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Review

Towards a metabolic therapy of cancer?

Martina Chiu, Laura Ottaviani, Massimiliano G. Bianchi, Renata Franchi-Gazzola, Ovidio Bussolati

Unit of General Pathology, Department of Biomedical, Biotechnological and Translational Sciences, University of Parma

Abstract. It is increasingly appreciated that cancer cells must be endowed with specific metabolic adaptations to support enhanced growth and to ensure survival under stressful conditions. On the other hand, many oncogenic mutations of protooncogenes and tumor suppressor genes directly cause metabolic derangements and, conversely, mutations of enzymes have been found to underlie several forms of cancer. Thus, cancer-specific metabolic alterations are now considered among the hallmarks of malignant tumors. Most commonly, cancer cells exhibit enhanced glycolysis under aerobic conditions (the Warburg effect) but alterations in the metabolism of amino acids, such as glutamine, serine and proline are increasingly described as important metabolic features of selected tumor types. In theory, all these deranged cancer-specific metabolic pathways may constitute novel therapeutic targets, although the only "metabolic" drug in clinical use is still represented by the enzyme L-asparaginase. However, the increasing amount of experimental evidence, as well as the number of trials in progress, suggests that metabolic drugs will soon complement standard anti-cancer chemotherapy and modern biological drugs. (www.actabiomedica.it)

Key words: cancer, metabolism, glycolysis inhibitors, glutamine analogues

Introduction

In 2001 Hanahah and Weinberg published their pivotal review on the hallmarks of cancer (1). That contribution contained a comprehensive recognition of the main advances in the understanding of the molecular aspects of cancer as well clear cut prospective indications for the future. The hallmarks identified were, needless to say, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.

After ten years, the same Authors decided to write a new review on the same subjects (2). In this contribution, which is also becoming a must of cancer bibliography, Hanahah and Weinberg propose two new hallmarks of cancer. One is evasion from immune destruction, the other being reprogramming of energy metabolism.

The "old" hallmarks were also proposed as likely therapeutic targets and the provision was correct: for instance, antiangiogenetic drugs have crossed the border between the bench and the bedside. Similarly, since also the "new" hallmarks are forecast as possible therapeutic targets, we should expect the introduction of metabolic drugs or metabolic therapy in clinical oncology (3).

This short review intends to briefly summarize the major advances in the cancer metabolism field as well as to recount the perspectives for a metabolic therapy of neoplastic diseases.

The Warburg effect and metabolic alterations in cancer

Why this renewed interest for cancer metabolism? Actually, the hypothesis that cancer is also a metabolic disease is all but new. At the beginning of

the 20th century an ingenious German biochemist, Otto Warburg, proposed that cancer is essentially a metabolic disease, which he attributed to a basic mitochondrial defect present in all the cancer cells. Consequently, cancer cells would rely entirely on glycolysis even in the presence of normal oxygen tension, producing large amounts of lactate (the so called "aerobic glycolysis" or "Warburg effect"). Warburg won the Nobel Prize for Biochemistry and Physiology in 1931 for the discovery of cytochrome C oxidase but, until his death in 1956, he remained intimately sure that his major contribution to science was indeed the discovery of metabolic alterations in cancer (see the enlightening historical perspective in (4)). Warburg's ideas led to bitter contrasts with other big names, such as, for instance, Sir Hans Krebs, and it was ascertained that most, but not all, of the cancer cells exhibit the Warburg effect and that not all tumors present defective mitochondria.

In the following years, Warburg hypothesis on the metabolic origin of cancer was rapidly overcome by the tumultuous progresses in viral carcinogenesis and molecular oncology, leading to the still dominant concept of cancer as a genetic disease. Yet, Warburg's estimation on glycolytic rates in cancer cells proved astonishingly correct, demonstrating that in several cancer types the consumption of glucose is of hundreds of folds higher than in normal counterparts. Although not all the tumors exhibit comparable dependence upon glycolysis (5), Warburg effect has provided the rationale for one of the most important devices in clinical oncology, the ^{18}F-deoxyglucose Positron Emission Tomography (PET), able to spot malignant lesions on metabolic rather than morphological grounds (6).

Why many tumors preferentially use glycolysis instead of Krebs cycle and oxidative phosphorylation, if their mitochondria are normal? The usual answer to this question is that glycolytic cancer cells are not too dependent upon oxygen and, hence, clearly favoured compared to ischemic normal counterparts. However, one may argue that, if glycolytic tumors are less dependent upon oxygen, at the same time they should be much more dependent upon glucose.

Recent studies have focussed on anaplerosis, a classical concept of intermediate metabolism (7, 8). Anaplerosis is the continuous fuelling of metabolic intermediates of glycolysis and Krebs cycle that prevents their depletion (and hence an energy shortage of the cell) notwithstanding the sustained flux of 3-, 4- and 5-C units diverted towards anabolic reactions, such as protein and fatty acid synthesis. Thus, glucose can behave as an anaplerotic substrate if its oxidative metabolism stops to pyruvate but it is simply an energetic fuel if it is completely oxidized to CO_2. For a cancer cell, large availability of anaplerotic substrates is a prerequisite to couple under safe conditions high proliferative activity (and, hence, high rates of anabolic reactions) with high energetic charge.

