System Biology, Metabolomics, and Breast Cancer: Where We Are and What Are the Possible Consequences on the Clinical Setting

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Abstract The discovery that the metabolism of cancer cells is different from non-malignant cells is not new, this finding was described more than a century ago by O. Warburg. Nevertheless, in the last decade the technologies such as capillary electrophoresis, mass spectroscopy (MS), and proton nuclear magnetic resonance spectroscopy (H-NMR) have allowed deciphering the complexity and heterogeneity underlying the cancer metabolism. These high-performance technologies are generating a large amount of data that requires conceptual schemes and approaches to efficiently extract and physiologically interpret the dynamic spectrum of the metabolome data in cancer samples. Breast cancer is a disease that highlights the need to develop computational schemes to systematically explore the metabolic alterations that support the malignant phenotype in human cells. Hence, systems biology approaches with capacities to integrate in silico modeling and high-throughput data are very attractive for clinicians to make oncological treatment decisions combined with static parameters such as clinical and histopathological variables. In this chapter we present a cutting-edge review, perspectives and scope of how metabolic approaches in breast cancer studies can be used not only to

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integrate the local and systemic response of the host, but also as a technique to look for metabolic biomarkers by non-invasive and simpler sample procedure in biofluids such as serum, saliva, urine, pleural fluid, breath, and ascites. We discuss how the "metabolic phenotype" approach could contribute to developing a personalized medicine by combining metabolome data and computational modeling to evaluate some clinical variables such as detection of relapses, monitoring and response prediction to treatment and toxicity prediction in patients. Even though some advances have been accomplished, in practice there are many challenges and limits that will have to be broken before the metabolomics can be integrated into the day-to-day clinic. Despite this situation, it is evident that the translational multidisciplinary approach combined with the rapid technological development and the correct data interpretation will bring in the future tools for improving outcomes in the clinical area.

Keywords Metabolism in cancer · Systems biology · Genome-scale metabolic reconstructions · Metabolome · Precision medicine

Abbreviations

ABC	Advanced breast cancer
AUC	Area under curve
AUROC	Area under the receiver operating characteristic curve
CE-TOF/MS	Capillary electrophoresis time-of-flight mass spectrometry
CE	Capillary electrophoresis
EBC	Early breast cancer
GC-MS	Gas chromatography-mass spectroscopy
HER2	Human epidermal growth factor receptor 2
H-NMR	Proton nuclear magnetic resonance spectroscopy
HR-MAS	High resolution magic angle spinning magnetic resonance spectrometry
LBC	Localized breast cancer
LC-MS	Liquid chromatography-mass spectroscopy
LTNBC	Localized triple negative breast cancer
LTPBC	Localized triple positive breast cancer
MS	Mass spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
no pCR	No pathologic complete response
OPLS-DA	Orthogonal least-squares discriminant analysis
OS	Overall survival
pCR	Pathologic complete response
PLS-DA	Partial least squares discriminant analysis
TNBC	Triple negative breast cancer
TNMc	Clinical tumor/nodes/metastasis

TNMp	Pathological tumor/nodes/metastasis
TPBC	Triple positive breast cancer
TT	Treatment toxicity
TTP	Time to disease progression

1 Introduction: A Brief Survey of Metabolic Alterations in Cancer

Metabolic alteration is a hallmark in cancer cells whose characterization and understanding have crucial implications to develop optimized drug design and identify feasible biomarkers to improve the diagnosis and prognosis of the disease. The most well-known metabolic alteration in cancer is the Warburg effect, which represents an aerobic glycolysis in cancer cells given by an excessive consumption of glucose and high rates of lactate production even at high concentrations of oxygen available in the microenvironment [1]. Besides glycolysis, glutaminolysis is another main pillar of tumor cells for energy production and an important source of nitrogen for proteins [2]. Glutamine is an abundant metabolite in blood and its consumption and degradation play an essential role to replenish a variety of metabolic functions and consequently, its control has been suggested as a potential metabolic therapeutical strategy again the uncontrolled proliferation in cancer [3]. For instance, glutamine is a fundamental piece in metabolism being responsible for many cellular functions, like nitrogen donor for purines, pyrimidines, and non-essential amino acids necessary for proliferation, also required in the generation of antioxidants to remove reactive oxygen species, as well as carbon donor for fatty acids synthesis [4]. Moreover, it is an indispensable piece in cell signaling, apoptosis, and drug resistance, as well as epithelial-mesenchymal transition, a considerable step in metastasis [5]. Furthermore, glutaminolysis contributes to tumor growth in two distinct but connected ways, by promoting cell proliferation and inhibiting cell death. Overall, both of these metabolic mechanisms, Warburg effect and glutaminolysis, are paired in cancer cells for completing each other the metabolic requirements for biomass production in cancer tissues [6, 7].

Despite these findings have been a milestone in cancer biology, currently there is evidence that these effects can be the tip of the iceberg, and new complex metabolic phenotypes can emerge due at least by two main factors: the genetic heterogeneity in cancer evolution and the specific physiological conditions prevailing in human tissues [8]. Thus, the understanding of how metabolic alterations emerge in different types of cancer and how these can be associated with clinical variables for diagnosis and prognosis is an active field that promises the development of precision medicine based on the personalized description of the genetic background and clinical history of the patients. In order to move toward this last direction, there will be a more close and permanent communication between clinical knowledge in oncology, data obtained from high-throughput technologies and systems biology

schemes to interpret them. Notably, significative advances have been achieved toward the integrative description of metabolome technologies and the development of computational schemes to integrate and interpret data. Thus, recent advances in technologies such as capillary electrophoresis (CE), mass spectroscopy (MS), and proton nuclear magnetic resonance spectroscopy (H-NMR) have allowed to characterize and differentiate the metabolic phenotype between patients with and without cancer by monitoring invasive and non-invasive clinical samples such as biopsies, urine, saliva, and plasma [9]. On the other hand, genome-scale metabolic reconstructions and constraint-based modeling have been a proper paradigm in systems biology to integrate high-throughput data, explore the metabolic phenotype, and predict metabolic targets to reduce proliferation in cancer cells and explore other strategies in human complex diseases. In this chapter, we present a deep discussion of the advances, challenges, and perspectives of how systems biology and metabolome technology is an appealing strategy to understand the metabolic alterations in clinical stages for breast cancer, a disease with a strong impact at worldwide scale. Given the different academic backgrounds between physicians and computational scientists, one of our purposes is to build a line of communication between both areas. To this end we discuss recent clinical applications of metabolome and systems biology in the study of breast cancer. With this idea in mind, we organize the chapter as follows. Section 2 is devoted to present some clinical applications of metabolome data to understand the metabolic alterations in breast cancer. In Sect. 3 we present the main ideas and principles that drive modeling in systems biology, mainly genome scale metabolic reconstructions. Here we highlight the need of mathematical and computational formalisms to get three aims: (1) integrate and interpret high-throughput data from a systemic point of view, (2) construct a framework for systematically building biological hypotheses; and (3) rational design of experiments to reduce the proliferative progress and malignancy of cancer cells. Finally, the last section is focused to analyze and evaluate the current limitations and challenges that we need to face in order to persuade and implement future systems biology schemes in the health-care sector.

