

Metabolic profiling of cell growth and death in cancer: applications in drug discovery

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Metabolic profiling using stable-isotope tracer technology enables the measurement of substrate redistribution within major metabolic pathways in living cells. This technique has demonstrated that transformed human cells exhibit profound metabolic shifts and that some anti-cancer drugs produce their effects by forcing the reversion of these metabolic changes. By revealing tumor-specific metabolic shifts in tumor cells, metabolic profiling enables drug developers to identify the metabolic steps that control cell proliferation, thus aiding the identification of new anti-cancer targets and screening of lead compounds for anti-proliferative metabolic effects.

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▼ A mammalian cell's genetic program directs the cell's response to specific regulatory ligands, resulting in a distinctive phenotype at any given point during the cell's life cycle. This phenotype is expressed in the context of the microenvironment, particularly substrate availability, which profoundly determines enzyme activities and substrate utilization and redistribution within and among major metabolic pathways [1–3]. Alterations in substrate flow in response to hormonal, environmental or carcinogenic stimuli reflect changes in metabolic enzyme activities. These changes occur through either protein phosphorylation or glycosylation, or metabolic enzyme gene expression regulated by transcription factors.

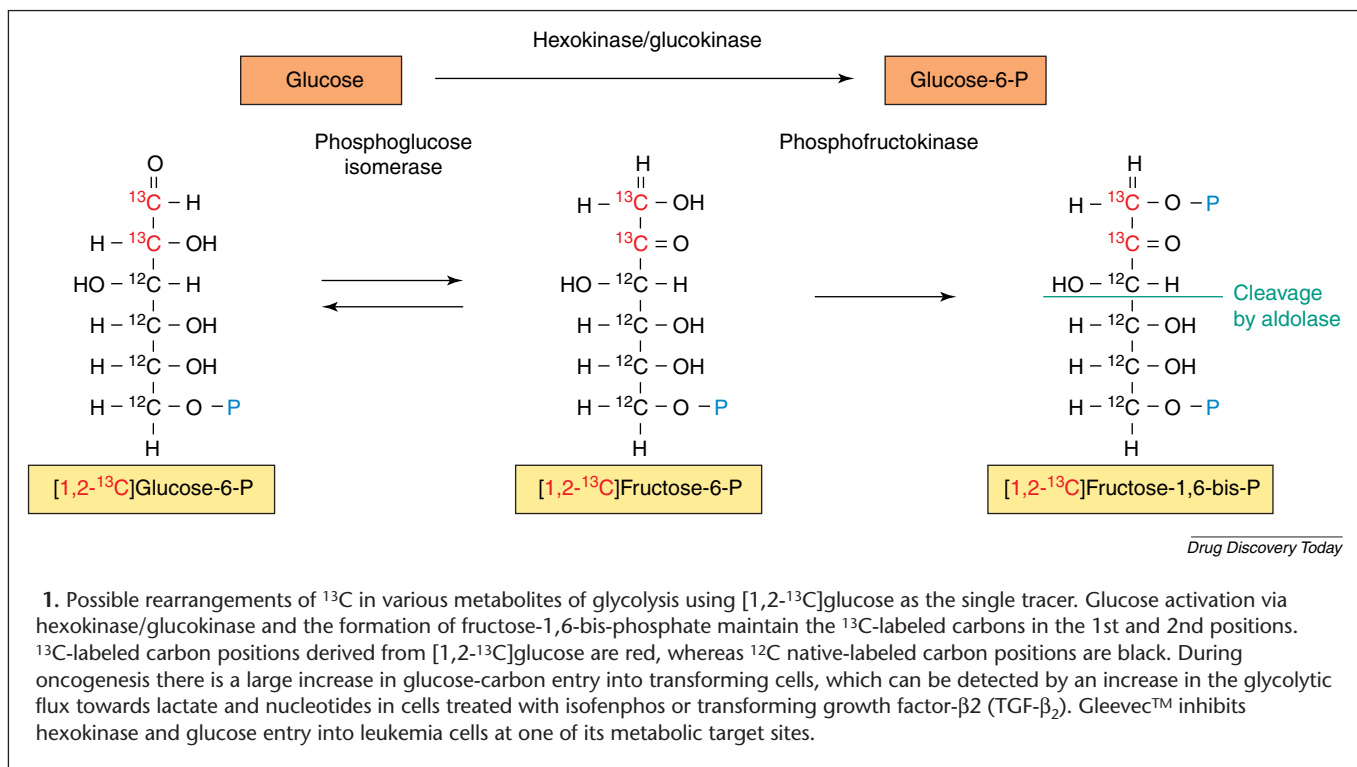
The metabolic profile of a given cell represents the integrated end-point of many growth modifying signaling events against the background of the cell's genetic makeup. Thus, metabolic profiling is the simultaneous assessment of substrate flux within and among major metabolic pathways of macromolecule synthesis and energy production under various physiological conditions, growth phases and

substrate environments. It can greatly assist the industrial drug discovery process by revealing metabolic adaptive changes that occur as a consequence of gene expression and changes in microenvironment, and is, therefore, complementary to genetic profiling. Obtaining metabolic profiles can both characterize the efficacy of new anti-proliferative drugs and provide a screening tool for drug development.

This article reviews the changes in substrate carbon flow and re-distribution among various metabolic pathways observed in the transformed proliferating-cell phenotypes observed in cancer, and illustrates how metabolic profiling can be used to identify crucial enzymes and their substrate analogues to facilitate drug development.

