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Metabolic Pathways as Targets for Drug Screening

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1. Introduction

In the drug development process, candidate compounds are first screened for desirable biological properties such as effects on gene expression, signal transduction, or enzyme activity. The genetic and metabolic pathways used in the readouts are known as targets of the drug screening process. Despite advances in molecular targeting, proteomics and metabolomics, drug screening with molecular or metabolic targets have not produced the results that meet the need of the pharmaceutical industry in the selection of small molecules leads/targets for clinical testing. The relative lack of success in applying the -omics in drug screening is partly due to the inability of the –omics to account for metabolic regulation, a property of the cellular metabolic network. More recently, tracer-based metabolomics has been developed as an experimental approach for the study of cellular metabolic networks. Interconversion of metabolites are measured in terms of "extreme pathways" of the metabolic network which can be used for drug screening purposes. In this paper, these approaches for drug screening targeting genetic pathways (transcriptomics), biochemical pathways (metabolomics and fluxomics) and 'extreme pathways" (tracer-based metabolomics) are compared. The advantages and limitations of these approaches for metabolic research and drug screening are discussed.

2. Genetic/signaling pathways as targets for drug screening

In the days of the genomic era, scientists are eager to apply the knowledge of genomics and the advances in genetic/molecular engineering in clinical and translational research. The general concept is that a genetic signal acts as an on-off switch in controlling metabolic processes. However, in order to successfully apply genetic pathways (gene switches) for drug screening, one has to establish genotype-phenotype correlation. The generally accepted dogma of genotype-phenotype correlation is that metabolism is the final expression of the genetic information, and peptide molecules act as signaling switches for

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the regulation of metabolism. This popular molecular genetic approach to drug screening is based on the assumption that the effect of drugs on metabolism and metabolic regulation is determined by gene transcription and translation alone.

The rationale for choosing gene switches as targets for drug screening can be illustrated by the example of the action of the tumor suppressor gene (P53) in cancer metabolism. Cancer cells have metabolic characteristics that are distinct from normal cells in that there is an overall increased macromolecular syntheses to sustain cell growth and proliferation. These metabolic characteristics are generally grouped under the Warburg effect which consists of increased anaerobic glycolysis, decreased glucose oxidation and increased glutamine utilization (1). A representation of the model of gene switches is depicted in Figure 1. The signals that orchestrate these metabolic changes originate from the balance between oncogenes (growth promoting factors) that turn on signaling pathways regulating the utilization of substrates for growth and tumor suppressor genes such as P53 that modulate energy utilization. The loss of a cancer suppressor gene or the over-expression of an oncogene may be sufficient to generate genetic signals to switch on or off (or modulate) metabolic pathways resulting in the cancer cell metabolic phenotype. The interaction between molecular pathways and metabolic pathways in cancer has recently been reviewed (1). At the molecular level, P53 regulates transcription of genes that modulate PI3K, Akt and mTOR pathways (growth promoting pathways) to reduce cancer growth. Excessive growth induces expression of P53 in cells keeping cell growth and cell death in balance. Independently, P53 inhibits glucose uptake, ribose synthesis and glycolysis thus modulating cellular metabolism. When the action of P53 is lost due to mutation, cells take up more glucose for ribose synthesis and glycolysis, the key elements of the Warburg effect. The fact that the actions of P53 can be used to explain the cancer metabolic phenotype suggests that any signaling pathway that interacts with P53 is a potential target for anticancer drug screening.

The use of genetic pathways for the understanding of metabolism and drug screening has its limitations. The interactions among signaling pathways are often based on demonstrations using artificial overexpression or underexpression of these pathways. The real actions of these signaling pathways in normal physiology are not exactly known. The quantitative relationship connecting gene expression to metabolism has not been worked out. Therefore, the genetic switch hypothesis is only one possible explanation for the expression of the cancer metabolic phenotype. Conceptual limitations of genetic switches in the understanding of metabolisms or the metabolic effect of drugs have been noted by D. E. Koshland Jr (2) almost half a century ago. He pointed out that overproduction or underproduction of enzymes by molecular manipulation may sometimes have dramatic effects on an organism and other times with only minor effects. The overall effect of genetic manipulation on cellular metabolism cannot always be predicted. The lack of observable effect when an enzyme concentration is changed is analogous to the "silent" phenotypes (3) of the carrier states of many recessive diseases when enzyme or protein concentrations of the affected genes can be substantially reduced.

Discrepancies in genotype phenotype correlation between signaling pathways and metabolism when it occurs may be explained by our incomplete knowledge of the feedback regulation of the signaling pathways as well as metabolic regulations of cellular metabolism. However, the lack of genotype-phenotype correlation in many cases can be attributed to conceptual difficulties of using genetic switches to the understand metabolism. First, metabolic regulation is rarely an "all-or-none" type of control. According to metabolic

control analysis, the regulation of metabolic pathway is distributed over many enzymes of the biochemical reaction. Transcriptional or post-translational modification of an enzyme potentially changes its Km and/or Vmax of the reaction. However, the change in Km or Vmax of one enzyme may be compensated by either a change in precursor substrate concentration or by a shift in the locus of control of the reaction to other enzymes such that net flux remains unchanged. Secondly, the model of metabolic switches does not take into account how the change in one metabolic pathway may impact on many other pathways that are connected by shared substrates or co-factors and vice versa. The lack of quantitative relationship between genotype and phenotype is the Achille's heel of the gene switching hypothesis† and the use of genetic pathways for drug screening.