2 Clinical Applications of Metabolome: Studies in Breast Cancer

For many years patients with breast cancer were treated with the concept that one treatment would fit to all. Treatment criteria were based only on anatomical and pathologic variables such as tumor, nodes, metastases (TNMc (clinical)/TNMp (pathologic) stage), negative versus positive nodes, and hormone receptors status. However, even though these variables are extremely relevant to determine a patient diagnosis, the overwhelming heterogeneity of cancer has made evident that the drug effectiveness depends on a set of factors such as the genetic context, treatment tolerance, toxicity, HER2 overexpression, and patient metabolism. In the last years

after the omics revolution, the discovery of new biomarkers, receptors, and intracellular mechanisms have allowed to treat in a personalized way and consequently benefiting the patients with the concept of "less is better." The correct selection of the optimal treatment allows to have the most effective and less toxic treatment. Personalized medicine from the therapeutic point of view is defined as a model that uses molecular profiling technologies to tailoring the correct treatment for the right person at the right time [10]. Breast cancer is one of the best cancer examples in which high-throughput technologies have transferred genetic knowledge into clinical practice through the association of different biological subtypes with differentiated prognostic or treatment response [11]. Altered metabolism is a hallmark to sustain the malignancy in cancer given that cancer cells simultaneously rewire their metabolism to increase the production of biomass, and induce genetic profiles that modify the checkpoint mechanism of the cell cycle. Uncovering and understanding the molecular mechanism by which metabolism, cell cycle, and microenvironment link together have important implications to explore the physiological responses that can emerge inside the tumor and design strategies for optimal outcome in the clinical field. The metabolic approach in breast cancer is attractive given that the genomic and transcriptional approach cannot analyze the dynamic metabolites that could be a key determinant of many malignant and non-malignant pivotal cell pathways [12]. Another practical advantage for the clinician and patient is the easier sample taking and the goodness of obtaining multiple samples during the disease evolution. The breast cancer clinical scenarios where the metabolomic approaches have been applied are summarized in Table 1.

2.1 Screening/Early Diagnosis

Despite human life expectancy has increased in the last century, the frequency of chronic diseases, such as cancer, have increased due a variety of factors that include the diet, the environmental and unhealthy lifestyles. Cancer is and will be a common disease that will confront resource-limited health systems; derived from this there is a great interest in screening susceptible population to detect in a preclinical manner which would make the possible cancer cure or reduce treatment costs. Cancer diagnosis in preclinical stages increases the possibilities of cure and evidently has a less expense on health systems. Like any screening maneuver, there is a possibility of damage finding false results (positives or negatives) or giving overtreatment to tumors that do not impact the patient survival. That is why the interest in developing fine diagnostic tools with great accuracy is a constant in cancer screening. The metabolome is theoretically envisaged as a great screening tool because of easiness in obtaining samples and their predictive accuracy. There are several studies in which it has been possible to demonstrate that the metabolic alterations differ markedly between malignant and non-malignant proliferative cells [13].

Table 1 Clinical applications of metabolome: studies in breast cancer

Clinical Scenario	Author/n	Study design	Sample type	Outcome(s)
Screening/early diagnosis	Slupsky et al./$n = 38/67$	Cases/controls	Urine	$R^2 = 0.75 Q^2 = 0.57$
	F-P7 METAcancer consortium/$n = 289/15$	Cases/controls	Breast cancer tissue/breast non-malignant tissue	
Staging	Oatman et al./EBC $n = 44$ ABC $n = 51$	Prospective cohort	Pre- and post-surgery serum	Sensitivity 75% Specificity 69% Accuracy 72%
	Jobard et al./validation cohort EBC $n = 61$ ABC $n = 51$	Prospective cohort	Pre-surgery and pre-chemotherapy serum	AUC 0.91 Sensitivity 78.2–95.6% Specificity 67.3–87.7%
	Memorial Sloan Kettering Cancer Center Biobank LBC $n = 61$ ABC $n = 51$	Prospective cohort	LBC pre-surgery and post-surgery ABC serum	Accuracy 84–87% ER negative Relapse AUC 0.82, sensitivity 82%, specificity 72% Accuracy 75%
	Hadi et al./$n = 152/155$	Cases/controls	Serum Cases pre-surgery	Sensitivity 96% specificity 100%
Classification by biological subtypes	Borgan et al./$n = 46$	Retrospective cohort	Breast cancer tissue	3 luminal subgroups identification ($p = 0.001$)
	Cao et al./$n = 75$ LTNBC LTPBC	Retrospective cohort	Breast cancer tissue	Accuracy 77% ($p = 0.001$)
Predictive markers/neoadjuvant context	Wei et al./$n = 28$ LBC	Prospective cohort	Serum	pCR vs no pCR Discrimination for no pCR 80% AUROC 0.72
Prediction/early detection of toxicity	COMET project (consortium for Metabonomic toxicology)	Murine model	Serum and urine	Sensitivity 41% specificity 77–100%
Measurement of residual disease	Asiago et al./EBC $n = 56$	Prospective cohort	Serum pre-surgery	Relapse prediction 13 months earlier than clinical methods in 55% of relapsed patients
	Oakman et al./$n = 44$ EBC	Prospective cohort	Pre- and post-surgery serum samples	Preoperative LBC Sensitivity 75%, specificity 69% predictive accuracy 72%

Table 1 (continued)

Clinical Scenario	Author/n	Study design	Sample type	Outcome(s)
Prognosis/treatment response prediction in ABC	Tenori et al. $n = 579$ ABC	Prospective cohort	Subgroup analysis HER2 positive	TTP accuracy 89.6% OS predictive accuracy 78%

