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Review

REVIEW

Metabolic reprogramming and dysregulated metabolism: cause, consequence and/or enabler of environmental carcinogenesis?

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Abstract

Environmental contributions to cancer development are widely accepted, but only a fraction of all pertinent exposures have probably been identified. Traditional toxicological approaches to the problem have largely focused on the effects of individual agents at singular endpoints. As such, they have incompletely addressed both the pro-carcinogenic contributions of environmentally relevant low-dose chemical mixtures and the fact that exposures can influence multiple cancer-

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associated endpoints over varying timescales. Of these endpoints, dysregulated metabolism is one of the most common and recognizable features of cancer, but its specific roles in exposure-associated cancer development remain poorly understood. Most studies have focused on discrete aspects of cancer metabolism and have incompletely considered both its dynamic integrated nature and the complex controlling influences of substrate availability, external trophic signals and environmental conditions. Emerging high throughput approaches to environmental risk assessment also do not directly address the metabolic causes or consequences of changes in gene expression. As such, there is a compelling need to establish common or complementary frameworks for further exploration that experimentally and conceptually consider the gestalt of cancer metabolism and its causal relationships to both carcinogenesis and the development of other cancer hallmarks. A literature review to identify environmentally relevant exposures unambiguously linked to both cancer development and dysregulated metabolism suggests major gaps in our understanding of exposure-associated carcinogenesis and metabolic reprogramming. Although limited evidence exists to support primary causal roles for metabolism in carcinogenesis, the universality of altered cancer metabolism underscores its fundamental biological importance, and multiple pleiomorphic, even dichotomous, roles for metabolism in promoting, antagonizing or otherwise enabling the development and selection of cancer are suggested.

Abbreviations

αKG	α-ketoglutarate
3-PG	3-phosphoglycerate
6PD	6-phosphogluconate dehydrogenase
ACC	acetyl-coA carboxylase
ACL	adenosine triphosphate–citrate lyase
AEC	adenylate energy charge
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMPK	adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
EPA	United States Environmental Protection Agency
ETC	electron transport chain
FA	fatty acid
FASN	fatty acid synthetase
GAPDH	glyceraldehyde phosphate dehydrogenase
Glc	glucose
Gln	glutamine
Glu	glutamate
GPx	glutathione peroxidase
GSH	reduced glutathione
HK	hexokinase
2HG	2-hydroxyglutarate
HIFα	hypoxia-inducible factor-α
HTS	high throughput screening
IDH	isocitrate dehydrogenase
LDH	lactate dehydrogenase
LPL	lipoprotein lipase
MAGL	monoacylglycerol lipase
NAD(P)H/ NAD(P)$^+$	nicotinamide adenine dinucleotides
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
PK	pyruvate kinase
PPP	pentose phosphate pathway
ROS	reactive oxygen species
SCD	stearoyl-coA desaturase
SDH	succinate dehydrogenase
Ser	serine
TAG	triacylglycerol
TCA	tricarboxylic acid
TIGAR	Tp53-induced glycolysis and apoptosis regulator
VDAC	voltage-dependent anion channel

Introduction

Environmental contributions to cancer development are widely recognized and involve factors as diverse as diet, tobacco and alcohol use, reproductive and sexual behaviors, occupational exposures, environmental pollutants, medical therapies, geophysical factors and infectious agents (1,2). Corresponding effects on intermediary metabolism and specific metabolic contributions to the development of cancer, however, have been incompletely explored. Little is known about the specific causal and spatiotemporal relationships between exposures, dysregulated metabolism and the development of cancer and its associated phenotypic hallmarks (Figure 1) (3), including the 'missing hallmark' of dedifferentiation (4).

Biochemical characterization of cancers in the early-to-mid 20th century established many of the fundamental metabolic characteristics of cancer cells (5–8). Interest in cancer metabolism subsequently waned with the advent of genetic sequencing and molecular biology, shifting instead to the study of mutagenic effects and the regulation of gene expression. Interest has subsequently rebounded over the course of the past few decades, however, as investigators sought to better delineate the mechanistic underpinnings and functional importance of demonstrable genetic and epigenetic changes associated with dysregulated cancer metabolism. Alterations in the expression of numerous genes encoding metabolic enzymes, transporters and regulatory effectors have been associated with cancer. Many address known biochemical features of cancer, whereas others may suggest novel unexplored or previously unappreciated associations. Warburg originally proposed that fixed mitochondrial defects were primarily responsible for both cancer development and its associated highly glycolytic phenotype, but his own data and that of his contemporaries (6,9,10) demonstrated not only preservation of oxidative metabolism in cancer (5,11), but also its persistence in the absence of exogenous substrates (5), suggesting an expanded metabolic repertoire and an intrinsic capacity to oxidatively utilize endogenous substrates when exogenous substrates are not available (6,12).