Moreover, several of the enzymes involved in glycolysis, such as hexokinase (9) are also important regulators of apoptosis. More in general, excess of cytosolic ATP production through glycolysis inhibits mitochondrial ATP synthase, induces a chemiosmotic backpressure and hyperpolarizes the mitochondrial membrane (10). This is the basis for the so-called "Crabtree effect", the net decrease of oxygen consumption upon glucose addition to the medium (11). Sustained hyperpolarization fixes mitochondria in an anti-apoptotic state, a phenomenon observed in both cancer and normal cells (12, 13), which has important consequences on the susceptibility of cancer cells to radiotherapy and chemotherapy.

Additional putative advantages yielded by aerobic glycolysis are lactate shuffling, through which highly glycolytic cancer cells may feed neighbouring cells (14, 15), and prevention of oxidative damage due to activation of the pentose phosphate pathway. Recent results suggest that the activation of this pathway may derive from the accumulation of phosphoenol pyruvate due to the increased expression in cancer cells of PKM2, the low-activity isoform of pyruvate kinase (16, 17).

Enzymes and mutations in cancer cells

What are the relationships between the large host of known oncogenic mutations and cell metabolism? Given the premises, it is by no means surprising that many common oncogenic mutations affecting both protooncogenes and cancer suppressor genes also affect metabolism, ensuring to the cancer cell high avail-

ability and enhanced rates of utilization of anaplerotic substrates. For instance, p53 mutations accelerate glucose influx and glycolysis and, conversely, the wild type protein enhances mitochondrial oxidative metabolism (18, 19), lowers the expression of GLUT transporters (20) and suppresses the activity of Glucose-6-Phosphate Dehydrogenase, the key enzyme for the pentose shunt pathway (21). The metabolic effects of p53, which recent work would indicate as the main responsible for the antitumor activity of the protein (22), are mostly dependent on its target TIGAR (TP53-induced glycolysis and apoptosis regulator) and synthesis of cytochrome c oxidase 2 (SCO2) (23). Also the metabolism of glutamine, the other important anaplerotic substrate, is modulated by p53 (2426) as well as by the product of the oncogene *MYC*, involved in a large number of human tumors (27), which also increases glycolytic flux (28). Although alterations in *MYC*, p53 and HIF-1 are considered the most common causes of metabolic alterations in cancer (29), the role of other pathways is also increasingly recognized. For instance, a diversion of glucose metabolism from energy-producing glycolysis to anabolic pathways is a powerful means through which *KRAS* mutations drive pancreatic tumorigenesis (30). Moreover, in virus-induced tumors metabolic alterations can be traced back to the expression of viral genome (31). Whenever a relevant oncogenic mutation has clear cut metabolic effects promoting the consumption of a particular substrate, the tumor cells would require large amounts of that substrate, an "insatiable appetite" (32) quite properly named "addiction" (27).

Recent data demonstrate that mutations of enzymes involved in intermediate metabolism, and not obviously linked to cell cycle regulation, apoptosis or DNA repair, can promote cancerogenesis behaving as tumor suppressors or oncogenic products. These mutations can be grouped in two main classes: i) germ line inactivating mutations of enzyme-coding genes (which should be thus considered tumor suppressor genes), leading to rare familial cancer syndromes and ii) somatic activating mutations of enzyme genes (which should be thus considered oncogenes), present in subsets of common sporadic cancers.

As for the first class of mutations, it was already known that metabolic effects of mutations in components of regulatory pathways such as HIF and VHL, which underlie familial pheomochromocytomas (33), have dramatic metabolic consequences. However, it was surprising understanding that inactivating mutations of TCA cycle enzymes, such fumarate hydratase (*FH*) and succinate dehydrogenase (*SDHB, SDHC,* and *SDHD*) may directly promote oncogenesis behaving as true oncosuppressor genes (34). *FH* mutations in the germinal line cause the rare familial syndrome Hereditary Leiomyomatosis Renal Cell Cancer (HLRCC) (35). Other hereditary cancer conditions caused with mutations of these enzymes are hereditary paraganglioma–pheochromocytoma syndrome (36), and hereditary paraganglioma (37). Consistent with the classic two-hit theory, patients present the inherited mutation in one allele, while the second allele is lost or inactivated in the tumors. Although a common mechanism underlying the oncogenic effect of these mutations consists in the perturbation of the HIF pathway, known to be activated in many forms of cancer, other metabolic alterations may be also involved. For instance, oncogenic FH mutations result in glycolytic drift, along with decreased AMPK and p53 activities, and enhanced anabolic pathways in the tumor cells (38).