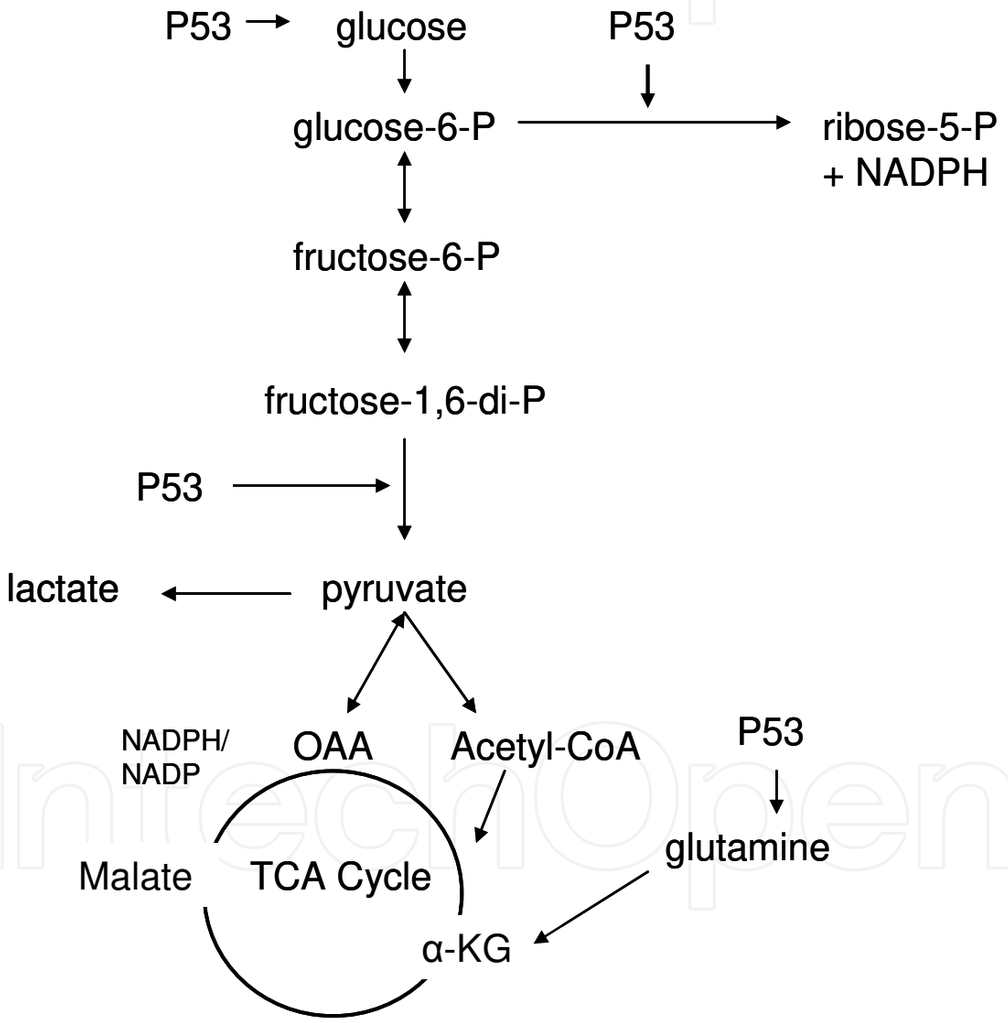

Fig. 1. A system of glucose metabolic pathways in a traditional format. The biochemical pathways potentially affected by P53 as a gene-switch are indicated.

†Gene expression can be quantitatively determined using RTPCR method. Results are reported in folds of change. Even though there may be a correlation between the fold of change and the observed metabolic effect, the correlation is not a quantitative one.

3. Biochemical pathways as targets for drug screening

In the past decades, metabolite profiling (metabolomics) and fluxomics have been developed to fill in the knowledge gaps of gene regulations of metabolism. Thus genomics, transcriptomics, metabolomics and fluxomics are the popular –omics of systems biology‡. The advances in mass spectrometry and nuclear magnetic resonance spectroscopy have enabled the new industry of metabolomics. These technologies provide quantitative and qualitative analyses of organic compounds in biological fluids and specimens. Quantitation of metabolites at different time points is the basis of fluxomics. Research in metabolite profiling and fluxomics is based on our understanding of metabolic control analysis (MCA). MCA provides quantitative measures of degree of influence of a change in enzyme kinetics or a change in substrate concentration can affect the consumption or production of a metabolite in terms of metabolic control coefficients (5, 6). Thus, measurements of substrate concentrations by metabolite profiling and flux analyses allow detail information regarding metabolic changes when the system is perturbed by drug treatment. In metabolomics and fluxomics, traditional biochemical pathways can be considered as targets for drug candidate screening. Such screening has the limitation in that the effect on the metabolic system as a whole is not evaluated in the screening process. Technically, current metabolomics technologies do not permit characterization of substrate concentrations at the subcellular level and reactions that are compartmentalized cannot be properly evaluated. The sampling processes usually do not separate the contribution from background environment such as the culture medium or neighboring cells to the metabolic processes of the cell. For these reasons, metabolomics has not been successfully used as targets for drug screening.

