoxia and acidity, all of which can influence the sensitivity of the tumor cells to drug treatment. Better understanding of how tumor microenvironment protects cancer cells, during and immediately after chemo- or radiotherapy is imperative to design new therapies aimed at targeting this tumor-protective niche [132,133]. The use of drug delivery systems can improve the pharmacological properties of traditional chemotherapeutics by altering pharmacokinetics and biodistribution to overcome the harsh conditions of the tumor microenvironment. Moreover, the co-administration of chemotherapeutics and tumor-associated stromal-depleting drugs helps to target the fibrous structure of the modified extracellular matrix, which can result in a less penetrable tumor microenvironment [134].

Another interesting approach considers therapies that interfere in the metabolic co-operation between cancer cells and stromal cells in their microenvironment [135] or between intratumoral subpopulations. The study of the metabolic coupling between different cellular populations as potential drug targets can be achieved by reconstructing an artificial tumor microenvironment by using GSMMs approach. To date several algorithms have been developed that integrate omic data into a GSMM reconstruction that permit to compute the secretion and uptake rates of nutrients (Table 1) and hence study the complementary secretomes within a heterogeneous cellular community. However, test and validation of a metabolic model becomes more complex if it considers a heterogeneous cellular population. Nevertheless, recent studies on artificial microbial ecosystems have demonstrated the potential of this type of approach to study synergies in heterogeneous cellular communities [136] that could be extrapolated to the study of cancer to unveil the mechanisms underlying the cooperation between tumoral and stromal cells, as well as between intratumoral subpopulations.

The intratumoral microenvironment also confers an extreme flexibility and adaptation capability to cancer cells that enhances tumor progression and represents a challenge for target-directed therapies [137]. The intratumoral heterogeneity is driven by two main processes: epithelial-to-mesenchymal transitions, by which epithelial cells gain invasive properties and lose at least part of their epithelial phenotypes [138]; and mesenchymal-to-epithelial transitions, by which mesenchymal cells can revert to an epithelial gene program displaying strong self-renewal and survival properties [138–140]. Drug targets that repress these processes have been proposed to significantly reduce tumoral progression.

Anti-angiogenic therapy has been proposed for a long time as an interesting approach to reduce tumor growth. Tumor blood vessels are surrounded by a very hostile environment, with a high amount of acidosis, low oxygen regions, weak pericyte–endothelial cell interaction, leading to its tortuous and leaky vessels with gaps that allow easy escape of invading tumor cells [141,142]. Additionally, restoring the blood vessels to a 'normal' state would get the tumor vessels back on track to its proper functional form, reducing hypoxia-induced metastasis and improving the effects of chemotherapy [143,144]. Also it is expected to reduce the spreading of cancer cells, because pericytes that are required to strengthen blood vessels would be acting more efficiently and hence prevent the intravasation of the cancer cells through the gaps found in the normally leaky tumor vessels.

Therapies based on both metastatic targets arresting cancer cells in a non-metastatic stage and angiogenic tar-

gets normalizing tumor vessels are promising strategies to design new anticancer therapies. Coupling this strategy with associated key metabolic pathways is a good approach in cancer treatment and requires computational tools to identify the putative targets. Recently developed methods, such as the 'metabolic transformation algorithm' allows the identification of the actors involved in metabolic transformations [145]. This methodology identifies targets that alter the metabolism retrieving the cells back from a given metabolic state to another metabolic state (Figure 3D). This method has been successfully used to find drug targets that revert disrupted metabolism focused on aging. However, this approach could be suitable to determine drug targets arresting tumor in a non-metastatic stage, normalize tumor vessels or prevent tumor intravasation, resulting in a reduction of tumor progression. Additionally, GSMM predictions could be refined by integrating information from dynamic ^{13}C FBA [146].

Moreover, combinatorial therapies, targeting angiogenesis and metastatic targets, have been proposed as a way to enhance anticancer therapies [27]. Traditionally, these approaches has been focused on targeting signaling pathways, such as the VEGF inhibition or VEGF receptors (R1/R2) blockade [147,148] and CXCR4 protein, which is involved in tumor colonization, or the cytokine PIGF, which prepares the metastatic niche in bone marrow for the cells invading from breast cancer [149]. However, studies on the metabolic reprogramming in endothelial cells have opened new avenues to explore the combinatorial therapies of targeting both tumors and their angiogenesis, in the context of metabolism.

The approaches reviewed here provide a guideline to improve the anticancer drug-target therapies focused on metabolic reprogramming. However, the lack of a proper model depicting the complete map of metabolic reactions, regulatory processes as well as tumor

Key term

Combinatorial therapies: Strategy that takes profit of the synergistic effects of two therapeutic treatments targeting different processes of the cellular biology.

Executive summary

Background

- Nowadays, it is widely recognized that metabolic reprogramming is essential to sustain tumor progression. These changes are promoted by genetic and epigenetic alterations producing mutations in key metabolic enzymes that modify flux distributions in metabolic networks, providing advantages to cancer cells in terms of energy production and synthesis of biomolecules.

Crosstalk between oncogenic signaling events & cancer cell metabolism

- Many key oncogenic signaling pathways, such as *HIF*, *Myc*, PI3K/AKT/mTOR or SREBPs, converge to adapt tumor cell metabolism in order to support their growth and survival. They are intimately involved in modulating glycolysis, mitochondrial oxidative phosphorylation, lipid metabolism and glutaminolysis.

Tumor microenvironment

- The tumor microenvironment is complex and comprises the extracellular matrix, tumor and stromal cells (e.g., epithelial cells, fibroblasts and inflammatory cells) that are embedded within this matrix and nourished by vascular network. The tumor heterogeneity, signaling molecules and chemicals, such as oxygen and protons, can influence tumor cell proliferation, survival, invasion, metastasis and energy metabolism reprogramming.

Isoenzymes: therapeutic targets in cancer

- Isoforms of many of the enzymes specific to important metabolic pathways are found to be overexpressed in tumor cells affecting important pathways of the energetic metabolism. These isoforms have a key role in mediating the aberrant metabolism of cancer cells and could serve as a promising source of novel drug targets.
- These tumor-specific isoforms can be involved in important pathways, such as glycolysis, tricarboxylic acid cycle, pentose phosphate pathway and glutamine metabolism, among other important energetic pathways

Genome-scale metabolic models as new tools emerging from systems biology approach to drug discovery

- Genome-scale metabolic models are emerging as a potential solution to decipher the molecular mechanisms underlying cancer in the context of systems biology. These models represent the metabolic reactions encoded by an organism's genome and summarize and codify information known about the metabolism of that organism.
- These models use constraint-based methods for the mathematical representation of biotransformations and metabolic processes occurring within the organism and offer an appropriate framework to integrate the increasing amount of 'omic' data generated by the different high-throughput technologies.
- Genome-scale metabolic models approaches have allowed to identify a number of tumor-specific biomarkers, anticancer drug-target and synthetic lethal genes opening a promising avenue in the development of new anticancer therapies.

heterogeneity and synergistic cooperation between cellular communities, makes selecting the best possible target combinations difficult. Thus, in order to develop more efficient anticancer therapies, more efforts need to be made in developing new methods to study tumor metabolism and obtain a better understanding of the molecular processes underlying tumor progression and invasion.

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