The most well known example of the second type is given by mutations of the two genes for Isocitrate Dehydrogenase isozymes (*IDH1* and *IDH2*), which are present in a large portion of Grade II and III astrocytomas, oligodendrogliomas and "secondary" glioblastomas, that is glioblastomas deriving from low-grade glial tumors (39). Mutated IDH1 and IDH2 were then found also in subsets of acute myelogenous leukemia (40) and in chondrosarcoma (41). Normally, IDH1 and IDH2 reversibly convert isocytrate into α-ketoglutarate (aKG) both in mitochondria (IDH-1) and in cytosol (IDH2), as a step of amino acid and fatty acid synthesis, while IDH3 is the enzyme responsible for the irreversible conversion of isocitrate to aKG in the second step of the Krebs cycle. The oncogenic, mutated variants of IDH1 and 2 acquire a new activity and, instead of producing aKG, use it as a substrate to synthesize large quantities of (*R*)-2-hydroxyglutarate (D-2HG), an uncommon metabolite in normal cells. The mechanisms underlying the oncogenic activity of D-2HG are still to be

defined, although the oncometabolite can interfere with aKG-dependent dioxigenases (34) thus altering both HIF-dependent pathways and methylation patterns (42). However, cells carrying these mutations have also profound changes in glutamine, fatty acid, and citrate synthesis pathways (43).

The list of enzymes the mutation of which associates with oncogenic transformation is likely expected to elongate. The last additions are glycine decarboxylase, originally found expressed in Tumor initiating Cells (TICs) of Non Small Cell Lung Cancer (NSCLC) but overexpressed in around 25% of the cancer cell lines tested (44), and prolyl oxidase (POX), which catalyzes the first step in the catabolism of proline. POX was originally thought a tumor suppressor (45) negatively regulated by MYC (46) but can behave as a pro-survival factor under hypoxic conditions (47). Great interest is also devoted to phosphoglycerate dehydrogenase (PHGDH), the first enzyme of the biosynthetic pathway for the non essential amino acids serine and glycine. *PHGDH* is amplified in almost half of the melanomas, in a smaller but significant portion of breast cancers and, overall, in more than 15% of the human cancers (48, 49). The metabolic role of PHGDH in cancer cells does not seem to be restricted to serine and glycine fuelling but may also provide an alternate source of aKG, thus fulfilling a classical anaplerotic function (49).

Therapeutic perspectives

Given that enhanced glycolysis is essential for the growth of many cancers, it is not surprising that most attempts have considered glycolysis control as an effective therapeutic target. Although restoration of metabolic anomalies is a tumor-specific result of p53 function reinstatement and may significantly contribute to the anti-tumor effects of the procedure (50), inhibitors of glycolysis provide a more direct mean to interfere with deranged cancer metabolism (51). The nonmetabolizable glucose analogues 2-deoxyglucose or 3-bromopyruvate have been used since many years to inhibit glycolysis and ATP production, leading to the suppression of cancer cell growth in vivo with relatively little damage to healthy organs (52, 53). The anticancer effects of 3-bromopyruvate have been recently reviewed (54), showing that the drug had fairly more complex activities than the straightforward inhibition of glycolysis. If these additive effects likely potentiate the antitumor effects of the drug, they may also complicate its clinical use. The inhibition of enhanced glucose transport (55) as well as of LDH activity (56) also exerts pro-apoptotic effects in tumor cells. Moreover, also the inhibition of carriers that provide the efflux route for lactate, such as the p53-regulated MCT1/*SLC16A1* (57) or the MCTA4/*SLC16A3* transporters (58), have remarkably effects on selected types of cancer cells.

Additional targets are also possible. For instance, ATP exerts a powerful feed-back inhibition on glycolysis so that, quite paradoxically, lowering intracellular ATP levels may provide an additional stimulus for high glycolytic flux and enhanced growth of cancer cells (see the discussion of this interesting item in (59)). Therefore, it is not surprising that many cancer cells have a high expression of UnCoupling Protein 2 (UCP2) as a means to suppress ATP synthesis and enhancing glycolytic flux (60). Thus, the inhibition of UCP2 may yield an approach to reprogram metabolism in cancer and to suppress tumor cell growth (61).

Also the widely used antidiabetic drug metformin, a well known AMPK activator, has recently gained much attention for its putative antineoplastic effects (62). For instance, the combination 2-deoxyglucose and metformin has dramatic antiproliferative and pro-apoptotic effects in prostate cancer cells (63). Even more interestingly, antiproliferative effects of metformin would be specific for p53-/- cells (64), a genetic condition often associated with apoptosis-resistant cancer phenotypes. Also jasmonates, a class of experimental antitumor agents derived from plant stress hormones, interfere with oxidative glucose metabolism and circumvent apoptopsis resistance, driving the tumor cell towards a non-apoptotic death (65).