Metabolic profiling of tumor cells: tools and applications

The excess accumulation of the ^{18}F tracer attached to deoxyglucose is a reliable indicator of increased metabolic activity in tumor cells, which also correlates with malignancy, growth rates and response to therapy [4,5]. Although indispensable as a diagnostic tool, this imaging technique does not permit the detailed characterization of the destinations or routes taken by glucose carbons in the macromolecule and energy producing metabolic reactions. However, with the use of mass spectrometry (MS) and glucose molecules labeled with stable (non-radiating) isotope (^{13}C), the distribution of labeled carbons in various intermediates during *de novo* macromolecule synthesis in cancer cells can be revealed. Metabolic profiling is designed to uncover detailed substrate-flow modifications in response to pathological processes or new



anti-tumor therapies. Here we discuss the specific application of $[1,2-^{13}\text{C}]$ glucose in metabolic profiling.

The changing pattern of distribution of ^{13}C from $[1,2-^{13}\text{C}]$ glucose in intracellular metabolic intermediates provides a measure of carbon flow towards the pentose cycle, glycolysis, direct glucose oxidation, the tricarboxylic acid (TCA) cycle and fatty acid synthesis, simultaneously. Metabolic profiling reveals specific flux changes in lactate, glutamate, nucleic acid ribose, palmitate and CO_2 during oncogenesis and during anti-proliferative treatments. Thus, it indicates major changes in glucose utilization for macromolecule synthesis in cancer, information that can also be used for drug target development.

Rationale for ^{13}C labeling

In general, $[1,2-^{13}\text{C}_2]$ glucose metabolism produces two isotope-labeled intermediary metabolite species (also called mass isotopomers): *m1*, with one ^{13}C substitution, and *m2*, with two ^{13}C substitutions. These isotopomers are readily separated and measured using gas chromatography–mass spectrometry (GC–MS) techniques described elsewhere [6–9].

Lactate is the main three-carbon product of glycolysis and is readily secreted into the cell culture medium; therefore, it can be used to measure label incorporation into the three-carbon metabolite pool. The possible arrangements of ^{13}C labels from $[1,2-^{13}\text{C}]$ glucose to lactate through glycolysis are shown in Fig. 1 and Fig. 2. Glucose oxidation through the pentose cycle, however, results in a loss of the

first ^{13}C of glucose (shown in Fig. 3). $^{13}\text{CO}_2$ is also released during glucose oxidation, which reflects glucose utilization for energy production in the pentose and TCA cycles. During metabolic profiling we determine not only the amount of ^{13}C isotope accumulation but also the positions of ^{13}C -labeled carbons within lactate. It is easy to see that the ratio between *m1* (recycled lactate from oxidized glucose via the oxidative branch of the pentose cycle) and *m2* (lactate produced by the Embden–Meyerhof–Parnas glycolytic pathway) is indicative of the activity of G6PD and glucose recycling in the pentose cycle. A detailed description of the reactions and calculations can be found elsewhere [6]. Anti-cancer drug treatment that affects direct glucose oxidation or glycolytic flux is expected to alter glucose-label rearrangement in lactate.

Ribose and deoxyribose are the building blocks of nucleotides and, therefore, ^{13}C incorporation from glucose into RNA ribose or DNA deoxyribose indicates changes in nucleic-acid synthesis rates through the respective branches of the pentose cycle. Singly labeled ribose molecules on the first carbon position (*m1*) represent ribose that is produced by direct glucose oxidation through G6PD (Fig. 3). This ribose can either be incorporated into nucleic acid or returned to glycolysis as shown in Fig. 4. The alternative pathway for ribose synthesis is through the non-oxidative steps of the pentose cycle using glycolytic metabolites. The non-oxidative synthesis of ribose from glucose is controlled by transketolase as shown in Fig. 5. There is