EBC early breast cancer, *LBC* localized breast cancer, *ABC* advanced breast cancer, *LTNBC* localized triple negative breast cancer, *LTPBC* localized triple positive breast cancer, *pCR* pathologic complete response, *no pCR* no pathologic complete response

With the purpose to move toward early diagnosis methods studies have been carried out on gastric, prostate, and pancreatic cancer [14–16]. For instance, Slupsky et al. collected urine samples in breast and ovarian cancer patients at different cTNM stages and in healthy women. They described 67 metabolites from 300 selected, 80% matched in the spectrum and could be dissected to the two study cohorts; women with cancer versus healthy controls [17]. Furthermore, the European FP7 METAcancer consortium analyzed 300 patients with breast tumor and non-malignant breast tissue. As a result of this study, there were identified 600 metabolites as complex lipids, primary metabolites, and unidentified metabolic signals. It was possible to identify significant differences in a heat map between no-malignant breast tissue and breast cancer. Notably, 15 non-malignant breast tissue and 289 breast cancer tissue were compared and the metabolites between the two groups were different with very few outliers [18, 19].

In a recent publication by Zhou et al., H-NMR spectroscopy was performed on urine and serum samples from patients with cancer and healthy controls; using orthogonal least-squares discriminant analysis (OPLS-DA) allowed to discriminate a metabolic profile for cancer. Nine serum metabolites and 3 metabolites in urine were significantly different between the two groups. In OPLS-DA score plots of serum sample there were significant biochemical differences between the two groups ($R^2X = 0.39$ y $Q^2 = 0.75$) [20].

2.2 Staging

The classic canons of the cancer staging are based on anatomical data (tumor size, node infiltration, and distant metastasis); however, it has been proven over time that this classification is not always associated with the prognosis or treatment benefit prediction because there are other variables in the equation. Thus, there are metabolomic studies in renal and bladder cancer which have demonstrated that the metabolomic profiles can differentiate between localized and advanced disease. In breast cancer, Oakman et al. [21] showed significant differences in the metabolic profiles between localized and advanced disease in 44 early breast cancer patients

in whom serum was collected pre- and post-surgery and in serum of 51 advanced breast cancer. The prediction of this metabolic profile for the advanced disease had a sensitivity of 75%, specificity of 69%, and predictive accuracy of 72%. On the other hand, Jobard et al. [22] described a metabolic fingerprint for metastatic disease in women with localized breast cancer and advanced disease, in the training cohort they used 46 preoperative samples in localized disease and 39 samples in advanced disease. In the validation cohort 61 samples in localized disease and 51 in metastatic disease showing a high power of discrimination between both entities. In the Memorial Sloan Kettering Cancer Center (MSK) biobank, the metabolic fingerprint was compared in 90 patients with advanced breast disease and 80 patients with localized breast disease. Patients with localized disease had a basal sample and another post-surgery. Clinical follow-up was 5 years and a training and validation cohort was included in the study. A high predictive accuracy was demonstrated between 84 and 87% to distinguish both entities and in localized disease where a risk score for relapse was generated. This score was compared with conventional clinical risk factors for relapse in ER negative disease and the metabolomic risk score was obtained in the validation cohort AUC 0.82, 82% sensitivity, 72% specificity, and 75% predictive accuracy [23]. Hadi et al. in the serum of 152 pre-surgery breast cancer patients and 155 controls compared by GC-MS (Gas chromatography-mass spectrometry) the metabolic profile followed by chemometric analysis. Partial Least Squares Discriminant Analysis (PLS-DA) model identified significant differences between the groups, sensitivity 96% and specificity of 100% on external validation. An increased lipogenesis and production of volatile organic metabolites indicates key metabolic alteration in breast cancer patients [24].

2.3   Classification by Biological Subtypes

Since the metabolomics approach has the ability to capture the entire picture of host–tumor interaction, it represents an attractive variable for their biological classification. Thus, Borgan et al. analyzed 46 biopsies of breast cancer patients with a combined transcriptomic (RNA extraction and microarray analysis) and metabolomic approach (high resolution magic angle spinning magnetic resonance spectroscopy) [25]. In 31 tumors classified as luminal A using unsupervised hierarchical clustering, three luminal subgroups A1, A2, A3 were identified. The major metabolites differed significantly between each of the profiles ($p = <0.001$) including alpha-glucose, beta-glucose amino acids, myo-inositol, and alanine. In the A2 subgroup were those samples with low glucose levels and high levels of alanine and lactate which results in a greater Warburg effect.

In addition, metabolome studies have contributed to distinguishing different subgroups in same cancer. For instance, the European FP7 METAcancer consortium has reported metabolic heterogeneity in breast cancer biological subtypes (hormone receptor positive and negative tumors) and between histological grade differenti-

ation (grade 1–2 versus grade 3) [11, 12]. Furthermore, Cao et al. characterized the metabolic profiles of triple negative breast cancer (TNBC) and hormonal positive plus HER2 positive breast cancer called triple positive breast cancer (TPBC positive). In order to explore the metabolic activity, high resolution magic angle spinning (HR MAS) magnetic resonance spectroscopy (MRS) was performed in 75 located infiltrating breast cancer tissues. Multivariate partial least squares discriminant analysis (PLS-DA) modeling and relative metabolite quantification were used. The PLS-DA showed high accuracy to separate TNBC and TPBC (77.7%, $p = 0.001$). The concentration of choline was higher in TNBC than TPBC ($p = 0.008$, $q = 0.041$), TNBC lower level of Glutamine ($p = <0.001$, $q = 0.001$) and higher Glutamate ($p = 0.002$, $q = 0.015$) compared to TPBC. In discrimination between HER2 positive and HER2 negative by PLS-DA CV accuracy was 69.1% ($p = <0.001$). High Glycine was found in HER2 positive ($p = 0.002$, $q = 0.012$) [26]. Even though these results represent good news for characterizing the malignant phenotype through some key metabolites, it is needed to study its clinical implications in the prognosis of the disease.