Measurements of flux (fluxomics) depend on the use of isotopes (7). Since ^{13}C labeled isotopes can be distributed widely among many metabolites, and not all of these metabolites can be measured in the same analytical method, there are always fewer data than needed to give precise quantification of flux. Nevertheless, this approach has its appeal in that once a mathematical model is constructed, literature values can be fitted into the model to give insight into possible changes in the system, and whether the model is robust or not can be tested (8). Such an approach was used by Selivanov et al. to model of the pathways of pentose phosphate cycle. The interconnection of these pathways is shown in Figure 2 (9). After incubating cells with [1, 2 $^{13}C_2$]-glucose, ribose was found to be labeled in many carbon positions. Using mass isotopomer distribution in ribose and known sugar phosphate concentrations and Km values of enzymes from the literature, these authors were able to simulate the fluxes of the pentose phosphate pathways. They were able to identify three reactions among other transketolase mediated reactions that were significantly inhibited when cells were treated with oxythiamine, a tranketolase inhibitor. These are xylulose-5p to glyceraldehyde-3-P, sedoheptulose-7-p to ribulose-5-P and xyluose-5-P to sedoheptulose-7-P (reactions 14, 15 and 13 in figure 2). The differences in response among tranketolase enzymes inhibited by oxythiamine are the consequence of stoichiometric constraints.

‡Systems biology as commonly defined is the enumeration of a collection of biologically related objects (genomics, proteomics and metabolomics) or characteristics (transcriptomics and fluxomics) within the boundary of a cell. However, in actuality the context of a cellular boundary i.e. how these objects or characteristics separate the cell from its environment is often absent in the definition of these systems (4).

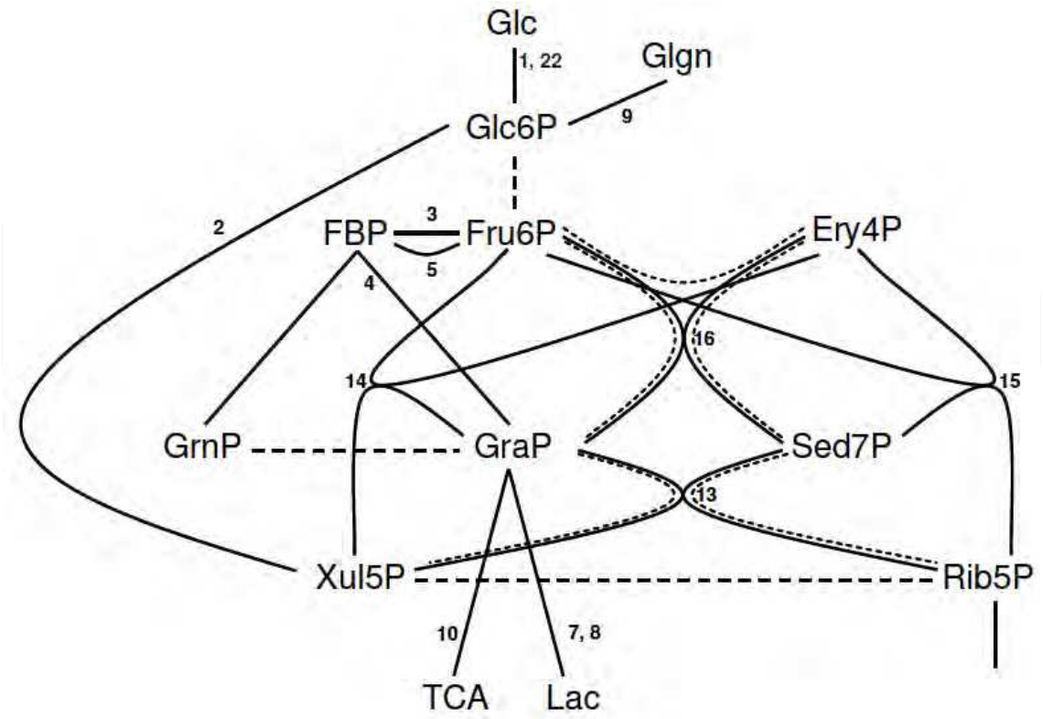

Fig. 2. A model of pentose phosphate cycle used for carbon tracing and flux analysis. Using experimentally determined isotopomer distribution in ribose and lactate, the fluxes of the numbered reactions can be calculated (from reference 9 with permission).

The fluxomic approach targeting traditional biochemical reactions provides more specific information regarding the metabolic system than metabolite profiling (metabolomics). The use of fluxomics allows the simultaneous assessment of the effect of a drug on multiple metabolic pathways and permits a better understanding of metabolism than the gene-targeting approach. However, in order to take into account futile cycling or stoichiometric constraints, stable isotope tracing (carbon tracing) is required as illustrated in the above example. Even though it is possible to construct a complex model for mammalian metabolic networks to take into account of futile cycles and stoichiometric constraints, such a model requires a very large data set and extensive programming. In the best case scenario, there is never sufficient data for solving all the parameters of the system and the results are model dependent and are difficult to verify for practical reasons (2). Nonetheless, the fluxomics approach definitely provides better correlation with phenotype than the gene switch targeting approach.