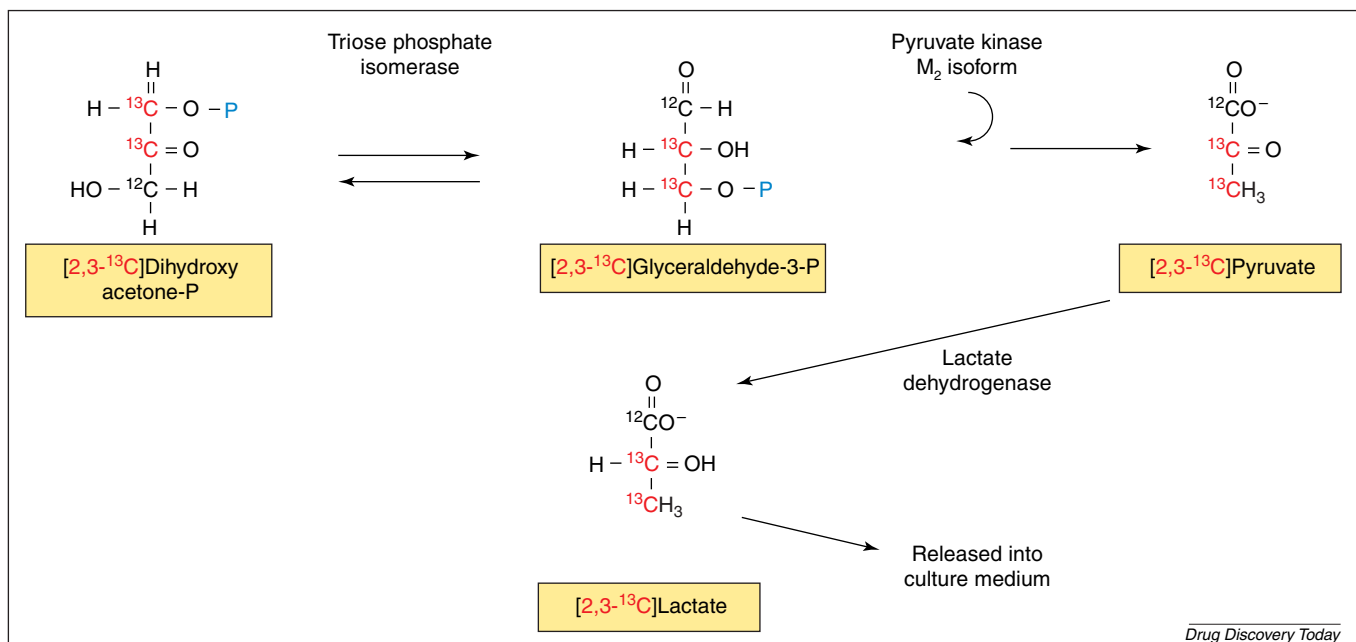


Figure 2. Formation of [2,3-¹³C]lactate through the Embden–Meyerhoff–Parnas pathway. The production of three-carbon metabolites by aldolase, glyceraldehyde and dihydroxyacetone phosphates transfers the labeled carbons into the 2nd and 3rd positions of glyceraldehyde. There are no subsequent positional changes in terms of ¹³C labeling by triose phosphate isomerase in the three-carbon metabolite pool that undergoes glycolysis, resulting in the release of lactate. Oncogenesis increases lactate production from glucose in all transformed cells, which can be detected as an increase ¹³C labeling in lactate in the medium. The M₂ isoform of pyruvate kinase inhibits glycolysis but expands the phosphometabolite pool for nucleic acid synthesis.