Dichloroacetate (DCA) has gained much attention as an "anti-tumor metabolite". DCA inhibits pyruvate dehydrogenase kinase isozymes, with a greater affinity for the isozyme II (which is commonly activated in glycolytic cancer cells), thus activating pyruvate dehydrogenase, increasing delivery of pyruvate into the mitochondria and enhancing oxidative

phosphorylation. Since DCA has been used in the treatment of lactic acidosis, its pharmacokinetic data are known, indicating a good tissue distribution (66). It has been claimed to exert antitumor activities in vitro and in xenografts (67) and to trigger apoptosis in non-small cell lung cancer, breast cancer, glioblastoma, endometrial cancer, and prostate cancer (66, 68, 69), although its activity may be restricted to tumor cells with mitochondrial low function (70). DCA has also other effects, such as the inhibition of HIF1alpha by both a PHD-(Prolyl hydroxylase-) dependent mechanism (through an increase of mitochondria-derived aKG) and a PHD-independent mechanism (through p53 activation and activation of GSK3beta) (71). However, until now, no clinical study has confirmed the antineoplastic activities of DCA. Given that the big media pressure on this issue and the experience with MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) patients treated with DCA (Kaufmann et al, 2006), which has indicated that DCA can cause symptomatic peripheral neuropathy, large and rigorous trials would be needed. It should be stressed, however, that since DCA is a simple, not patented, chemical no funding for such trials has to be expected from industry.

Also "old" drugs or drugs used for other indications are being evaluated as possible anti-tumor agents due to their effects on glycolysis. For instance, the antifungal agent clotrimazole decreases hexokinase (HK) binding to the outer mitochondrial membrane (72) and detaches phosphofructokinase-1 (PFK-1) and aldolase from the cytoskeleton (73, 74), is known to hamper glioma growth in vivo (75), to trigger apoptosis in breast and lung cancer cells in vitro (76, 77), and to specifically inhibit glycolysis in breast cancer tissue (78).

The metabolism of glutamine has also been targeted for therapeutic approaches (79). Actually, three glutamine analogues, acivicin, 6-diazo-5-oxo-L-norleucine (L-DON) and azaserine, were demonstrated many years ago to have a significant cytotoxic activity in vitro but attempts to translate these effects in vivo were hampered by significant multi-organ toxicity (80). One of the first effective antileukemic drugs to be introduced, the enzyme L-asparaginase (81, 82), works as a metabolic drug, depleting extracellular asparagine with particularly severe consequences for those tumor cells that express low levels of Asparagine Synthetase (83) such as ALL blasts (84) and NK lymphomas (85). However, most of the commercially available forms of L-asparaginase have also a glutaminolytic activity and, hence, cause glutamine, as well as, asparagine depletion (86). The renewed interest for glutamine role in cancer cells is leading to the reconsideration of L-asparaginase in non haematological tumors with some encouraging results in vitro (87). Since glutaminase is requested for glutamine metabolism in tumor cells, glutaminase is also a potential therapeutic target, with promising results in vitro and in xenografts (88).

Besides exhibiting direct anticancer activities, metabolic interference with anaplerotic fuelling may sensitize tumor cells to chemotherapy, radiotherapy or biological agents that promote apoptosis and, conversely, metabolic effects of oncogenic mutations strongly contribute to resistant phenotypes. For instance, the antiapoptotic activities of AKT, which underlie several drug resistant tumor phenotypes, need adequate glucose availability, so that glucose shortage abolishes pro-life effects of the kinase (89). 2DG powerfully synergizes the antineoplastic effects of inhibitors of histone deacetylases on glioblastoma cells (90) and of doxorubicin, 5 FU, cyclophosphamide, and herceptin in breast cancer cells (91). Both 2DG and the fatty acid beta-oxidation inhibitor etomoxir synergize a panel of anticancer drugs (92). 2DG also sensitizes melanoma cells to TRAIL – (93) and TNFα-induced cell death (94), while 3-Bromopyruvate potentiates the anti-proliferative effects of low-dose platinum, even in resistant p53-deficient cells (95). Significant synergy with chemotherapeutic drugs is also exhibited by DCA (96) and phloretin, an inhibitor of GLUT transporters (97).

Finally, the possibility to modulate cancer growth with diet should be mentioned. Indeed, Ketogenic Diet (KD), a low-carbohydrate, high-fat regimen particularly rich in medium chain fatty acids currently in use in selected neurological disorders (98, 99), has the capability to reproduce the effects of glycolysis inhibitors, at least in term of reducing glucose availability and hence glycolytic flux in cancer cells. Most of

the evidence comes from work on brain tumors (100, 101). When treated with ketogenic diet, murine glioma models exhibit prolonged survival of the host, markedly decreased growth rates, reduced ROS production, and overall reversion of tumor-associated gene expression pattern (102). Anecdotic evidence in humans is consistent with those findings (103, 104) with the supplementary benefit of an improved quality of life (105). However, KD is substantially ineffective in the control of tumour growth in patients with tuberous sclerosis (106) and data from specific clinical trials are not yet available. The antitumor effects of KD, which are not restricted to gliomas (107), may involve the activation of PPAR by fatty acids, suggesting that components of KD may directly affect metabolic alterations in tumors (108).

Conclusions

Specificity for cancer cells, significant antitumor efficacy and failure to promote resistant phenotypes are the goals for any antitumor drug. No metabolic drug developed thus far fulfils these requirements. This is likely due to the relevance of the genetic context to determine which enzyme (pathway) is essential for a given cancer cell and to the remarkable ability of tumor cells to adapt to alterations in critical metabolic pathways. These caveats indicate that accurate selection of cancer phenotypes (and hence of patients in the future) will be needed to identify what enzyme(s) is (are) to be targeted in a given tumor (109).