4. "Extreme pathways" and metabolic network

The "extreme pathways" of a metabolic network can also be used as targets for drug screening. "Extreme pathways" are elements of the well known constraint-based modeling (8) which has been applied to the study of cellular homeostasis. (The definition of "extreme pathway" is given in the next section.) Living organisms (cells) are metabolic systems (networks) continuously exchanging energy substrates with their environments to maintain the biological systems in a homeostatic state. The main metabolic function of a cell is to

utilize substrates from its environment to produce energy and building material for the synthesis of macromolecules. Excess intermediates are returned to the surrounding environment to maintain a relatively constant internal environment. The boundaries of metabolic activities represented by "extreme pathways" within which the cell functions define the homeostatic state (10, 11). These boundaries are the result of constraints by the stoichiometry of competing reactions, synchronization of shared pathways and/or intermediates, and balance of energy production and utilization.

The role of "extreme pathways" in the maintenance of homeostasis can be illustrated by the example of glucose metabolism via the TCA cycle. Pyruvate from glycolysis is metabolized via pyruvate carboxylation leading to the conservation of 3-carbon species or pyruvate decarboxylation leading to production of 2-carbon species (via acetyl-CoA) and energy production (beta-oxidation and tricarboxylic acid (TCA) cycle) (16). These two processes are concurrent in cells and the activity of one pathway constrains the activity of the other. For a given homeostatic state, the observed utilization of pyruvate via these pathways is the optimal[§] pyruvate utilization and can be represented by a vector in the pyruvate phenotypic phase plane. The operation of the TCA cycle is an example of metabolic constraint due to synchronization of shared pathways or intermediates. A full turn of the TCA cycle oxidizes a mole of acetate into two moles of carbon dioxide with production of reducing equivalents and/or high energy phosphates. At the same time each of the TCA cycle intermediate may have its respective substrate cycle such as the malate cycle and the citrate lyase cycle. These individual substrate cycles perform separate metabolic functions in conveying reducing equivalents (malate shuttle) and acetyl-CoA (citrate lyase cycle) from the mitochondria to the cytosol. The operations of these cycles are usually synchronized for efficiency. When there is a lack of synchrony of these cycle, abnormal substrate and energy balance can result and a loss of homeostasis in the cell occurs. The imbalance of energy metabolism in the mitochondria due to imbalance of substrate cycles is a frequent cause for reactive oxygen species generation and apoptosis. Changes in these boundaries consisting of "extreme pathways" are sensitive to metabolic or therapeutic perturbations and are excellent markers of therapeutic effects.

The differnces between a metabolic network and a traditional biochemical reaction model can best be shown by representing a metabolic network as an engineering system. The working of such a system is illustrated in figure 3 in which pathways shown in Figure 2 are represented as belts and wheels connecting glycolytic/gluconeogenic substrates to those of the pentose cycle. The enzymes that drive the belts are indicated and the role of energy production and utilization are included. Figure 2 is model of pentose cycle intermediates linked by enzymatic reaction. The fluxes of these reactions can be modeled mathematically using a set of ordinary differential equations. Figure 3 shows the production and consumption of different classes of compounds connected to the production and consumption of ATP and reducing equivalents. These models are conceptually different. The input-output model of cellular homeostasis of tracer-based metabolomics can account for stoichiometric constraints and synchronization of substrate cycles thus overcoming limitations of the previous approaches in metabolic studies.

[§]Optimality is sometimes thought of as a teleological concept. The optimal metabolic function of a cell is not for its purpose to survive, but is defined by the internal organization of the metabolic network.