Cancer-associated changes in metabolism may reflect alterations in either metabolic capacity or control—or both. Changes in capacity are well described, although altered control may ultimately be of greater relative importance (13). Since control does not reside at a single point in any metabolic pathway (13) and controlling factors differ between intact cells and *in vitro* assays, observed changes in individual pathway elements do not always translate into metabolic flux changes and *vice versa*. Cancer cell phenotypes are also neither fixed nor specific for cancer (4,14,15),

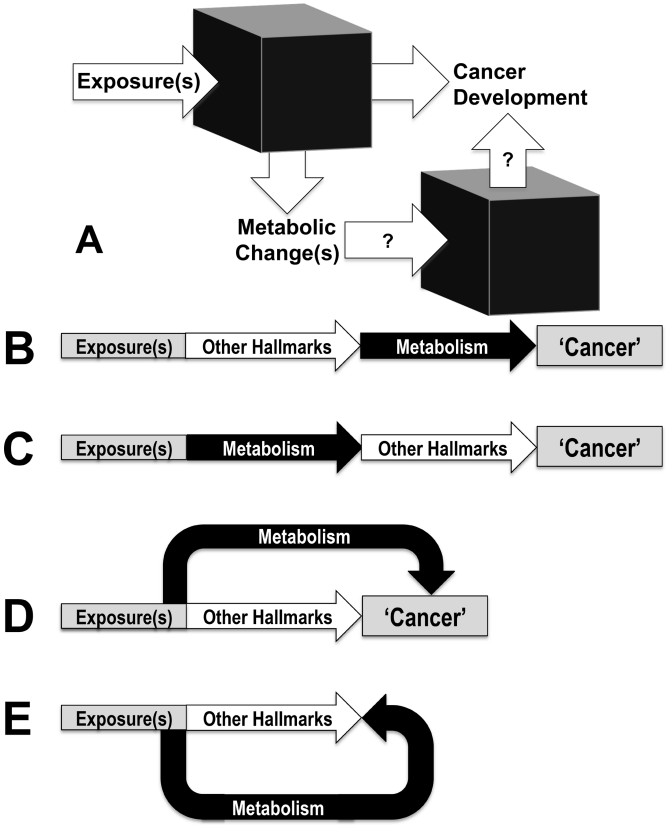

Figure 1. Dysregulated metabolism in cancer development due to environmental exposures and potential relationships to other cancer hallmarks. The specific sequence, priority and relevance of reprogramming and dysregulated metabolism in the (often decades-long) carcinogenic continuum between environmental exposures and cancer development are incompletely understood. Specific relationships between altered metabolism and other cancer hallmarks are also poorly delineated. Much of our specific knowledge of cancer metabolism is largely associative in nature, and a deeper understanding of the numerous remaining mechanistic 'black boxes' (**A**) is needed before specific metabolic changes can be optimally exploited for preventative or therapeutic benefit. For example, it is not clear whether altered metabolism is a cause or a consequence of cancer development—or both. In principle, the contributions of metabolism to carcinogenesis may operate in series (**B**, **C**), in parallel (**D**, **E**) or even in opposition (**E**) to the contributions of other hallmarks of cancer (e.g. via modulation of oxidative stress). Temporally, changes in metabolism may also precede (C), follow (B) or coincide with (D, E) other key determinants of the carcinogenic program. Since metabolism is not a singular entity, the specific type of relationship observed for a given aspect of metabolism is not mutually exclusive of different types of relationships with other aspects of metabolism.

and it is a basic biological truism that distinct cell types or tissues respond differently to common extrinsic stimuli, including hormones, physical stimuli, environmental stress or chemical exposures (16,17). Although metabolic derangements in cancer are widely recognized and accepted as fundamental to the nature of cancer, much, if not most, of the literature in this domain is descriptive or associative in nature. At present, there are limited data directly supporting a primary metabolic link between environmental exposures and cancer development. The continually 'evolving, dynamic, and heterogeneous' nature of cancer (4,15) thus poses problems for the treatment, as well as the study, of cancer, so a better understanding of the determinants and functional consequences of such heterogeneity is needed (16).