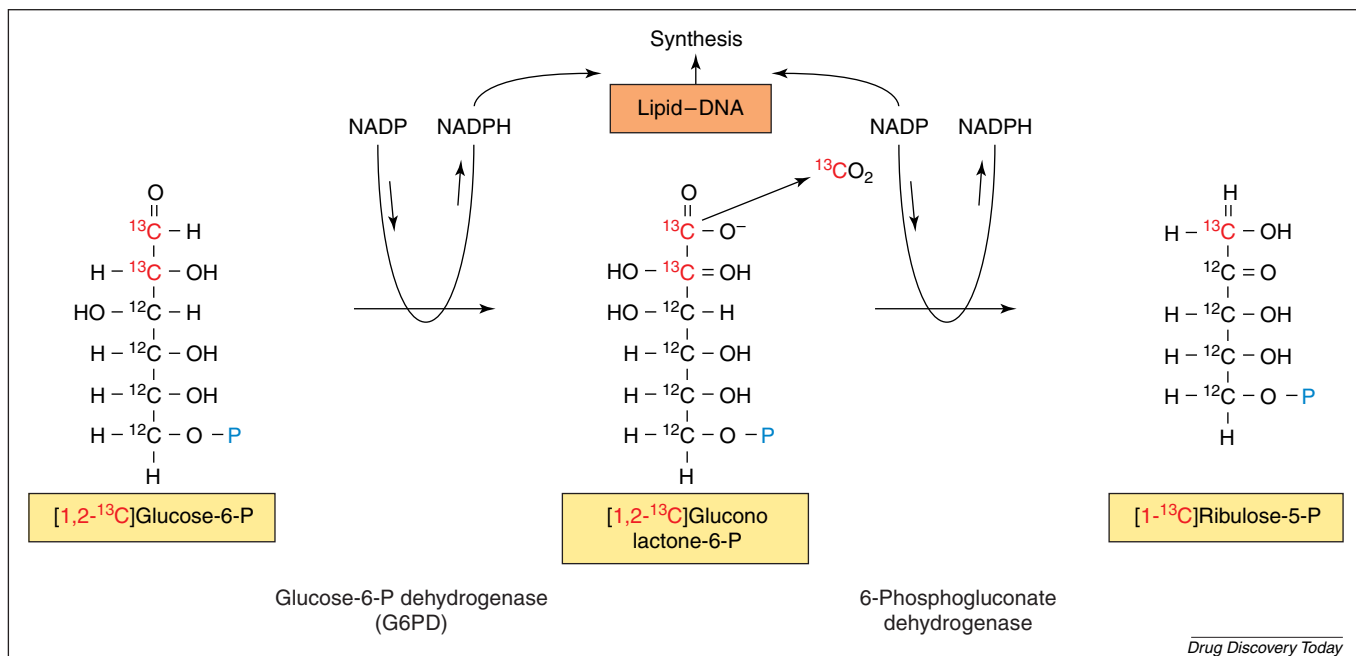
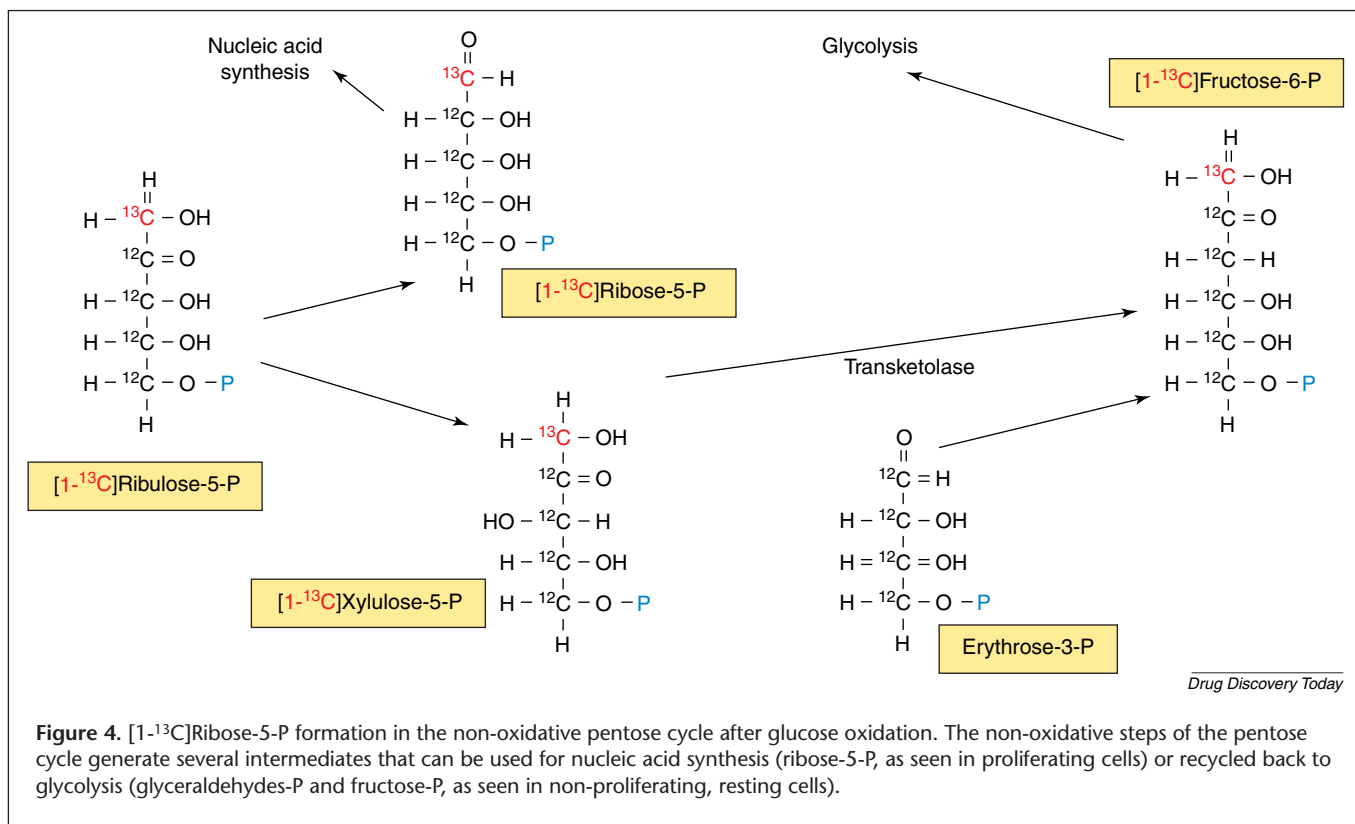
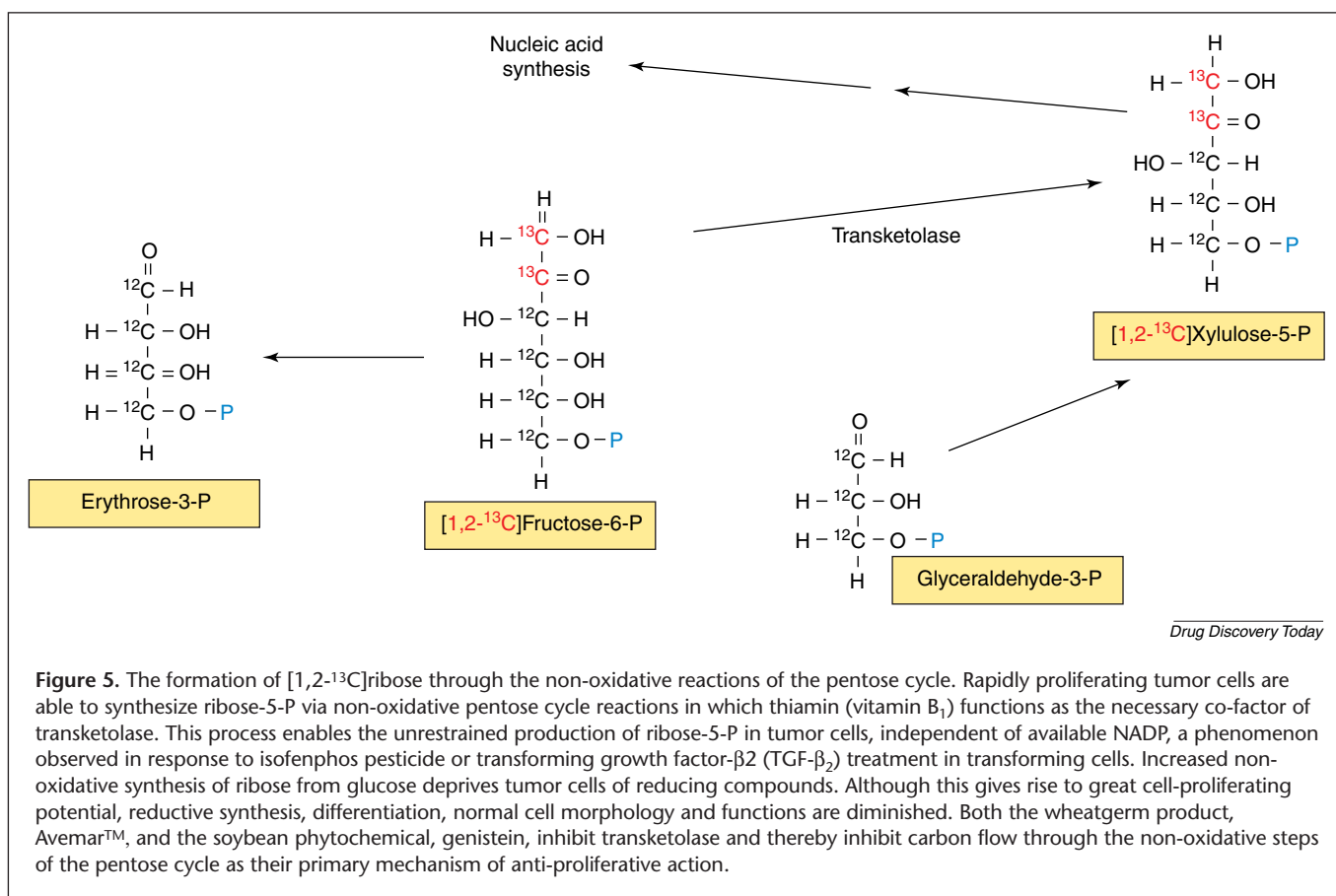