The two most important reasons for the poor performance of antineoplastic drugs are the high adaptability of cancer cells and the molecular heterogeneity even within a single tumour. For this reason, it is highly unlikely that the metabolic approaches to cancer therapy cited above will ever yield a monotherapy, although intensive research work is trying to connect specific metabolic features to specific molecular alterations in a growing number of cancer types.

Rather, metabolic therapies will likely add to other therapeutic approaches. Indeed, metabolic alterations often constitute the cause of tumour resistance to radiotherapy and chemotherapy, so that normalization of tumor cell metabolism would probably also revert its resistance to therapy. However, the extent to which metabolic alterations contribute to cancer phenotype, and even to cancerogenesis, is no more underestimated and drugs aimed to selectively address these alterations are expected to enter soon in clinical practice.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100: 57-70.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-74.
3. Tennant DA, Duran RV, Gottlieb E. Targeting metabolic transformation for cancer therapy. *Nat Rev Cancer* 2010; 10: 267-77.
4. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* 2011; 11: 325-37.
5. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? *Biochim Biophys Acta* 2011; 1807: 552-61.
6. Mankoff DA, Bellon JR. Positron-emission tomographic imaging of cancer: glucose metabolism and beyond. *Semin Radiat Oncol* 2001; 11: 16-27.
7. Thompson CB, Bauer DE, Lum JJ, et al. How do cancer cells acquire the fuel needed to support cell growth? *Cold Spring Harb Symp Quant Biol* 2005; 70: 357-62.
8. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 2008; 7: 11-20.
9. Pastorino JG, Hoek JB, Shulga N. Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity. *Cancer Res* 2005; 65: 10545-54.
10. Naviaux RK. Oxidative Shielding or Oxidative Stress? *J Pharmacol Exp Ther* 2012; 342: 608-18.
11. Crabtree HG. Observations on the carbohydrate metabolism of tumours. *Biochem J* 1929; 23: 536-45.
12. Bonnet S, Archer SL, Allalunis-Turner J, et al. A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 2007; 11: 37-51.
13. Behera MA, Dai Q, Garde R, Saner C, Jungheim E, Price TM. Progesterone stimulates mitochondrial activity with subsequent inhibition of apoptosis in MCF-10A benign breast epithelial cells. *Am J Physiol Endocrinol Metab* 2009; 297: E1089-1096.
14. Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. *Cell* 2008; 134: 703-7.
15. Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; 11: 85-95.
16. Gruning NM, Rinnerthaler M, Bluemlein K, et al. Pyruvate kinase triggers a metabolic feedback loop that controls

redox metabolism in respiring cells. *Cell Metab* 2011; 14: 415-27.
17. Anastasiou D, Poulogiannis G, Asara JM, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* 2011; 334: 1278-83.
18. Matoba S, Kang JG, Patino WD, et al. p53 regulates mitochondrial respiration. *Science* 2006; 312: 1650-3.
19. Chen JQ, Russo J. Dysregulation of glucose transport, glycolysis, TCA cycle and glutaminolysis by oncogenes and tumor suppressors in cancer cells. *Biochim Biophys Acta* 2012; 1826: 370-84.
20. Kawauchi K, Araki K, Tobiume K, Tanaka N. p53 regulates glucose metabolism through an IKK-NF-kappaB pathway and inhibits cell transformation. *Nat Cell Biol* 2008; 10: 611-8.
21. Jiang P, Du W, Wang X, et al. p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. *Nat Cell Biol* 2011; 13: 310-6.
22. Li T, Kon N, Jiang L, Tan M, et al. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. *Cell* 2012; 149: 1269-83.