The identification and characterization of specific causal relationships between common environmental exposures, carcinogenesis and associated metabolic changes is methodologically challenging, in part, because exposures typically occur in the context of complex mixtures at concentrations not commonly examined in standard toxicity or carcinogenicity testing. Biological effects of individual 'low dose' exposures also frequently reflect biphasic dose–response relationships, sometimes with directionally opposite biological responses that would not be anticipated on the basis of traditional testing (17,18). The term 'low dose' can also easily—and inappropriately—be misconstrued as suggesting an absence of biological effects. In contradistinction to conventional toxicological dogma, however, there may be no basal exposure threshold below which is completely bereft of biological effects (17–19).

The present review—reflecting the efforts of 30 authors representing 21 institutions in 8 countries—broadly addresses these issues and is a direct outgrowth of 'The Halifax Project', an international initiative launched in 2011 by the non-profit organization Getting to Know Cancer (http://gettingtoknowcancer.org/) with the explicit aim of producing a series of overarching reviews assessing the contributions of environmentally relevant exposures to the development of cancer and its associated phenotypic hallmarks. This review was specifically undertaken to explore what is—and is not—presently known about the roles of dysregulated metabolism in environmental carcinogenesis, and it was conducted with the hope of stimulating additional interest in cancer metabolism and identifying critical knowledge gaps and unmet research needs to help direct future research. The authors were also specifically tasked to identify key metabolic targets for disruption or dysregulation, as well as a corresponding list of prototypical environmental exposures with the potential to act on these targets. Prototypical exposures were selected on the basis of environmental ubiquity and the demonstrated ability to act on selected targets to mimic specific cancer-associated phenotypes. To focus efforts

on the identification of novel and underexplored exposures, both lifestyle-related exposures and chemicals known as 'Carcinogenic to Humans' (e.g. Group 1 carcinogens, International Agency for Research on Cancer) were specifically excluded from primary consideration (see the accompanying capstone article in this issue for details (20)). The focus on environmentally relevant exposures was also intentionally restrictive to provide insights that would be of value to cancer researchers interested in the effects of complex environmental chemical mixtures, as well as investigators and policymakers involved in environmental risk assessment and management.

Given the importance and complexity of the subject matter and to obviate common misconceptions, this review briefly addresses our present understanding of cancer metabolism before tackling its potential roles in exposure-associated carcinogenesis. The metabolism of carbohydrates, lipids and proteins are individually considered for characteristic changes associated with cancer, as well as catabolic and anabolic contributions to its highly proliferative phenotype. Dichotomous roles for metabolism in both the promotion and amelioration of cellular stress (e.g. oxidative, hypoxic, nutritional and physical stress) are also considered. Finally, individual relationships between dysregulated metabolism and other hallmarks of cancer (e.g. apoptotic resistance, genomic mutability, replicative immortality, sustained proliferation, angiogenesis, tissue invasion and metastasis) are briefly addressed.

Metabolic reprogramming and dysregulation in cancer

Metabolic dysregulation is one of the most common and recognizable features of cancer (21,22), although associated metabolic phenotypes are not necessarily fixed (4) and can change in response to substrate availability and the metabolic demands of proliferation, growth and cell survival. Proliferative cancer cells alter their ability to metabolize carbohydrates, lipids and peptides to meet increased energy demands and provide anabolic precursors needed to support obligatory nucleic acid and protein biosynthesis and membrane biogenesis (21,23,24). These processes are intimately intertwined and result in an expanded metabolic repertoire that affords increased flexibility to adapt to increased cellular demands, changing environmental conditions and fluctuating substrate availability.

Carbohydrate metabolism in cancer

All mammalian cells require amphibolic glucose (Glc) metabolism via glycolysis and the tricarboxylic acid (TCA) cycle to meet catabolic demands and support anabolic carbon needs (Figures 2 and 3). It has been recognized for nearly a century that cancer cells increase glycolytic lactate production independent of O_2 availability (5,6,8,11,23). Glycolytic capacity and Glc flux rates, however, greatly exceed the anabolic and catabolic needs of both normal and cancer cells (13,25). In normal cells, lactate production is reduced in the presence of O_2, a suppressive response commonly known as the Pasteur effect. Although partially preserved in cancer (7), increased lactate generation is still observed in the presence, as well as absence, of O_2 (5,6). This so-called aerobic glycolysis probably reflects simultaneous NAD^+/NADH coupling between glyceraldehyde phosphate dehydrogenase (GAPDH) and both lactate dehydrogenase (LDH) and the mitochondrial malate–aspartate shuttle system (Figure 3, *right panel*), which is not typically observed in normal cells (12,26). Mitochondrial uncoupling associated with cancer may contribute to cytosolic NADH recycling to