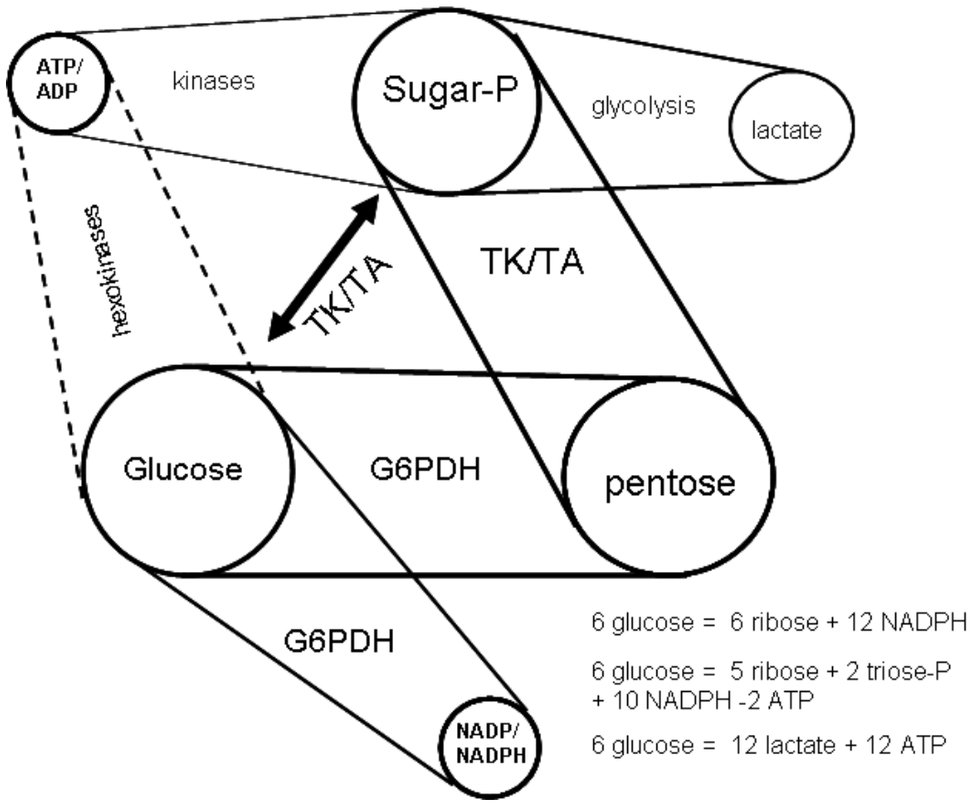

Fig. 3. An engineering model of the system of reactions depicted in Figure 1 and Figure 2. The relationship among the different substrate pools is represented by different circles. Stoichiometric relationships are provided by the mass balance equations. The productions of these metabolites from one another are indicated by respective drive belts. Energy substrate consumption and production is also included in the model. The metabolic network and its function is shown as a factory production model with sources and sinks of the raw materials and products.

The basic concept and tracer methodology of tracer-based metabolomics have been reviewed (4, 10-12). A key feature that distinguishes tracer-based metabolomics from metabolite profiling (metabolomics) and fluxomics is the inclusion of a system boundary that permits input-output analysis and a balance of flux** model in which substrate input is link to its output (products) by "extreme pathways" (12, 13, 14). "Extreme pathways" are pathways that elements (carbon, oxygen and nitrogen) from compounds (precursors) introduced into the system travel over to the final products. The basic elements of "extreme pathways" form the axes of a high dimension phenotypic space, any two of these axes forms a phenotypic phase plane and the line of optimality which is a vector within the space (or a plane) representing the metabolic phenotype. The relationship among any three "extreme

**A balance of flux analysis requires a steady state or quasi-steady state assumption. For most cellular processes involving cell growth and division, these processes are slow relative to the experimental study period and quasi-steady state of metabolic reactions may be safely assumed. However, in biological processes that are fast such as muscle contraction, or nerve conduction the balance of flux model cannot be applied and a dynamic model is required.

pathways" can be described by isoclines (15). Therefore, tracer-based metabolomics offers a graphical representation of a quantitative metabolic phenotype not available by gene-switching model or fluxomic model.

Instead of measuring substrate fluxes over specific biochemical reactions, the experimental model focuses on fluxes over "extreme-pathways" which are pathways linking the precursor substrates to the specific products. There may be many "extreme pathways" for the formation of a product depending on how many interconnecting reactions between precursor and end-product. In the synthesis of glutamic acid from glucose, there are at least two "extreme pathways" namely the pyruvate carboxylase and pyruvate dehydrogenase reactions (4). Pentose is synthesized from glucose at least through two "extreme pathways" either by oxidative (G6PDH) or non-oxidative (TK/TA) pathways (10).

5. Measuring "extreme pathways" – Carbon tracing in tracer-based metabolomics

A unique feature of tracer-based metabolomics as the name implies is the application of stable isotope labeled metabolites and mass spectrometry or magnetic resonance (NMR) spectroscopy. The ^{13}C or ^{2}H label from the labeled substrate is distributed into metabolic intermediates in specific positions according to the "extreme pathways". Tables 1a and 1b show some of the examples of labeling in amino acids, glycogen, ribose and lactate from uniformly labeled glucose [$U^{13}C_6$]-glucose (carbon tracing from glucose) (16-24). The tables show the potential mass isotopomers that can be generated, the positions that are labeled in the products, and the corresponding glucose carbon that the ^{13}C originates. For example, three mass isotopomers (M3, M2 and M1 can be found in alanine or lactate from an experiment with [$U^{13}C_6$]-glucose. M3 of alanine comes from glycolysis of glucose. The ^{13}C in carbon 1 of alanine comes from carbon 3 of glucose (G3). There are two M2 isotopomers with ^{13}C on either C3 and C2 or C2 and C1 of alanine. The sources of ^{13}C's are from glucose carbon 2 (G2) and carbon 1 (G1). X represents a ^{12}C carbon originated from exchange at the level of the TCA cycle. The mass isotopomers and positions with ^{13}C label in these glucose metabolic intermediates are indicated. These tables can be used as a guide to design tracer studies or interpret results from such studies. The mass and position isotopomers in these metabolites represent individual "extreme pathways" from glucose carbon to the respective products. It should be noted that some of the isotopomers are products of the same "extreme pathways" thus providing redundancy in the information on the "extreme pathways". In the example of labeled glucose forming labeled amino acids (Table 1a), we can gain insight into the simultaneous reactions of pyruvate carboxylation, pyruvate dehydrogenase, malate cycle, gluconeogenic cycle relative to TCA cycle flux. When the distribution of isotopomers is determined using mass spectrometry or NMR, we can use these isotopomer ratios to construct phenotypic phase planes (11, 15). Such a database of mass isotopomers can easily be managed with computational algorithm (subroutines) which can compare distances between individual phenotypes on different phenotypic phase planes. Phenotypic differences can also be quantitatively compared using isocline analysis. Thus, tracer-based metabolomics is a quantitative experimental approach to the study of metabolism and metabolic regulation.