23. Won KY, Lim SJ, Kim GY, et al. Regulatory role of p53 in cancer metabolism via SCO2 and TIGAR in human breast cancer. *Hum Pathol* 2012; 43: 221-8.
24. Suzuki S, Tanaka T, Poyurovsky MV, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. *Proc Natl Acad Sci U S A* 2010; 107: 7461-6.
25. Shen L, Sun X, Fu Z, Yang G, Li J, Yao L. The fundamental role of the p53 pathway in tumor metabolism and its implication in tumor therapy. *Clin Cancer Res* 2012; 18: 1561-7.
26. Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z. Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proc Natl Acad Sci U S A* 2010; 107: 7455-60.
27. Wise DR, DeBerardinis RJ, Mancuso A, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A* 2008; 105: 18782-7.
28. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clin Cancer Res* 2009; 15: 6479-83.
29. Yeung SJ, Pan J, Lee MH. Roles of p53, MYC and HIF-1 in regulating glycolysis - the seventh hallmark of cancer. *Cell Mol Life Sci* 2008; 65: 3981-99.
30. Ying H, Kimmelman AC, Lyssiotis CA, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 2012; 149: 656-70.
31. Noch E, Khalili K. Oncogenic viruses and tumor glucose metabolism: like kids in a candy store. *Mol Cancer Ther* 2012; 11: 14-23.
32. Locasale JW, Cantley LC, Vander Heiden MG. Cancer's insatiable appetite. *Nat Biotechnol* 2009; 27: 916-7.
33. Favier J, Briere JJ, Burnichon N, et al. The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS One* 2009; 4: e7094.
34. Mullen AR, Deberardinis RJ. Genetically-defined metabolic reprogramming in cancer. *Trends Endocrinol Metab* 2012; 23: 552-9.
35. Toro JR, Nickerson ML, Wei MH, et al. Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am J Hum Genet* 2003; 73: 95-106.
36. Astuti D, Douglas F, Lennard TW, et al. Germline SDHD mutation in familial phaeochromocytoma. *Lancet* 2001; 357: 1181-2.
37. Baysal BE, Ferrell RE, Willett-Brozick JE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000; 287: 848-51.
38. Tong WH, Sourbier C, Kovtunovych G, et al. The glycolytic shift in fumarate-hydratase-deficient kidney cancer lowers AMPK levels, increases anabolic propensities and lowers cellular iron levels. *Cancer Cell* 2011; 20: 315-27.
39. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009; 360: 765-73.
40. Ward PS, Patel J, Wise DR, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 2010; 17: 225-34.
41. Amary MF, Bacsi K, Maggiani F, et al. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. *J Pathol* 2011; 224: 334-43.
42. Turcan S, Rohle D, Goenka A, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 2012; 483: 479-83.
43. Borodovsky A, Seltzer MJ, Riggins GJ. Altered cancer cell metabolism in gliomas with mutant IDH1 or IDH2. *Curr Opin Oncol* 2012; 24: 83-9.
44. Zhang WC, Shyh-Chang N, Yang H, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. *Cell* 2012; 148: 259-72.
45. Liu Y, Borchert GL, Donald SP, Diwan BA, Anver M, Phang JM. Proline oxidase functions as a mitochondrial tumor suppressor in human cancers. *Cancer Res* 2009; 69: 6414-22.
46. Liu W, Le A, Hancock C, et al. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proc Natl Acad Sci U S A* 2012; 109: 8983-8.
47. Liu W, Glunde K, Bhujwalla ZM, Raman V, Sharma A, Phang JM. Proline oxidase promotes tumor cell survival in hypoxic tumor microenvironments. *Cancer Res* 2012; 72: 3677-86.
48. Locasale JW, Grassian AR, Melman T, et al. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat Genet* 2011; 43: 869-74.
49. Possemato R, Marks KM, Shaul YD, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011; 476: 346-50.
50. Zawacka-Pankau J, Grinkevich VV, Hunten S, et al. Inhibition of glycolytic enzymes mediated by pharmacological-