It has been previously described that breast cancer requires glutamine for cell proliferation, in TNBC cell lines has recently been reported that the expression of a Mucin 1 (MUC1). MUC1 is a glycoprotein that causes alterations in metabolic pathways involved in tumor growth. Goode et al. observed a cellular dependence of glutamine with MUC1 expression which facilitates metabolic reprogramming to use glutamine and generate chemoresistance. MUC1 overexpression significantly increased glucose and glutamine uptake in MDA-MB-231 cells and MUC1 knock-down reduced glucose and glutamine uptake in MDA-MB-468 cells [27].

2.4 Predictive Markers/Neoadjuvant Context

The context of neoadjuvant treatment in breast cancer is for the clinician the perfect scenario to test biologically sustainable hypotheses for the search of prognostic or predictive markers that allow treating each patient in a personalized and precise way [28]. Undoubtedly, this enterprise has strong impact in identifying early markers of response to treatment in the understanding of taking better and more dynamic therapeutic decisions. Even though studies evaluating metabolomics in the neoadjuvant context of breast cancer treatment are very scarce, some breakthroughs have been reported. For example, Wei et al. described in serum samples the metabolic profile of 28 patients with non-metastatic breast cancer who received sequential neoadjuvant chemotherapy (Epirubicin 990 mg/m^2 + Cyclophosphamide 600 mg/m^2, 4 three weekly cycles) followed by taxane (Docetaxel 100 mg/m^2, 4 three weekly cycles) [29]. In HER2 positive tumors, trastuzumab was added. They reported a complete pathological response ($n = 8$), partial response ($n = 14$), and no response ($n = 6$) to chemotherapy. A prediction model with LC-MS and NMR was used. Threonine, Isoleucine, Glutamine from NMR and linolenic acid from LC-

MS were significantly different ($p = <0.05$) and ($p = <0.01$), respectively, between the pathological response groups. Combining NMR and LC-MS analysis had the capacity to discriminate up to 80% of the patients who would not have a complete pathological response to neoadjuvant chemotherapy (AUROC = 0.72).

2.5 Prediction or Early Detection of Toxicity

For many years it has been known that the toxicity derived from the oncological treatment varies from patient to patient. Because of this fact, there is a need to identify metabolic biomarkers that help us to select the optimal treatment with the adequate doses for each patient. With this purposes in mind, the COMET project (Consortium for Metabonomic Toxicology) has studied murine models and organ-specific toxicity (liver, kidney) in urine and serum samples with NMR-based technology that has shown that the metabolomic signature can have a sensitivity of 41% and specificity of 77–100%, which is a practical tool for toxicity screening [30].

2.6 Measurement of Residual Disease

Breast cancer, even when clinically appears to be localized to the breast and axillary nodes, is a systemic disease at the time of diagnosis (micrometastatic disease). Notably, the metabolomic profile would allow distinguishing patients with the micrometastatic disease who are at increased risk of relapse. Asiago et al. analyzed serum samples of women with early breast cancer in surveillance following surgical resection to test if metabolomic profile could be used to predict relapse. 257 samples from 56 breast cancer patients, 20 patients relapsed and 36 had no relapse evidence. With a follow-up of at least 2 years Combination NMR (nuclear magnetic resonance spectroscopy) and MS (mass spectroscopy) found a relevant metabolites panel to discriminate relapse versus no relapse; the metabolites predict the relapse 13 months earlier than clinical methods did in 55% of relapsed patients [29].

In another study, Oakman et al. in forty-four early breast cancer patients with pre- and postoperative serum samples analyzed a metabolomic fingerprint by NMR, fifty-one metastatic breast cancer patients were used as control group. This fingerprint was contrasted with the Adjuvantonline calculator for relapse risk measurement. Preoperative localized breast cancer was identified (75% sensitivity, 69% specificity, and 72% predictive accuracy). The comparison with Adjuvantonline was discordant, high risk by Adjuvantonline, pre- and post-metabolomic were 21, 10, and 6 respectively [19].

2.7 Prognosis/Prediction of Treatment Response in Advanced Breast Cancer

579 patients with metastatic breast cancer were randomized to receive pacli-taxel + lapatinib or placebo. Serum metabolomic profiles were analyzed using NMR spectroscopy. The outcomes were time to progression (TTP), overall survival (OS), and treatment toxicity (TT). In a subgroup analysis in HER2-positive treated with paclitaxel + lapatinib, metabolomic profiles (in the upper and lower thirds of dataset) showed significant differences for TTP ($n = 22$, predictive accuracy = 89.6%) and OS ($n = 16$, predictive accuracy = 78%) [31]. This work illustrates that the metabolomic profile can be used to predict the response and prognosis in a specific type of anti-HER2 target drug (lapatinib) in a specific subgroup of advanced breast cancer.

In summary, metabolome technology applied in cancer clearly opens a window with opportunities to develop new methods of diagnosis and prognosis in breast cancer with potential benefits to improve the outcomes of the treatments applied to patients. However, in order to reach this practice in clinical stages, yet there are challenges that should be overcome such as the proper biochemical interpretation and the biological implications of metabolome measurements. Thus, having obtained the data, a set of practical questions emerge such as what is the activity of the metabolic network associated with the measurements, what are their biological implications, and how this knowledge will be useful in diverse clinical scenarios. These questions define one frontier in personalized medicine and in order to build possible responses one should build computational schemes and methods capable to analyze the data by taking into account the complex nature of the human metabolic networks through a systems biology perspective. Given the inherent complexity of the disease, understanding the mechanisms by which metabolism supports cancer overcomes our intuition and in this context systems biology schemes supply with computational frameworks to integrate the high-throughput data and build testable hypothesis in a systematic and systemic fashion.

The next section is devoted to discussing a paradigm in systems biology that has the capacities to contribute to tackling the previous questions by the simultaneous combination between metabolome data and genome-scale metabolic reconstructions.