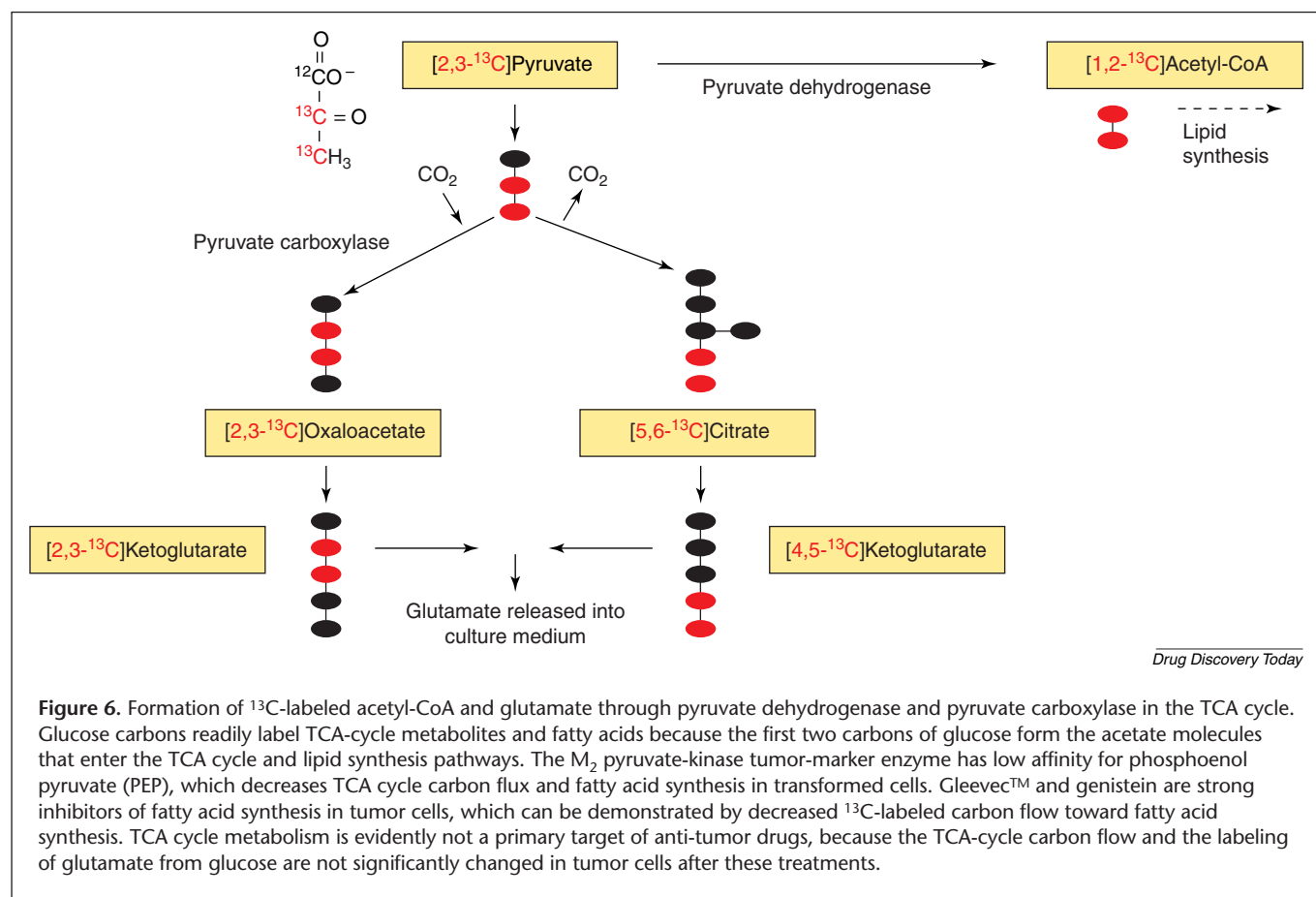
Figure 3. The rearrangement of ¹³C in pentose cycle metabolites resulting from direct glucose oxidation. The loss of the first labeled carbon of glucose resulting from direct oxidation produces ribulose molecules that are labeled only on the first position with ¹³C. During the oxidation of glucose, ¹³CO₂ is released, which can easily be detected using isotope ratio mass spectrometry (IRMS). Reducing equivalent NADP⁺ is also produced that can be used in lipid synthesis, DNA nucleotide production or to maintain reductive–oxidative reactions throughout metabolism. New anti-cancer treatment modalities such as Gleevec™ and Avemar™ primarily target glucose oxidation and ribose synthesis in the pentose cycle by decreasing G6PD and glucose carbon flow towards nucleic acid synthesis.



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no net carbon-loss throughout the non-oxidative steps of the pentose cycle; therefore, ribose molecules labeled on the first two carbon positions with ^{13}C (m_2) represent nucleic acid ribose synthesis through the non-oxidative route [6]. The ratio between m_1 and m_2 of nucleic acid ribose and deoxyribose closely reflects the involvement of glucose oxidation and non-oxidative ribose synthesis in tumor cells. These reactions are effectively modulated by novel and effective cancer treatment modalities during *de novo* nucleic acid synthesis and cell growth, as described later.

$^{13}\text{CO}_2$ release is a reliable marker of glucose oxidation (Fig. 3). $^{13}\text{CO}_2$ production from $[1,2-^{13}\text{C}]$ glucose takes place in both the pentose and TCA cycles and is measured as part of the metabolic profiling process to determine the rate of glucose oxidation in response to anti-cancer therapies. Decreased glucose oxidation with increased glucose uptake is always a reliable sign of increased anabolism, as seen in transformed cells.

Glutamate, a non-essential amino acid, is partially produced from mitochondrial α -ketoglutarate, which is a central intermediate of the TCA cycle. Glutamate is readily released into the culture medium after synthesis, which represents one of the routes for glucose carbon utilization.