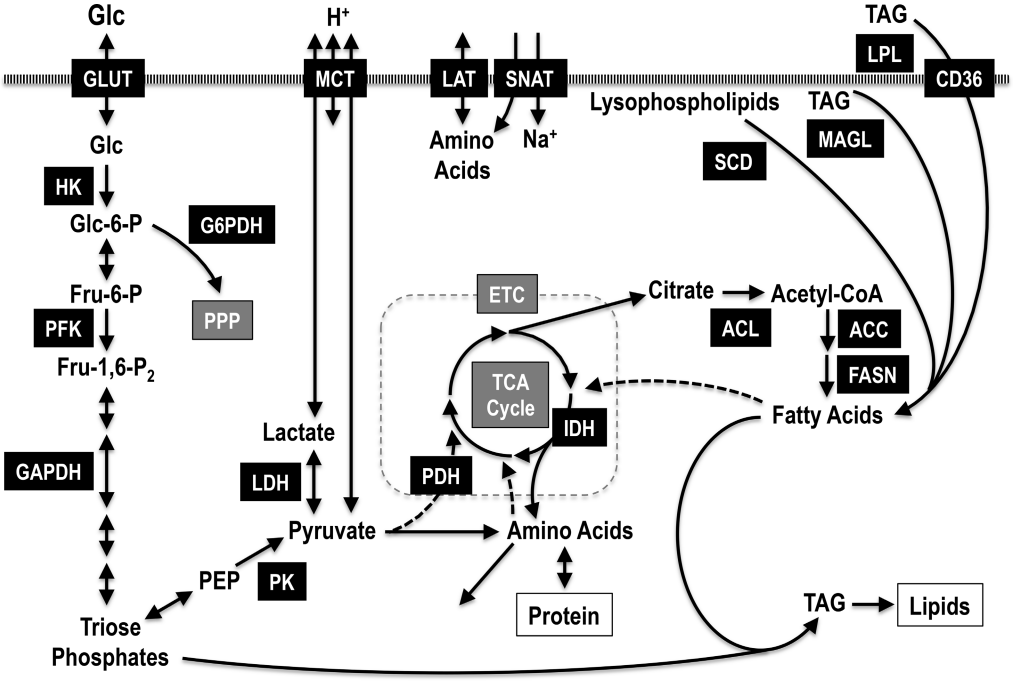

Figure 2. Selected metabolic pathways and targets implicated in cancer development and progression. Major interactions between Glc and lipid metabolism are highlighted, and the fundamental interchangeability of corresponding metabolic intermediates with amino acid metabolism via the major amphibolic pathways, glycolysis and the TCA cycle, is indicated. Gln and Ser metabolism and coupled processes such as glyceroneogenesis and one-carbon metabolism are not depicted but are addressed in the text. Major anaplerotic inputs needed to counterbalance cataplerotic carbon losses from the TCA cycle are indicated by dashed arrows. Major transport mechanisms for the transcellular movement of Glc (GLUT), amino acids (L-type amino acid transporters [LAT], A-type Na^+-linked amino acid transporters [SNAT]), FA (CD36) and monocarboxylates such as pyruvate and lactate (monocarboxylate transporters [MCT]) are also depicted. Both intracellular (MAGL, SCD) and extracellular (LPL) lipases are responsible for the liberation of FA moieties from more complex intracellular and extracellular lipids such as TAG and lysophospholipids.