3 Systems Biology. Integrative Schemes Between Computational Modeling and High-Throughput Data

With the advent of high-throughput technologies the possibility to extend our molecular knowledge of how human tissues maintain its functional capacities has been significantly extended. Thus "omics" technologies have contributed to uncovering a set of genes, proteins, or metabolites that simultaneously change together for

sustaining the physiological states that we observe in multifactorial diseases such as cancer. Thus, combining these technologies important breakthroughs have been reported to uncover the genetic and epigenetic mechanisms in a variety of cancer and distinguish their metabolic alterations in cancer cell lines and biopsies on human tissues. However, the integrative description and biological interpretation of high-throughput data are not easy and in order to complete this task we should develop computational algorithms that let us to integrate, interpret, and hypothesize the underlying biological mechanisms in the system of study, this latter point being a central aim in Systems Biology [32]. Systems biology deals with the development of computational methods to explore the relationship between the genotype and the phenotype, and old question in biology, but now taking into account that living systems are composed by biological entities such as genes, proteins, and metabolites that interact simultaneously to sustain the phenotype. In the more fundamental essence, the principle in this science is based on the fact that we are a system, it means our body is integrated by a variety of components whose integration and interaction induce states of wellness and disease such as cancer, in other words, seen the physiological states as emergent properties. Thus, in the case of metabolism a variety of breakthroughs have been reported to describe the phenotypic behavior in microorganisms and human tissues in cancer [33]. Even though there is no consensus on defining systems biology, we can highlight three properties in the field: (1) an integrative scheme to integrate and interpret high-throughput data; (2) a mathematical framework to explore and model biological networks, and (3) a computational framework to build testable hypothesis for associating the genotype and phenotype relationship. Thus, systems biology has become a proper description to analyze and explore the biological mechanisms that guide a complex disease such as cancer. An interesting field in this area has been the analysis of the metabolic alterations that guide the phenotype in cancer [6, 34–36]. As we have described in previous sections, metabolic alterations are a hallmark of cancer whose description is currently an active line of research to design new strategies in the therapeutic field [37]. In the next section, we present a mathematical framework to link the metabolome data and the genome-scale metabolic reconstruction, a powerful tool to analyze the metabolic phenotype in cancer [33].

3.1 K-cone: A Mathematical Scheme to Explore the Phenotype from Metabolome Data

Living organisms orchestrate their functions through the constant activity of biochemical reactions, these required to evolve and survive under external environments. With the publication of the human metabolic reconstruction and the high-throughput technologies, the association between the metabolic pathways and the emergence of a disease have been an appealing activity in basic biomedical research and potentially in translational medicine. In this scenery, the development of computational methods for analyzing and interpreting the metabolic activity of a

sample starting from their metabolome profile is one goal in systems biology. In this section, we present one computational scheme that contributes this latter point, how to interpret the different metabolic phenotypes obtained from two or more samples associated with different physiological states. As we will see, the method can be useful to identify the metabolic alterations in cancer studies by taking into account genome-scale metabolic reconstructions and metabolome profile of the samples. The scheme is based on the consideration that we have a metabolic reconstruction defined by a certain number of metabolic reactions, see Fig. 1. Without loss of generality, the dynamical behavior of the metabolites conforming the metabolic network is entirely described by

$$\frac{dx}{dt} = S \cdot v \tag{1}$$

where S is the stoichiometric matrix, x is the vector that contains the concentrations of the metabolites included in the metabolic reconstruction, and v represents the vector with the metabolic fluxes. Then, by considering that the metabolic fluxes for each transformation are ruled by the mass action law, it means the metabolic flux is the rate constant of the reaction multiplied by the concentrations of the substrates

$$v_i = k_i \prod_j^m x^{S_{i,j}} \tag{2}$$

By substituting Eq. (2) into Eq. (1) it is possible to show that Eq. (1) at the steady state condition is written as:

$$S \cdot \Gamma \cdot k = 0 \tag{3}$$

where Γ is a diagonal matrix whose entries contain the mass action terms for the flux of each metabolic reaction (mass action matrix) and k is a vector with all the rate constants.

$$\Gamma = \begin{pmatrix} \prod_j^m x^{S_{i,j}} & 0 & \dots 0 \\ 0 & \prod_j^m x^{S_{i,j}} & \dots 0 \\ 0 & 0 \dots 0 & \prod_j^m x^{S_{i,j}} \end{pmatrix} \quad k = \begin{pmatrix} k_1 \\ \dots \\ k_n \end{pmatrix} \tag{4}$$

This equation now defines a space for all feasible kinetic constants in the steady state, the k-cone. Hence, by assuming that the metabolic reactions follow a mass action law, Eq. (1) at the steady-state condition allows us to write reduced expression. Notably, Eq. (3) is built by three components: the stoichiometric matrix which contains the specific metabolic capacities in the organisms; the matrix Γ which depends only on the concentrations of the metabolites, and the vector k which represents the set of parameters associated with the rate constant for each metabolic reaction. Thus, having measured the metabolome profiles associated with the concentrations of metabolites in a metabolic reconstruction, the solution of
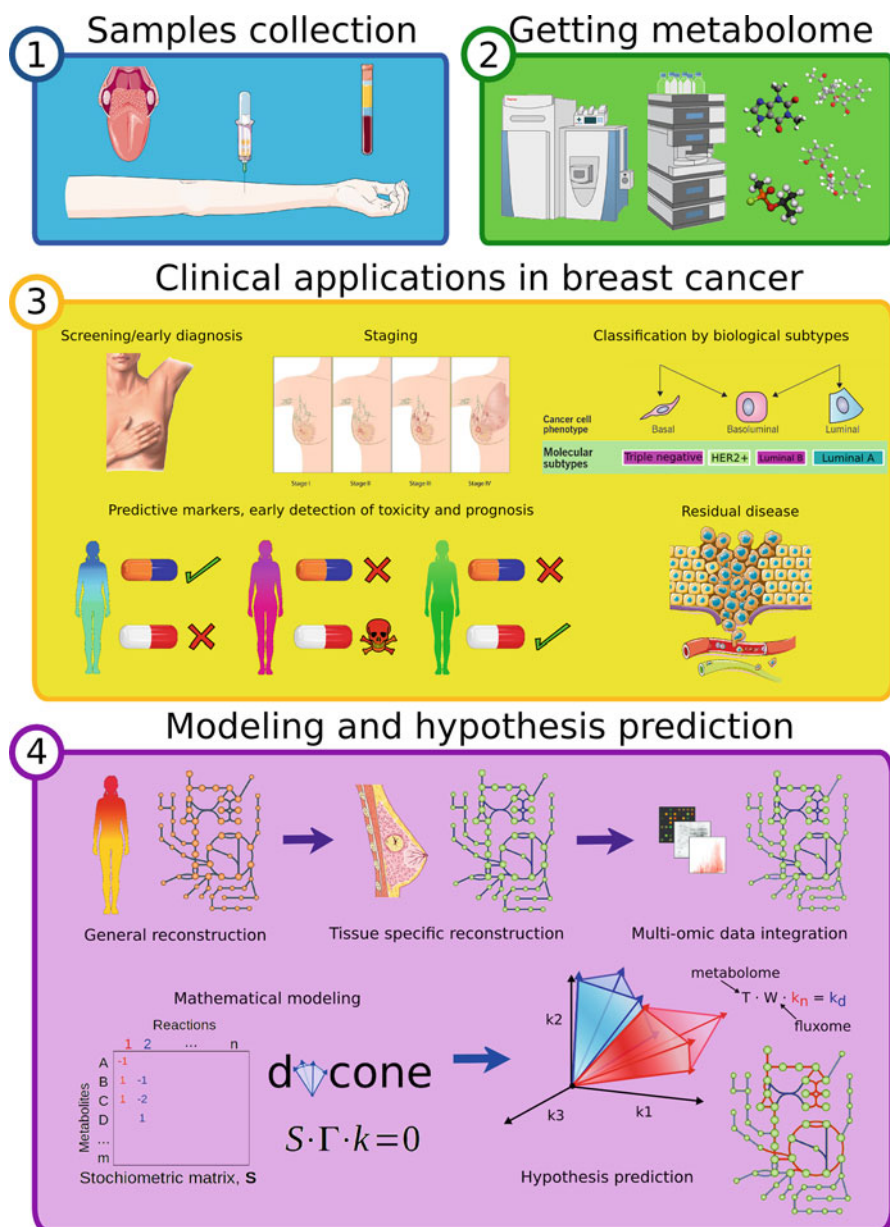
Fig. 1 A possible pipeline to establish the relationship between clinic and metabolome data through the pass of four levels. (**a**) The collection of biofluids (urine, blood, plasma, saliva, etc.) is a central step to obtain the profile of concentrations of metabolites associated with the phenotype among different biological conditions and patients. (**b**) The metaboloma analysis of the samples through different techniques such as NMR and CE-TOF/MS. (**c**) Clinical studies and classification of the samples to store and evaluate metabolome data in prevention, detection, diagnosis, and prognosis. For prediction of a new hypothesis, one of the new approaches is systems biology, specifically mathematical modeling. In this chapter, we describe a specific algorithm called k-cone, developed to analyze differences in metabolic fluxes of metabolic networks in different conditions. Panel (**d**) is depicted the implementation of computational model that contributes to the classification and interpretation of the data at a genome-scale for each patient