ly activated p53: targeting Warburg effect to fight cancer. *J Biol Chem* 2011; 286: 41600-15.
51. El Mjiyad N, Caro-Maldonado A, Ramirez-Peinado S, Munoz-Pinedo C. Sugar-free approaches to cancer cell killing. *Oncogene* 2011; 30: 253-64.
52. Mack P, Ahren B, Jeppsson B, Kan Z, Bengmark S. Influence of 2-deoxy-D-glucose and arterial ischaemia on glucose oxidation and growth of liver cancer in the rat. *Eur J Cancer Clin Oncol* 1988; 24: 1433-7.
53. Geschwind JF, Ko YH, Torbenson MS, Magee C, Pedersen PL. Novel therapy for liver cancer: direct intraarterial injection of a potent inhibitor of ATP production. *Cancer Res* 2002; 62: 3909-13.
54. Cardaci S, Desideri E, Ciriolo MR. Targeting aerobic glycolysis: 3-bromopyruvate as a promising anticancer drug. *J Bioenerg Biomembr* 2012; 44: 17-29.
55. Liu Y, Zhang W, Cao Y, Liu Y, Bergmeier S, Chen X. Small compound inhibitors of basal glucose transport inhibit cell proliferation and induce apoptosis in cancer cells via glucose-deprivation-like mechanisms. *Cancer Lett* 2010; 298: 176-85.
56. Fiume L, Manerba M, Vettraino M, Di Stefano G. Impairment of aerobic glycolysis by inhibitors of lactic dehydrogenase hinders the growth of human hepatocellular carcinoma cell lines. *Pharmacology* 2010; 86: 157-62.
57. Boidot R, Vegran F, Meulle A, et al. Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors. *Cancer Res* 2012; 72: 939-48.
58. Gerlinger M, Santos CR, Spencer-Dene B, et al. Genome-wide RNA interference analysis of renal carcinoma survival regulators identifies MCT4 as a Warburg effect metabolic target. *J Pathol* 2012; 227: 146-56.
59. Locasale JW, Cantley LC. Metabolic flux and the regulation of mammalian cell growth. *Cell Metab* 2011; 14: 443-51.
60. Derdak Z, Mark NM, Beldi G, Robson SC, Wands JR, Baffy G. The mitochondrial uncoupling protein-2 promotes chemoresistance in cancer cells. *Cancer Res* 2008; 68: 2813-9.
61. Baffy G, Derdak Z, Robson SC. Mitochondrial recoupling: a novel therapeutic strategy for cancer? *Br J Cancer* 2011; 105: 469-74.
62. Pierotti MA, Berrino F, Gariboldi M, et al. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. *Oncogene* 2012. doi: 10.1038/onc.2012.181.
63. Ben Sahra I, Laurent K, Giuliano S, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res* 2010; 70: 2465-75.
64. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 2007; 67: 6745-52.
65. Fingrut O, Reischer D, Rotem R, et al. Jasmonates induce nonapoptotic death in high-resistance mutant p53-expressing B-lymphoma cells. *Br J Pharmacol* 2005; 146: 800-8.
66. Michelakis ED, Webster L, Mackey JR. Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. *Br J Cancer* 2008; 99: 989-94.
67. Michelakis ED, Sutendra G, Dromparis P, et al. Metabolic modulation of glioblastoma with dichloroacetate. *Sci Transl Med* 2010; 2: 31ra34.
68. Wong JY, Huggins GS, Debidda M, Munshi NC, De Vivo I. Dichloroacetate induces apoptosis in endometrial cancer cells. *Gynecol Oncol* 2008; 109: 394-402.
69. Cao W, Yacoub S, Shiverick KT, et al. Dichloroacetate (DCA) sensitizes both wild-type and over expressing Bcl-2 prostate cancer cells in vitro to radiation. *Prostate* 2008; 68: 1223-31.
70. Stockwin LH, Yu SX, Borgel S, et al. Sodium dichloroacetate selectively targets cells with defects in the mitochondrial ETC. *Int J Cancer* 2010; 127: 2510-9.
71. Sutendra G, Dromparis P, Kinnaird A, et al. Mitochondrial activation by inhibition of PDKII suppresses HIF1a signaling and angiogenesis in cancer. *Oncogene* 2012. doi: 10.1038/onc.2012.198.
72. Majewski N, Nogueira V, Bhaskar P, et al. Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol Cell* 2004; 16: 819-30.
73. Glass-Marmor L, Beitner R. Detachment of glycolytic enzymes from cytoskeleton of melanoma cells induced by calmodulin antagonists. *Eur J Pharmacol* 1997; 328: 241-8.
74. Zancan P, Rosas AO, Marcondes MC, Marinho-Carvalho MM, Sola-Penna M. Clotrimazole inhibits and modulates heterologous association of the key glycolytic enzyme 6-phosphofructo-1-kinase. *Biochem Pharmacol* 2007; 73: 1520-7.
75. Khalid MH, Tokunaga Y, Caputy AJ, Walters E. Inhibition of tumor growth and prolonged survival of rats with intracranial gliomas following administration of clotrimazole. *J Neurosurg* 2005; 103: 79-86.
76. Furtado CM, Marcondes MC, Sola-Penna M, de Souza ML, Zancan P. Clotrimazole preferentially inhibits human breast cancer cell proliferation, viability and glycolysis. *PLoS One* 2012; 7: e30462.
77. Penso J, Beitner R. Clotrimazole decreases glycolysis and the viability of lung carcinoma and colon adenocarcinoma cells. *Eur J Pharmacol* 2002; 451: 227-35.
78. Coelho RG, Calaca Ide C, Celestrini Dde M, Correia AH, Costa MA, Sola-Penna M. Clotrimazole disrupts glycolysis in human breast cancer without affecting non-tumoral tissues. *Mol Genet Metab* 2011; 103: 394-8.
79. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci* 2010; 35: 427-33.
80. Ahluwalia GS, Grem JL, Hao Z, Cooney DA. Metabolism and action of amino acid analog anti-cancer agents. *Pharmacol Ther* 1990; 46: 243-71.
81. Broome JD. Evidence that the L-asparaginase of guinea pig serum is responsible for its antilymphoma effects. I. Properties of the L-asparaginase of guinea pig serum in relation to those of the antilymphoma substance. *J Exp Med* 1963; 118: 99-120.