Pathway	Isoto-pomer	Alanine C3-C2-C1	Serine C3-C2-C1	Glycine C2-C1	Asparate C4-C3-C2-C1	Glutamate C5-C4-C3-C2-C1
Glycolysis Plus TCA Cycle	M5	---	---	---	---	G2-G1-G1-G2-G3[@]
	M4	---	---	---	G2-G1-G1-G2[@]	G2-G1-G1-G2-X; G2-G1-G1-G1-X
	M3	G1-G2-G3	G1-G2-G3	---	X-G1-G2-G3	
	M2	G2-G1-X X-G2-G1	G2-G1-X	G2-G1	X-G1-G2-X G2-G1-X-X	G2-G1-X-X-X
	M1	G1-X-X X-G1-X X-X-G1	X-X-G1	---	G1-X-X-X, X-G1-X-X X-X-G1-X	X-X-G1-X-X X-X-X-G1-X X-X-X-X-G1
1-Carbon Metabolism	M3	---	G1-G2-G3	---	---	---
	M2	---	G2-G3	G2-G3	---	---
	M1	---	G3	G3	---	---

The orientations of the amino acid molecules are shown in the top row. Mass isotopomers are designated as M1 to M5 indicating the number of ^{13}C per molecule of the amino acid. The corresponding position of glucose carbon within the amino acid is designated as G1 to G3. The glucose molecule is symmetrical around C3-C4. In the table, G1-G2-G3 is the same as G6-G5-G4, if these positions are labeled equally. X represents ^{12}C from exchange within the TCA cycle.
[@] When glucose enrichment is high, there is a likelihood of labeled OAA condensing with labeled acetyl-CoA resulting in M5 α–ketoglutarate and subsequently M5 glutamate and M4 aspartate.

Table 1a. Examples of Position and Mass Isotopomer Distribution in Gluconeogenic Amino Acids from [$U^{13}C_6$]-glucose (16-24)

Pathway	Isotopomer	Glycogen C6-C5-C4-C3-C2-C1	Ribose C5-C4-C3-C2-C1	Lactate C3-C2-C1
Hexose-P cycles	M6	G6-G5-G4-G3-G2-G1	---	---
Glycolysis Glycolysis Plus TCA Cycle	M3	G6-G5-G4-X-X-X X-X-X-G3-G2-G1		G1-G2-G3
	M2	G6-G5-X-X-X-X X-X-X-G3-G2-X		G2-G1-X X-G2-G1
	M1	G1-X-X X-X-X X-G1-X-X-X-X X-X-G1-X-X-X		G1-X-X X-G1-X X-X-G1
Oxidative	M5	Glycogen glucose are labeled as in glycolysis/ gluconeogenesis shown above	G6-G5-G4-G3-G2	
Non-oxidative	M4		G3-G2-X-G3-G2	
Oxidative plus Non-oxidative	M3		G3-G2-X-X-G3	
	M2		X-X-X-G3-G2 G3-G2-X-X-X	
	M1		G3	

The orientations of the glycogen, ribose and lactate molecules are shown in the top row. Mass isotopomers are designated as M1 to M6 indicating the number of ^{13}C per molecule of the amino acid. The corresponding position of glucose carbon within the glycogen, ribose and lactate is designated as G1 to G6. The glucose molecule is symmetrical around C3-C4. In the table, G1-G2-G3 is the same as G6-G5-G4, if these positions are labeled equally. X represents ^{12}C from exchange within the TCA cycle.

Table 1b. Examples of Position and Mass Isotopomer Distribution in Glycogen, Ribose and Lactate from [$U^{13}C_6$]-glucose (16-24)

6. "Extreme pathways" as targets for drug screening

The application of phenotypic phase plane (PPP) analysis of balance of flux data from tracer-based metabolomics is a graphical way of presenting experimental data that is unique to tracer-based metabolomics. Since metabolic phenotype of a cell is characterized by the pattern of its utilization of substrates, the phenotype of a cell is represented by the input/output characteristics which can be measured as fluxes through the "extreme pathways". Any two of these "extreme pathways" can form a phenotypic phase plane. The metabolic phenotype of a cell is given by a vector in the plane and the vector divides the plane into two regions representing regions of relative excess and relative deficiency of substrate utilization (4). The use of phenotypic phase plane analysis together with isocline analysis allows quantitative comparison of different treatment effects. An example of the use of isocline analysis is illustrated by the study of effects of fructose and glutamine on the glycolytic/gluconeogenic pathways (11).