Eq. (3) supplies with information about the numerical range of the rate constants that ensure that at steady state the system will reach the metabolic concentrations experimentally measured. These numerical intervals for the kinetic parameters are useful because contribute to define the feasible metabolic space of the reconstructed network. The characterization of the metabolic phenotype is entirely specified by Eq. (3) whose solution, in general, is not unique but it falls into a space of possible solutions, the *k-cone* space [38, 39], given by the null space of the matrix resulted by $S \cdot M$, see Fig. 1. In general, as the metabolic reconstruction increases in number of reactions, the solution of Eq. (3) is not unique and as the feasible space increases, it represents the variety of metabolic mechanisms by which the network can ensure a steady-state condition where the metabolites concentrations are the ones measured experimentally. As shown in Fig. 1, this space of solutions is visually represented as cones in the space of kinetic rate constants. Notably, this method supplies with a pipeline to represent the metabolic capacities of a network based on a set of metabolome measurements, and consequently, a corollary of the method is the study of metabolic alterations that can guide the metabolic phenotype between two or more samples [38, 40]. For instance, we focus our method on the analysis of two physiological samples such as a cancer cell line and control cell line. In this case, the profiles associated with each metabolic phenotype are mathematically specified as follows:

$$S \cdot M_n \cdot k_n = 0$$
$$S \cdot M_d \cdot k_d = 0 \tag{5}$$

The solutions of the set of equations shown in (5) allow us to tacked a set of questions that uncover the potential metabolic alterations that distinguish each physiological condition. As shown in Fig. 1, the feasible space for each condition has some differences, this indicates the metabolic alteration in kinetic parameters required to ensure each physiological condition. In general terms, both *k-cone* spaces are different and both can be seen as a linear transformation from one to the other. Thus, writing Eq. (1) in vector notation for each set of metabolome data, the kinetic feasible space in each case can be written as follows:

$$k_d = M_d^{-1} \cdot V_d \tag{6}$$

which can be rewritten as follows:

$$
\begin{aligned}
k_d &= M_d^{-1} \cdot \operatorname{diag}\left(\frac{V_d}{V_n}\right) \cdot V_n \\
&= M_d^{-1} \cdot \operatorname{diag}\left(\frac{V_d}{V_n}\right) \cdot M_n \cdot M_n^{-1} \cdot V_n \\
&= M_d^{-1} \cdot M_n \cdot \operatorname{diag}\left(\frac{V_d}{V_n}\right) \cdot M_n^{-1} \cdot V_n \\
&= M_d^{-1} \cdot M_n \cdot \operatorname{diag}\left(\frac{V_d}{V_n}\right) \cdot k_n
\end{aligned}
$$

Concluding that each point in *k-cone* from disease space can be transformed to the other *k-cone* space:

$$k_d = T \cdot W \cdot k_n \tag{7}$$

with

$$T = M_d^{-1} \cdot M_n$$
$$W = \mathrm{diag}\left(\frac{V_d}{V_n}\right)$$

Equation (7) quantifies the metabolic transformation in the kinetic parameters that guide the metabolic profiles measured experimentally in both conditions, interestingly the difference between the normal and the disease state of the entire space of steady-state kinetic parameters is completely defined by the mass-action terms which can be obtained from metabolome data. The feasible space of kinetic parameters, associated with normal and disease samples, help us to identify differentiated enzymatic activity between both physiological states. This method recently has been applied to explore the metabolic alterations between HaCat and the cancer cell line HeLa, the most studied cell line for cervix cancer [36]. Applying the method to the metabolome data obtained for HeLa cancer cell and HaCat independently, it has been possible to uncover the metabolic differences that guide the malignancy in HeLa with respect to a control system. By selecting the log2-fold between the average of the range for each kinetic parameter, this study concludes that among the enzymes with significative alterations are phosphofructokinase, phosphoglycerate mutase, pyruvate kinase, 6-phosphogluconate dehydrogenase, pyruvate dehydrogenase, and aconitase. More importantly, these findings are in agreement with previous reports [41–45]. In addition to these predictions obtained from our formalism, it has capacities to identify global changes in classical pathways between the comparative analysis of both physiological samples. For instance, the analysis with *k-cone* predicts a strong dysregulation of TCA cycle enzyme activities as well as increased ATP usage and lactate export in HeLa cells, all consistent with the Warburg effect [46]. The set of necessary regulations for proliferation consisted of the up-regulation of four glycolytic enzymes, the up-regulation of ATP synthesis, and the increased export of lactate. Thus, the Warburg effect in HeLa cells seems to be a consequence of maintaining a high proliferation rate. In general, the strongest regulation was observed for phosphofructokinase, which showed an 8-fold increase in enzyme activity in HeLa compared to the HaCaT cell line. Because phosphofructokinase is allosterically regulated by ATP, citrate, and pH, this regulation is consistent with the observed lower concentrations of ATP, citrate, and lactate.