Therefore, label incorporation from glucose into glutamate is a good indicator of TCA cycle anabolic metabolism for amino acid synthesis instead of glucose oxidation (Fig. 6) [7,8]. Surprisingly, cell-transforming agents and anti-cancer treatments only weakly influence TCA cycle carbon flow as demonstrated by unaltered glutamate ^{13}C accumulation from glucose in tumor cell cultures.

Fatty acid synthesis is also strongly dependent on glucose carbons through the formation of acetyl-CoA via pyruvate dehydrogenase. The incorporation of ^{13}C from $[1,2-^{13}\text{C}]$ glucose gives key information about the fraction of *de novo* lipogenesis in tumor cells and about glucose carbon contribution to acetyl-CoA for fatty acid synthesis (Fig. 6) [9]. Almost all cancer treatment modalities alter fatty acid synthesis, and changes in the flow of carbon toward fatty acid synthesis are important in tumor growth control.

Studying metabolic profiles in response to cell-transforming agents or cancer growth-controlling compounds using stable isotopes in cancer cell cultures or *in vivo*, can reveal how the growth signaling and metabolic processes are linked and to what extent metabolic pathway flux influences cell growth. Effective anti-cancer therapeutics are

Table 1. Metabolic profiles during carcinogenesis

Transforming agent	Target pathways	Metabolic profile	Phenotype	Ref.
Transforming growth factor b2	Pentose cycle, glycolysis, TCA cycle	Increased glucose carbon flow through the non-oxidative pentose cycle; decreased glycolysis and glucose oxidation	Poorly differentiated lung carcinoma	[11]
Isofenphos organophosphate pesticide	Pentose cycle, TCA cycle, fatty acid synthesis	Increased glucose carbon flow through the non-oxidative pentose cycle; severely diminished fatty acid synthesis and glucose oxidation	Rapidly progressing leukemia	[12]
Bcr/Abl tyrosine kinase oncogene (leukemia)	Glucose phosphorylation	High increase in glucose intake resulting from the expression of a high affinity glucose transporter GLUT-1	Highly proliferative, differentiated	[19]
M2 isoform pyruvate kinase	Glycolysis	Decreased glycolytic flux, increased levels of glucose-phosphate intermediates available for nucleotide precursor synthesis	Transformed, highly proliferative	[13]

Abbreviation: TCA, tricarboxylic acid.

expected to limit carbon flow towards nucleic acid synthesis and shift glucose toward oxidation or fatty acid synthesis. These changes can easily be revealed by metabolic profiling, making it an excellent tool for screening potential new drugs to treat cancer. Examples of how metabolic profiles change in cells, how cancer therapeutics reverse them, and how metabolic profiling can reveal both types of change as they happen, are provided in the next section.

Increased pentose cycle carbon flow and cell transformation

Cancer progression is enhanced by the autonomous growth promoting tyrosine kinase signaling ligand, transforming growth factor- β_2 (TGF- β_2) [10]. This process primarily depends on non-oxidative glucose conversion into ribose as the end-result of this signaling pathway [11]. Similarly, the carcinogen pesticide isofenphos directs glucose carbon-flow towards nucleic acid ribose synthesis in myeloid cells, resulting in a proliferative phenotype [12]. As depicted in Table 1, cell-transforming agents uniformly induce carbon-flow changes consistent with increased pentose cycle metabolism. The primary direction of glucose carbon flow in transformed human cells is toward nucleic acid ribose synthesis. By contrast, decreased carbon flow is observed towards amino acid and fatty acid synthesis pathways. The phenotypic consequences of this include rapidly proliferating, poorly differentiated cells with aneuploidy.

This increased carbon flow towards nucleic acid synthesis requires the unremitting expansion of six, five and three-carbon phospho-metabolite pools. This is expedited by the oncogenic expression of the dimer M₂ isoform of pyruvate kinase (M₂PK) [13]. The oncogenic transition lowers the

affinity of substrate phosphoenolpyruvate for pyruvate kinase, thus decreasing glycolytic flux but expanding all glucose phosphate intermediary metabolites in tumor cells that promote nucleotide synthesis (Fig. 2; Table 1). The expression of M₂PK has consistently been observed in kidney [14], lung [15], gastrointestinal [16], and breast malignancies [17], indicating the presence of malignant cells with sufficiently high specificity and sensitivity that it is now recommended as a reliable tumor marker.

Decreased pentose cycle carbon-flow as a cell growth controlling mechanism

Oncogenic transformation of myeloid cells in chronic myeloid leukemia (CML) results from the expression of a constitutively active tyrosine-kinase signaling protein construct, generated by the re-alignment of the breakpoint cluster region and Ableson leukemia virus proto-oncogene sequences (Bcr/Abl) [18]. This construct stimulates glucose transport in multipotent hematopoietic cells [19]. The inhibition of the Bcr/Abl tyrosine kinase by the anti-cancer drug, STI571 (Gleevec™), has been shown to effectively inhibit glucose utilization towards nucleic acid synthesis and leukemia cell proliferation [20]. At doses comparable to those used in the treatment of myelogenous leukemia, STI571 suppresses hexokinase and G6PD activities in K562 myeloid leukemia cells. As a result, STI571 limits pentose cycle carbon-flow towards nucleic acid ribose synthesis, as depicted in Table 2.