Other applications of phenotypic phase plane analysis to investigate metabolic mechanisms of a therapeutic intervention are illustrated in Figure 4. Panel (i) of the figure shows the metabolic response of a cell to two therapeutic interventions (e.g. drugs, receptor inhibitor,

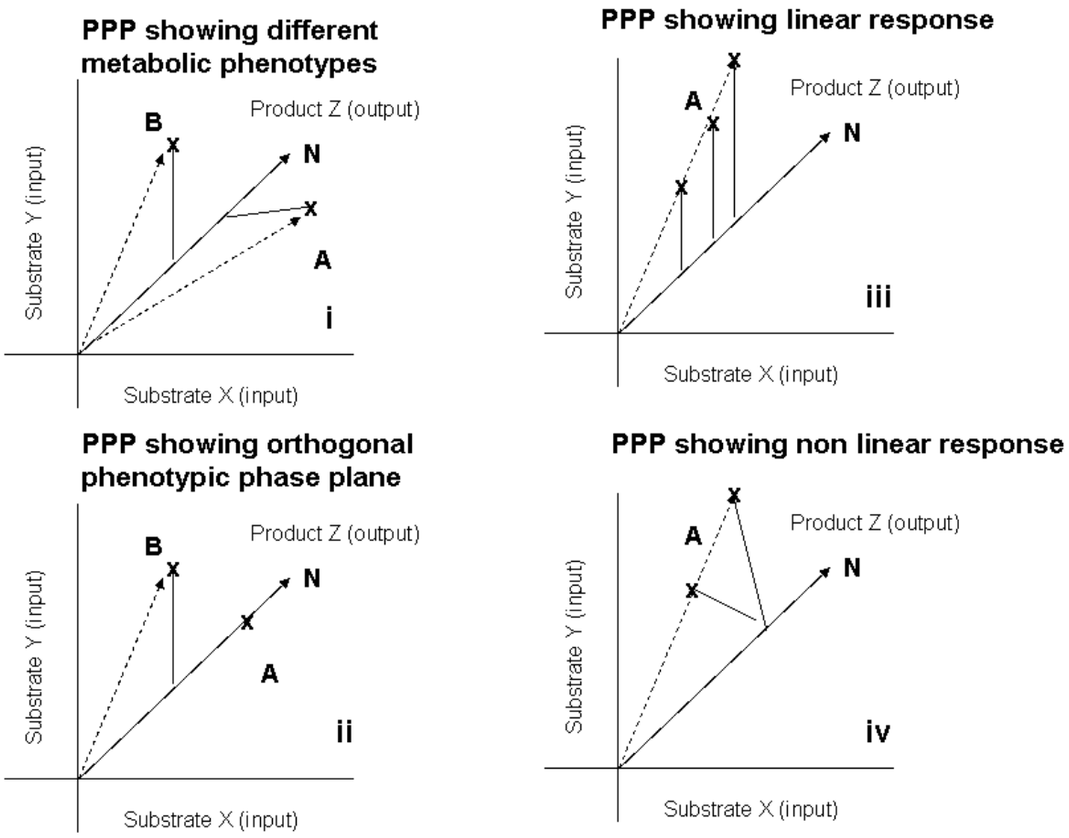

Fig. 4. Examples of phenotypic phase plane analysis showing the quantitative relationship among phenotypes by isocline analysis. Panel (i) shows effects of two drugs (A and B) with different mechanisms on metabolic pathways X and Y. N represents the normal phenotype. Panel (ii) shows the effect of A is orthogonal to the X-Y plane. Panel (iii) shows dose dependent effect of A. Panel (iv) shows non-linear response to two different doses of A.

or siRNA) A and B. These treatments result in changes in phenotypes (decrease in production of Z) accompanied by different metabolic compensations in substrate utilization. Treatment B results in a decreased utilization of substrate X (or its metabolic pathways) which is compensated by a slight increase in the utilization of substrate Y. Whereas treatment A results in the increase utilization of X and decreased utilization of Y as compared to control (N). If the mechanism of action of treatment B is known (such as inhibition of a specific kinase), one can conclude that treatment A must act on a different set of metabolic and/or signaling pathways. Such an approach will allow an iterative approach to the discovery of new treatment or new pathways. Additional examples of using phenotypic phase plane to understand phenotype changes of fibroblasts from a patient with thiamine responsive megaloblast anemia (TRMA) and pancreatic cancer cell (MIA) and normal fibroblasts using PPP have been provided by Lee and Go in their review (15). When TRMA cells were treated with high doses of thiamine, the phenotype approached that of the normal fibroblasts. On the other hand, MIA cells had a high pentose synthesis phenotype which was corrected when they were treated with oxythiamine. (15) We have recently used phenotypic phase plane analysis to show that inhibition of histone acetylation by genetic

intervention or by chemical inhibitor of the reaction had similar metabolic phenotypes (25). Panel (ii) shows result of treatment A which is orthogonal to the phenotypic phase plane of X and Y. This means that treatment A affects a different part of the metabolic system which is not linked to the utilization of substrates X and Y. Metformin and rosiglitazone, two antihyperglycemic drugs, have been shown to alter de novo lipogenesis. While inhibition of de novo lipogenesis by metformin is in the plane of ribose metabolism, meaning changes in pentose cycle metabolism is related to the decrease in lipogenesis. On the other hand, the increase in fatty acid synthesis by rosiglitazone is orthogonal to the ribose phenotypic phase plane suggesting very different mechanism of actions by these two drugs (26). The ability to detect orthogonal phenotypic phase plane is important because there are potentially many of these orthogonal phenotypic phase planes which can be discovered using tracer-based metabolomics, and each of the orthogonal pair would suggest different mechanism of action by different drugs. The finding of orthogonal planes is one of the unique capability of the metabolomics approach in generating mechanistic hypothesis. Panel (iii) shows the proportional response to inhibitor of substrate X where all of the isoclines are parallel to each other. An example of this type of response is provided by our study on the response of a methotrexate resistant colon cancer cell line (HT29) to the effect of DHEAS, oxythiamine and methotrexate treatment alone and in combination (27). Panel (iv) shows response to two inhibitors of substrate X with non-linear compensation of substrate Y. The application of PPP analysis has allowed a far better understanding of metabolic adaptation in cellular homeostasis using tracer-based metabolomics. Using PPP and isocline analysis, we can directly exploit the large dataset accumulated from tracer-based metabolomics studies for target discovery and lead identification in pharmaceutical industry.