Overall, this first study suggests that *k-cone* analysis can contribute to building hypothesis around the regulatory and metabolic alterations that can support a malignant phenotype such as cancer from metabolome profiles. In the case of cancer cell lines, this method has proven to be a proper description to identify metabolic alterations between control cells and cancer cell lines, this latter point is a valuable goal to design metabolic strategies to control the malignant phenotype in cancer.

Even though this approach represents an important breakthrough in modeling metabolism, an immediate application of the method into samples more complex such biopsies will claim for overcome some challenges such as the heterogeneity of the samples, the physiological interpretation of the metabolome data, and the experimental assessment for the application in precision medicine.

4 Metabolome and Cancer: Critics, Perspectives, and Challenges in the Clinical Application

The advances in breast cancer prognosis and treatment in the last decades have been just to recognize different biological phenotypes and it has allowed in the present and in a near promising future to develop personalized and precision medicine with a great clinical impact. Theoretically, the metabolic approach is a great idea for clinicians who make oncological treatment decisions based on static parameters (clinical and histopathological variables without considering the interaction between tumor and host). However, some challenges should be overcome before metabolome technologies can be utilized in the clinical practice. (Among these challenges we can list the following: small cohorts, number, time and type of sampling, definition lack of a reference standard, complex algorithms to interpret the daunting amount of data, variability and reproducibility, external validation, expensive and sophisticated technology not accessible around the whole world, a clear superiority demonstration with regard to clinical-pathological parameters or complementarity to other approaches like circulating tumor cells or identification of tumor DNA in liquid biopsies, etc.) but the metabolic approach allows to integrate the local and systemic response of the host (interaction between tumor and host). From the clinical perspective, the metabolomic approach is extremely attractive not only for obtaining a non-invasive and simpler sample (serum, saliva, urine, pleural fluid, breath, ascites, etc.) but it allows what other technologies have not been able so far, make possible integrate the local and systemic response of the host. In particular, the characterization of the breast cancer "metabolic phenotype" approach could contribute to an even more personalized and precise treatment which would allow having fewer supra or infra-treated patients and toxicity saving. This aim is not an easy task and will require to evaluate if some fundamental metabolic organizing principles observed in more simple organisms could contribute to understand how cancer acquires robustness under drug action [47–49]. Overall, many challenges and limits will have to be broken before the metabolomics can be integrated into the day-to-day clinic but it is evident that the translational multidisciplinary approach combined with the rapid technological development and the correct dates interpretation will in the future allow the metabolomics applications for precision medicine [50].

References

1. Resendis-Antonio O, González-Torres C, Jaime-Muñoz G, Hernandez-Patiño CE, Salgado-Muñoz CF (2015) Modeling metabolism: a window toward a comprehensive interpretation of networks in cancer. Semin Cancer Biol 30:79–87
2. Rajagopalan KN, DeBerardinis RJ (2011) Role of glutamine in cancer: therapeutic and imaging implications. J Nucl Med 52:1005–1008
3. Wise DR, Thompson CB (2010) Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci 35:427–433
4. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB (2008) Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev 18:54–61
5. Yang L, Venneti S, Nagrath D (2017) Glutaminolysis: a hallmark of cancer metabolism. Annu Rev Biomed Eng 19:163–194
6. Resendis-Antonio O, Checa A, Encarnación S (2010) Modeling core metabolism in cancer cells: surveying the topology underlying the Warburg effect. PLoS One 5:e12383
7. Hernández Patiño CE, Jaime-Muñoz G, Resendis-Antonio O (2012) Systems biology of cancer: moving toward the integrative study of the metabolic alterations in cancer cells. Front Physiol 3:481
8. McGranahan N, Swanton C (2017) Clonal heterogeneity and tumor evolution: past, present, and the future. Cell 168:613–628
9. Ishikawa S, Sugimoto M, Kitabatake K, Sugano A, Nakamura M, Kaneko M et al (2016) Identification of salivary metabolomic biomarkers for oral cancer screening. Sci Rep 6:31520
10. Ocana A, Pandiella A (2010) Personalized therapies in the cancer "omics" era. Mol Cancer 9:202
11. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61–70
12. Claudino WM, Goncalves PH, di Leo A, Philip PA, Sarkar FH (2012) Metabolomics in cancer: a bench-to-bedside inter\ion. Crit Rev Oncol Hematol 84:1–7
13. Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: cancer's achilles' heel. Cancer Cell 13:472–482
14. Wu H, Xue R, Tang Z, Deng C, Liu T, Zeng H et al (2010) Metabolomic investigation of gastric cancer tissue using gas chromatography/mass spectrometry. Anal Bioanal Chem 396:1385–1395
15. Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J et al (2009) Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature 457:910–914
16. OuYang D, Xu J, Huang H, Chen Z (2011) Metabolomic profiling of serum from human pancreatic cancer patients using 1H NMR spectroscopy and principal component analysis. Appl Biochem Biotechnol 165:148–154
17. Slupsky CM, Steed H, Wells TH, Dabbs K, Schepansky A, Capstick V et al (2010) Urine metabolite analysis offers potential early diagnosis of ovarian and breast cancers. Clin Cancer Res 16:5835–5841
18. Denkert C, Bucher E, Hilvo M, Salek R, Orešič M, Griffin J et al (2012) Metabolomics of human breast cancer: new approaches for tumor typing and biomarker discovery. Genome Med 4:37
19. METAcancer: home [Internet]. [cited 10 Jul 2017]. http://www.METACANCER-fp7.eu