Decreased glucose carbon-flow towards nucleic acid synthesis has also been observed in cancer cells after treatment with other agents, including phytochemicals (Avenmar™, genistein) [21,22] and natural hormones such as dehy-

Table 2. Metabolic profiles in response to anticancer treatments

Treatment	Target pathways	Metabolic profile	Phenotype	Ref.
Gleevec (STI571) (Bcr/Abl tyrosine kinase inhibitor)	Glucose intake, pentose cycle, fatty acid synthesis	Decreased glucose intake and carbon flow through the oxidative pentose cycle; decreased fatty acid synthesis; inhibitor of hexokinase and G6PD	Decreased cell proliferation	[20]
Genistein (soy bean phytochemical)	Pentose cycle, fatty acid synthesis	Decreased glucose intake and carbon flow through the non-oxidative pentose cycle; decreased fatty acid synthesis	Apoptosis	[21]
AVEMAR (fermented wheat germ extract; G6PD and transketolase inhibitor)	Pentose cycle, fatty acid synthesis	Decreased glucose intake and carbon flow through the non-oxidative pentose cycle; increased fatty acid synthesis	Decreased proliferation; cancer patients gain weight	[22]
DHEA-S(G6PD inhibitor)	Pentose cycle	Decreased oxidative pentose cycle flux	G1 cell cycle arrest	[23]
Oxythiamine (transketolase inhibitor)	Pentose cycle	Decreased non-oxidative pentose cycle flux	G1 cell cycle arrest	[23,31]

Abbreviation: DHEA-S, dehydroepiandrosterone sulphate; G6PD, glucose-6-phosphate dehydrogenase.

droepiandrosterone sulfate (DHEA-S) [23] (Table 2). Because ribose is a structurally similar metabolite of glucose and ribose nucleotides are essential for *de novo* DNA and RNA synthesis, it is evident that metabolic profiling using specifically labeled glucose isotopes will have far reaching applications in the discovery of anti-proliferative drugs. Metabolic profiling has been much appreciated for its role in uncovering the leukemic effect of anti-cholinesterases by revealing what happens metabolically when cells shift from a normal to a tumorigenic cell cycle [24]. It is rather surprising that the TCA cycle is not a primary target of the cell transformation process [11] and anti-cancer modalities. Treatments that effectively limit carbon flow in the pentose cycle do not induce the same effect in the TCA cycle [21,22].

Metabolic control coefficients in metabolic profiling

It is evident that high levels of pentose cycle enzymes are associated with cell aging, uncontrolled cell proliferation and prolonged cell survival [25]. Pentose cycle enzymes provide the necessary ribose substrate for *de novo* nucleic acid synthesis as well as the reducing compound NADP⁺ for deoxyribose and fatty acid syntheses. A shortage in glucose carbon flow towards nucleic acid synthesis, as well as decreased NADP⁺ production, are strong metabolic signs of cell cycle withdrawal and apoptosis in rapidly dividing cells [26,27] (Table 2). To understand why pentose cycle enzymes have such a strong influence on cell physiology and how they control cell growth and transformation, one needs to consider the concept of metabolic control analysis (MCA). MCA provides a quantitative description of substrate

flux in response to changes in various system parameters in complex enzyme systems [28]. Therefore, MCA and metabolic profiling are closely related fields, because carbon flow changes are the combined results of enzyme activity changes and substrate availability and redistribution.

In MCA, the control exerted over substrate flux or any systemic parameter (e.g. cell proliferation) can be quantitatively described as a control coefficient for each and every enzyme in a metabolic network. Pentose cycle enzymes have been characterized in terms of their flux control coefficients in various mammalian cells, including tumor cells. The growth control coefficients of G6PD and transketolase in the Ehrlich's tumor model were recently reported to be high [29] and these enzymes are ranked as new promising anti-cancer drug targets [30]. The expanded understanding of crucial proliferative processes provided by MCA and metabolic profiling make it clear that target metabolic enzymes for new anti-cancer therapies have to be those that are demonstrated to have high control coefficients to limit substrate flow for nucleic acid ribose synthesis. This is, of course, also a crucial criterion in drug development efforts in which the efficacy of potential anti-proliferative drugs is determined by metabolic screening.

Applying metabolic profiling in drug discovery

Current drug discovery places heavy emphasis on finding new anti-cancer compounds that work by interfering with cell growth by targeting specific signal transduction pathways or genes. The use of metabolic profiles in the drug discovery process enables the determination of the crucial enzymatic steps that control carbon flow into proliferation-

related macromolecules. It is possible, using metabolic profiling, to identify new targets for anti-cancer compounds that selectively disrupt the unique metabolic networks of cancer cells. Our previous studies have mainly focused on glucose metabolic enzymes including hexokinase, G6PD and transketolase. Other potential targets remain to be explored with the notion that advanced, therapy-resistant tumor growth probably depends on constitutively active metabolic networks, which can be reached only by using drugs that inhibit metabolic enzymes. A combined approach of studying signaling, genetic and metabolic events on the same bench enables us to better define the metabolic processes of cancer cell growth and death. Using such an approach, a much larger set of anti-cancer compounds could be screened according to their signaling properties and metabolic profiles during the industrial drug development process.

The need for metabolic profiling using stable isotope-labeled substrates is demonstrated by the fact that although molecular genetic studies can anticipate changes in metabolism, they can not fully reveal whether metabolic network enzymes with strong control properties are active and their substrates abundantly present.

Conclusion

Metabolic profiling provides vital information beyond the reach of signal transduction and genetic studies, showing whether the adjustments in carbon flow and pentose cycle metabolism that are essential for rapid cell proliferation are taking place. Furthermore, the use of metabolic profiling to both identify crucial targets and fully establish whether a candidate anti-cancer drug actually produces a growth-limiting effect makes molecular profiling a valuable new tool in the efficient development of effective mechanism-based anti-cancer drugs.

Acknowledgements

We acknowledge help received from volunteers of the Inflammatory Breast Cancer Research Foundation, a non-profit-making organization aimed at increasing awareness of inflammatory breast cancer. The text of this article was co-edited by Dale Chenoweth (Austin, TX, USA) and Marvin Lee Davis (Long Beach, CA, USA). This work was supported by grants from the General Clinical Research Unit, the UCLA Clinical Nutrition Research Unit Stable Isotope Core, the Spanish Government Science and Technology Ministry, the Health Ministry and NATO Science Program.