82. Hill JM, Roberts J, Loeb E, Khan A, MacLellan A, Hill RW. L-asparaginase therapy for leukemia and other malignant neoplasms. Remission in human leukemia. *JAMA* 1967; 202: 882-8.
83. Broome JD. Studies on the mechanism of tumor inhibition by L-asparaginase. Effects of the enzyme on asparagine levels in the blood, normal tissues, and 6C3HED lymphomas of mice: differences in asparagine formation and utilization in asparaginase-sensitive and -resistant lymphoma cells. *J Exp Med* 1968; 127: 1055-72.
84. Haskell CM, Canellos GP. l-asparaginase resistance in human leukemia - asparagine synthetase. *Biochem Pharmacol* 1969; 18: 2578-80.
85. Matsumoto Y, Nomura K, Kanda-Akano Y, e al. Successful treatment with Erwinia L-asparaginase for recurrent natural killer/T cell lymphoma. *Leuk Lymphoma* 2003; 44: 879-82.
86. Covini D, Tardito S, Bussolati O, e al. Expanding targets for a metabolic therapy of cancer: L-asparaginase. *Recent Pat Anticancer Drug Discov* 2012; 7: 4-13.
87. Tardito S, Chiu M, Uggeri J, et al. L-Asparaginase and inhibitors of glutamine synthetase disclose glutamine addiction of beta-catenin-mutated human hepatocellular carcinoma cells. *Curr Cancer Drug Targets* 2011; 11: 929-43.
88. Katt WP, Ramachandran S, Erickson JW, Cerione RA. Dibenzophenanthridines as inhibitors of glutaminase C and cancer cell proliferation. *Mol Cancer Ther* 2012; 11: 1269-78.
89. Coloff JL, Mason EF, Altman BJ, et al. Akt requires glucose metabolism to suppress puma expression and prevent apoptosis of leukemic T cells. *J Biol Chem* 2011; 286: 5921-33.
90. Egler V, Korur S, Failly M, et al. Histone deacetylase inhibition and blockade of the glycolytic pathway synergistically induce glioblastoma cell death. *Clin Cancer Res* 2008; 14: 3132-40.
91. Zhang F, Aft RL. Chemosensitizing and cytotoxic effects of 2-deoxy-D-glucose on breast cancer cells. *J Cancer Res Ther* 2009; 5 Suppl 1: S41-43.
92. Hernlund E, Ihrlund LS, Khan O, et al. Potentiation of chemotherapeutic drugs by energy metabolism inhibitors 2-deoxyglucose and etomoxir. *Int J Cancer* 2008; 123: 476-83.
93. Liu H, Jiang CC, Lavis CJ, et al. 2-Deoxy-D-glucose enhances TRAIL-induced apoptosis in human melanoma cells through XBP-1-mediated up-regulation of TRAIL-R2. *Mol Cancer* 2009; 8: 122.
94. Halicka HD, Ardelt B, Li X, Melamed MM, Darzynkiewicz Z. 2-Deoxy-D-glucose enhances sensitivity of human histiocytic lymphoma U937 cells to apoptosis induced by tumor necrosis factor. *Cancer Res* 1995; 55: 444-9.
95. Ihrlund LS, Hernlund E, Khan O, Shoshan MC. 3-Bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. *Mol Oncol* 2008; 2: 94-101.
96. Cairns RA, Papandreou I, Sutphin PD, Denko NC. Metabolic targeting of hypoxia and HIF1 in solid tumors can enhance cytotoxic chemotherapy. *Proc Natl Acad Sci U S A* 2007; 104: 9445-50.
97. Cao X, Fang L, Gibbs S, et al. Glucose uptake inhibitor sensitizes cancer cells to daunorubicin and overcomes drug resistance in hypoxia. *Cancer Chemother Pharmacol* 2007; 59: 495-505.
98. Baranano KW, Hartman AL. The ketogenic diet: uses in epilepsy and other neurologic illnesses. *Curr Treat Options Neurol* 2008; 10: 410-9.
99. Kossoff EH, Hartman AL. Ketogenic diets: new advances for metabolism-based therapies. *Curr Opin Neurol* 2012; 25: 173-8.
100. Seyfried TN, Kiebish MA, Marsh J, Shelton LM, Huysentruyt LC, Mukherjee P. Metabolic management of brain cancer. *Biochim Biophys Acta* 2011; 1807: 577-94.
101. Seyfried TN, Marsh J, Shelton LM, Huysentruyt LC, Mukherjee P. Is the restricted ketogenic diet a viable alternative to the standard of care for managing malignant brain cancer? *Epilepsy Res* 2012; 100: 310-26.
102. Stafford P, Abdelwahab MG, Kim do Y, Preul MC, Rho JM, Scheck AC. The ketogenic diet reverses gene expression patterns and reduces reactive oxygen species levels when used as an adjuvant therapy for glioma. *Nutr Metab (Lond)* 2010; 7: 74.
103. Nebeling LC, Miraldi F, Shurin SB, Lerner E. Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: two case reports. *J Am Coll Nutr* 1995; 14: 202-8.
104. Zuccoli G, Marcello N, Pisanello A, et al. Metabolic management of glioblastoma multiforme using standard therapy together with a restricted ketogenic diet: Case Report. *Nutr Metab (Lond)* 2010; *7: 33.*
105. Schmidt M, Pfetzer N, Schwab M, Strauss I, Kammerer U. Effects of a ketogenic diet on the quality of life in 16 patients with advanced cancer: A pilot trial. *Nutr Metab (Lond)* 2011; 8: 54.
106. Chu-Shore CJ, Thiele EA. Tumor growth in patients with tuberous sclerosis complex on the ketogenic diet. *Brain Dev* 2010; 32: 318-22.
107. Otto C, Kaemmerer U, Illert B, et al. Growth of human gastric cancer cells in nude mice is delayed by a ketogenic diet supplemented with omega-3 fatty acids and medium-chain triglycerides. *BMC Cancer* 2008; 8: 122.
108. Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol* 2012; 3: 59.
109. Nilsson LM, Forshell TZ, Rimpi S, et al. Mouse genetics suggests cell-context dependency for Myc-regulated metabolic enzymes during tumorigenesis. *PLoS Genet* 2012; 8: e1002573.

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Correspondence: Ovidio Bussolati
Unit of General Pathology, Department of Biomedical, Biotechnological and Translational Sciences
University of Parma
Via Volturno, 39 - 43125 Parma (Italy)
E-mail: ovidio.bussolati@unipr.it