20. Zhou J, Wang Y, Zhang X (2017) Metabonomics studies on serum and urine of patients with breast cancer using ^{1}H-NMR spectroscopy. Oncotarget. https://doi.org/10.18632/oncotarget.16210

21. Oakman C, Tenori L, Claudino WM, Cappadona S, Nepi S, Battaglia A et al (2011) Identification of a serum-detectable metabolomic fingerprint potentially correlated with the presence of micrometastatic disease in early breast cancer patients at varying risks of disease relapse by traditional prognostic methods. Ann Oncol 22:1295–1301

22. Jobard E, Pontoizeau C, Blaise BJ, Bachelot T, Elena-Herrmann B, Trédan OA (2014) Serum nuclear magnetic resonance-based metabolomic signature of advanced metastatic human breast cancer. Cancer Lett 343:33–41

23. Tenori L, Oakman C, Morris PG, Gralka E, Turner N, Cappadona S et al (2015) Serum metabolomic profiles evaluated after surgery may identify patients with oestrogen receptor negative early breast cancer at increased risk of disease recurrence. Results from a retrospective study. Mol Oncol 9:128–139

24. Hadi NI, Jamal Q, Iqbal A, Shaikh F, Somroo S, Musharraf SG (2017) Serum Metabolomic profiles for breast cancer diagnosis, grading and staging by gas chromatography-mass spectrometry. Sci Rep 7(1):1715. https://doi.org/10.1038/s41598-017-01924-9

25. Borgan E, Sitter B, Lingjærde OC, Johnsen H, Lundgren S, Bathen TF et al (2010) Merging transcriptomics and metabolomics–advances in breast cancer profiling. BMC Cancer 10:628

26. Cao MD, Lamichhane S, Lundgren S, Bofin A, Fjøsne H, Giskeødegård GF et al (2014) Metabolic characterization of triple negative breast cancer. BMC Cancer 14:941

27. Goode G, Gunda V, Chaika NV, Purohit V, Yu F, Singh PK (2017) MUC1 facilitates metabolomic reprogramming in triple-negative breast cancer. PLoS One 12:e0176820

28. Damia G, Broggini M, Marsoni S, Venturini S, Generali D (2011) New omics information for clinical trial utility in the primary setting. J Natl Cancer Inst Monogr 2011:128–133

29. Wei S, Liu L, Zhang J, Bowers J, Gowda GAN, Seeger H et al (2013) Metabolomics approach for predicting response to neoadjuvant chemotherapy for breast cancer. Mol Oncol 7:297–307

30. Ebbels TMD, Keun HC, Beckonert OP, Bollard ME, Lindon JC, Holmes E et al (2007) Prediction and classification of drug toxicity using probabilistic modeling of temporal metabolic data: the consortium on metabonomic toxicology screening approach. J Proteome Res 6:4407–4422

31. Tenori L, Oakman C, Claudino WM, Bernini P, Cappadona S, Nepi S et al (2012) Exploration of serum metabolomic profiles and outcomes in women with metastatic breast cancer: a pilot study. Mol Oncol 6:437–444

32. Ebrahim A, Brunk E, Tan J, O'Brien EJ, Kim D, Szubin R et al (2016) Multi-omic data integration enables discovery of hidden biological regularities. Nat Commun 7:13091

33. Zielinski DC, Jamshidi N, Corbett AJ, Bordbar A, Thomas A, Palsson BO (2017) Systems biology analysis of drivers underlying hallmarks of cancer cell metabolism. Sci Rep 7:41241

34. Bordbar A, Yurkovich JT, Paglia G, Rolfsson O, Sigurjónsson ÓE, Palsson BO (2017) Elucidating dynamic metabolic physiology through network integration of quantitative time-course metabolomics. Sci Rep 7:46249

35. Yurkovich JT, Yang L, Palsson BO (2017) Biomarkers are used to predict quantitative metabolite concentration profiles in human red blood cells. PLoS Comput Biol 13:e1005424

36. Diener C, Muñoz-Gonzalez F, Encarnación S, Resendis-Antonio O (2016) The space of enzyme regulation in HeLa cells can be inferred from its intracellular metabolome. Sci Rep 6:28415

37. Locasale JW, Vander Heiden MG, Cantley LC (2010) Rewiring of glycolysis in cancer cell metabolism. Cell Cycle 9:4253–4253

38. Famili I, Mahadevan R, Palsson BO (2005) K-cone analysis: determining all candidate values for kinetic parameters on a network scale. Biophys J 88:1616–1625

39. López-Moyado IF, Resendis-Antonio O (2013) Dynamic metabolic networks, k-cone. In: Encyclopedia of system biology. Springer, New York, pp 624–629

40. Resendis-Antonio O (2009) Filling kinetic gaps: dynamic modeling of metabolism where detailed kinetic information is lacking. PLoS One 4:e4967

41. Yi W, Clark PM, Mason DE, Keenan MC, Hill C, Goddard WA et al (2012) Phosphofructoki-nase 1 glycosylation regulates cell growth and metabolism. Science 337:975–980
42. Webb BA, Forouhar F, Szu F-E, Seetharaman J, Tong L, Barber DL (2015) Structures of human phosphofructokinase-1 and atomic basis of cancer-associated mutations. Nature 523:111–114
43. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R et al (2008) The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 452:230–233
44. Chan B, VanderLaan PA, Sukhatme VP (2013) 6-Phosphogluconate dehydrogenase regulates tumor cell migration in vitro by regulating receptor tyrosine kinase c-met. Biochem Biophys Res Commun 439:247–251
45. Vander Heiden MG, Locasale JW, Swanson KD, Sharfi H, Heffron GJ, Amador-Noguez D et al (2010) Evidence for an alternative glycolytic pathway in rapidly proliferating cells. Science 329:1492–1499
46. Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11:85–95
47. Zepeda-Mendoza ML, Resendis-Antonio O (2013) Hierarchical agglomerative clustering. In: Encyclopedia of systems biology. Springer, New York, pp 886–887
48. Resendis-Antonio O, Hernández M, Mora Y, Encarnación S (2012) Functional modules, struc-tural topology, and optimal activity in metabolic networks. PLoS Comput Biol 8:e1002720
49. Kitano H (2004) Cancer as a robust system: implications for anticancer therapy. Nat Rev Cancer 4:227–235
50. Diener C, Resendis-Antonio O (2016) Personalized prediction of proliferation rates and metabolic liabilities in cancer biopsies. Front Physiol 7:644