References

- 1 Sachs, L. (1980) Constitutive uncoupling of the controls for growth and differentiation in myeloid leukemia and the development of cancer. *J. Natl. Cancer Inst.* 65, 675–679

- 2 Yamaji, Y. *et al.* (1994) Reciprocal alterations of enzymic phenotype of purine and pyrimidine metabolism in induced differentiation of leukemia cells. *Adv. Exp. Med. Biol.* 370, 747–751
- 3 Moley, K.H. and Mueckler, M.M. (2000) Glucose transport and apoptosis. *Apoptosis* 5, 99–105
- 4 Stokkel, M.P. *et al.* (2001) Positron emission tomography with 2-[18F]-fluoro-2-deoxy-D-glucose in oncology. Part IIIb: therapy response monitoring in colorectal and lung tumours, head and neck cancer, hepatocellular carcinoma and sarcoma. *J. Cancer Res. Clin. Oncol.* 127, 278–285
- 5 Smith, T.A. (2001) The rate-limiting step for tumor [18F] fluoro-2-deoxy-D-glucose (FDG) incorporation. *Nucl. Med. Biol.* 28, 1–4
- 6 Lee, W.N. *et al.* (1998) Mass isotopomer study of the non-oxidative pathways of the pentose cycle with [1,2-¹³C] glucose. *Am. J. Physiol.* 274, E843–E851
- 7 Lee, W.N. *et al.* (1996) Mass isotopomer study of glutamine oxidation and synthesis in primary culture of astrocytes. *Dev. Neurosci.* 18, 469–477
- 8 Leimer, K.R. *et al.* (1977) Complete mass spectra of N-TAB esters of amino acids. *J. Chromatogr.* 141, 121–144
- 9 Lee, W.N. *et al.* (1995) Isotopomer study of lipogenesis in human hepatoma cells in culture: contribution of carbon and hydrogen atoms from glucose. *Anal. Biochem.* 226, 100–112
- 10 Hojo, M. *et al.* (1999) Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature* 397, 530–534
- 11 Boros, L.G. *et al.* (2000) Transforming growth factor β_2 promotes glucose carbon incorporation into nucleic acid ribose through the nonoxidative pentose cycle in lung epithelial carcinoma cells. *Cancer Res.* 60, 1183–1185
- 12 Boros, L.G. and Williams, R.D. (2001) Isofenphos induced metabolic changes in K562 myeloid blast cells. *Leukemia Res.* 25, 883–890
- 13 Zwerschke, W. *et al.* (1999) Modulation of type M₂ pyruvate kinase activity by the human papillomavirus type 16 E7 oncoprotein. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1291–1296
- 14 Oremek, G.M. *et al.* (1999) The pyruvate kinase isoenzyme tumor M₂ (Tu M₂-PK) as a tumor marker for renal carcinoma. *Anti-cancer Res.* 19, 2599–2601
- 15 Schneider, J. *et al.* (2000) Quantitative detection of tumor M₂-pyruvate kinase in plasma of patients with lung cancer in comparison to other lung diseases. *Cancer Detect. Prev.* 24, 531–535
- 16 Hardt, P.D. *et al.* (2000) Tumor M₂-pyruvate kinase: a promising tumor marker in the diagnosis of gastro-intestinal cancer. *Anti-cancer Res.* 20, 4965–4968
- 17 Luftner, D. *et al.* (2000) Tumor type M₂ pyruvate kinase expression in advanced breast cancer. *Anti-cancer Res.* 20, 5077–5082
- 18 Gyger, M. *et al.* (1985) Chronic myeloid leukemia with an unusual simple variant translocation: t(2;22)(q13;q11). *Acta Haematol.* 73, 193–195
- 19 Bentley, J. *et al.* (2001) Glucose transport regulation by p210 Bcr-Abl in a chronic myeloid leukaemia model. *Br. J. Haematol.* 112, 212–215
- 20 Boren, J.A. *et al.* (2001) Gleevec (STI571) influences metabolic enzyme activities and glucose carbon flow towards nucleic acid and fatty acid synthesis in myeloid tumor cells. *J. Biol. Chem.* 276, 37747–37753
- 21 Boros, L.G. *et al.* (2001) Genistein inhibits nonoxidative ribose synthesis in MIA pancreatic adenocarcinoma cells: a new mechanism of controlling tumor growth. *Pancreas* 22, 1–7
- 22 Boros, L.G. *et al.* (2001) Wheat germ extract decreases glucose uptake and rna ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells. *Pancreas* 23, 141–147
- 23 Boros, L.G. *et al.* (1997) Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res.* 57, 4242–4248
- 24 Perry, C. and Soreq, H. (2001) The leukemic effect of anticholinesterases. *Leukocyte Res* 25, 1027–1028
- 25 Orgel, L.E. (1973) Ageing of clones of mammalian cells. *Nature* 243, 441–445

- 26 Tian, W.N. *et al.* (1999) Importance of glucose-6-phosphate dehydrogenase activity in cell death. *Am. J. Physiol.* 276, C1121–1131
- 27 Tuttle, S. *et al.* (2000) Glucose-6-phosphate dehydrogenase and the oxidative pentose phosphate cycle protect cells against apoptosis induced by low doses of ionizing radiation. *Radiat. Res.* 153, 781–787
- 28 Schuster, S. *et al.* (1999) Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. *Trends Biotechnol.* 17, 53–60
- 29 Comín-Anduix, B. *et al.* (2001) The effect of thiamine supplementation on tumor proliferation: a metabolic control analysis study. *Eur. J. Biochem.* 268, 4177–4182
- 30 Cornish-Bowden, A. (1999) Metabolic control analysis in biotechnology and medicine. *Nat. Biotechnol.* 17, 641–643
- 31 Rais, B. *et al.* (1999) Oxythiamine and dehydroepiandrosterone induce a G1 phase cycle arrest in Ehrlich's tumor cells through inhibition of the pentose cycle. *FEBS Lett.* 456, 113–118