7. Concluding comments

Study of metabolism in the post-genomic era differs from the traditional biochemistry in that the study is focused on the function of the system of biochemical reactions in a cell (or the cellular metabolic network) and its regulation. Metabolic function of a living organism (cell) is what mediates the the genetic potential of a cell and its interaction with its environment to maintain homeostasis. The maintenance of homeostasis by the cellular metabolic network in a living organism is the basis of normal physiology and histology (28). When the metabolic environment of a living organism or a cell is altered such as in diabetes or metabolic diseases, maladaptation or the lack of homeostasis in the living organism is the underlying cause for pathophysiology and histopathology (29).

Metabolic phenotype of a cell is the result of genetic and environmental interaction. Understanding metabolic phenotyping changes is important to our understanding of how cells maintain homeostasis by metabolic regulation. We have reviewed three approaches that are used in such investigations based on three different models. Of these different approaches, the gene-switch approach is the most extensively used in the pharmaceutical industry. In the gene-switch model, metabolic regulation begins with the interaction between genes and signaling pathways which eventually impact on biochemical reactions known as down-stream effects (30). This model ignores the fact that many of these signaling pathways or transcriptional factors are altered through post-translational events such as phosphorylation, acetylation, glycosylation and methylation. Since all these post-translational modifications are basic biochemical reactions, they are all subject to the stoichiometric and energy substrate constraints.

Once the downstream events are initiated, the interconversion of metabolic intermediates is subject to all the constraints as described in preceding paragraphs. We have previously shown that altered metabolic pathways can be the initiating events in gene transcription and post-translational modification of signaling pathways and enzymes (31, 32). Therefore, gene-switch model is an incomplete model to understand metabolic regulation. Because of these conceptual problems, application of gene-switch approach has had disappointing results in identifying drug candidates or targets and appalling failures in clinical trial due to unexpected toxicity or lack of efficacy. Of the two remaining approaches, tracer-based metabolomics is a practical experimental approach that does not require complicated mathematical modeling. Furthermore, the results can be graphically presented and the quantitative difference of metabolic phenotypes can be compared. Such features make the tracer-based metabolomics a powerful approach for drug screening in pharmaceutical research. Since the model does not assume any signaling pathways, it is most suitable for studies of nutriceuticals such as phytochemicals (33) or for screening of compounds in a chemical library that have no known molecular targets (34). It is also applicable to investigate the metabolic effect of drug combinations, in which the interaction of drugs can be studied. Most important of all, tracer-based metabolomics approach provides the understanding of cellular homeostasis and its changes under the influence of nutrient conditions or pharmaceuticals.

Since our first publication on metabolic profiling (35), progress in tracer-based metabolomics has been slow because there are few investigators who are trained in tracer technology. The current tracer model mainly addresses the area of glucose metabolic pathways. Methods for the investigation of other metabolic systems that may be distantly connected to glucose metabolism (orthogonal systems) are not represented. These systems include the systems of glutamine metabolism which connects glucose metabolism to nucleic acid synthesis; arginine metabolism which is part of the urea cycle and nitric oxide synthesis system; and the methyl donor pathways which are important in nucleic acid synthesis and choline synthesis. The complete development of tracer-based metabolomics is probably a decade away provided the development has the attention and adequate funding to complete the tasks to cover the metabolic pathways of the whole cellular metabolic network.

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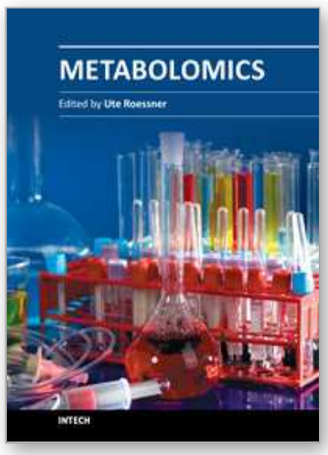

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Metabolomics is a rapidly emerging field in life sciences, which aims to identify and quantify metabolites in a biological system. Analytical chemistry is combined with sophisticated informatics and statistics tools to determine and understand metabolic changes upon genetic or environmental perturbations. Together with other 'omics analyses, such as genomics and proteomics, metabolomics plays an important role in functional genomics and systems biology studies in any biological science. This book will provide the reader with summaries of the state-of-the-art of technologies and methodologies, especially in the data analysis and interpretation approaches, as well as give insights into exciting applications of metabolomics in human health studies, safety assessments, and plant and microbial research.

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