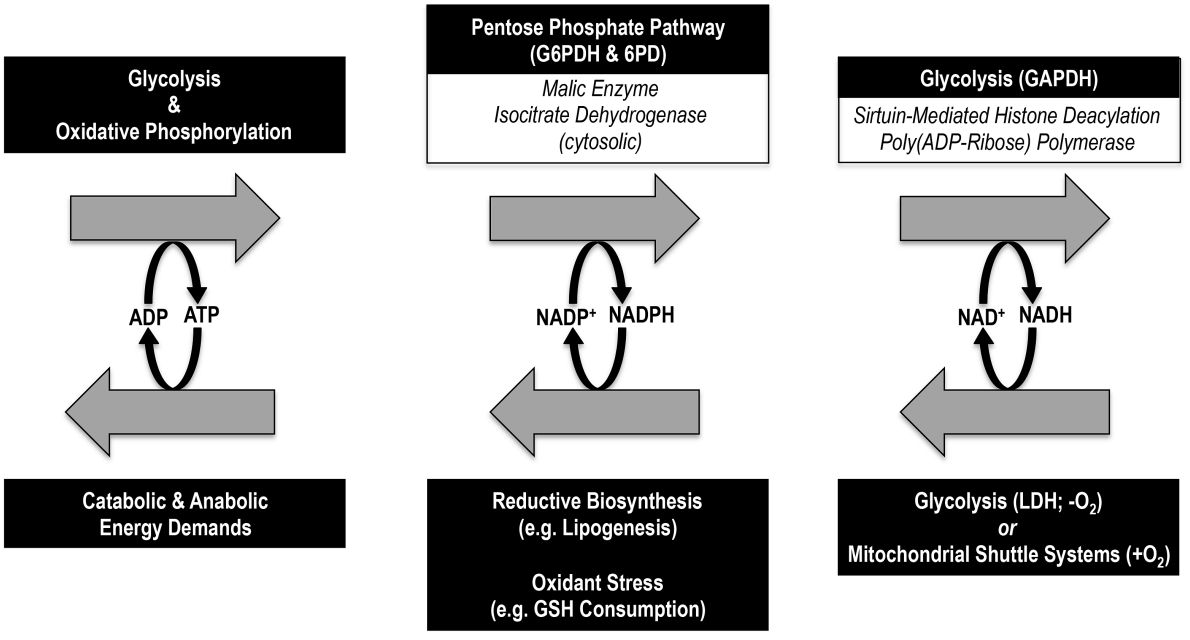

Figure 3. Major cellular metabolic coupling mechanisms. Energetic coupling between ATP generating mechanisms (i.e. glycolysis and the TCA cycle) and cellular adenosine triphosphatase (ATPase) activity is depicted (*left panel*). General redox coupling mechanisms for both the PPP (G6PDH and 6PD; *upper center panel*) and glycolytic (GAPDH, *upper right panel*) flux are similarly depicted alongside representative competing NAD(P)H-regenerating mechanisms (unshaded boxes). Ongoing metabolic flux through these pathways and cellular energy homeostasis are critically dependent upon the maintenance of these coupling mechanisms.

NAD^+ to support glycolytic flux in the setting of persistent oxidative metabolism (Figure 3) (27,28). However, given the heterogeneity and pleiomorphic nature of cancer (4,29,30), it is likely that no single mechanism fully accounts for this effect (6,24). The corresponding Crabtree (or reverse Pasteur) effect—the converse ability of glycolysis to inhibit respiration—plays a reciprocal role in the bidirectional coordination of oxidative metabolism and glycolysis in both normal cells and cancer cells (6,31,32). The Crabtree effect has been attributed to competition between glycolysis and oxidative phosphorylation for available adenosine diphosphate (ADP) and inorganic phosphate (6,8,32) and may also involve feedback inhibition of hexokinase (HK) activity (8,32) or HK–mitochondria interaction (23,33,34). The precise mechanisms underlying both effects remain incompletely delineated, however, and neither the Pasteur effect nor the Crabtree effect may have a single mechanistic explanation (8).

HK catalyze the first committed step of Glc metabolism, and thereby promote cellular Glc uptake and catalyze the initial step of all major pathways of Glc utilization (23). The high-affinity HK1 and HK2 isoforms also physically and functionally interact with mitochondria (33,35) to coordinate intra- and extramitochondrial metabolism, promote cell survival and directly antagonize apoptogenic signals converging on mitochondria (23,33). HK1 is constitutively expressed in most cells, whereas inducible HK2 is commonly overexpressed in cancer (23). Both isoforms compete for mitochondrial interaction (35), but the functional determinants and implications of this competition and the relative contributions of individual isoforms are still unknown. HK1 and HK2 are kinetically suited for distinct functional roles and are well positioned to direct both location-specific (33) and isoform-specific metabolic channeling. For example, HK1 is suited to direct Glc metabolism in a catabolic direction, whereas HK2 is better suited to channel Glc flux into anabolic pathways (35–38). Increased HK2 expression in cancer thus probably affords increased metabolic flexibility to respond to increases in both the catabolic and anabolic demands of rapid proliferative growth (36).

Pyruvate conversion to lactate by LDH is fully reversible, whereas its oxidative decarboxylation by the pyruvate dehydrogenase (PDH) complex irreversibly commits it to TCA cycle metabolism. PDH thus represents an important point of integration for regulatory feedback by its principal reaction products, acetyl-coA and NADH. As such, PDH plays a key role in coordinating intra- and extramitochondrial metabolism that can be disrupted by a variety of factors, including thiamine availability (39). Cancer cells also utilize exogenous lipids and proteins, as well as carbohydrates, but exhibit a hierarchy of substrate preferences. Cancers generally show a preference for Glc if multiple substrates are available (5,6,10,40), illustrating the extent to which substrate metabolism is intertwined at the cellular level (Figure 2).

Branched pathway flux via the pentose phosphate pathway (PPP) directly supports cancer proliferation via provision of ribose moieties and reducing equivalents needed for nucleotide and nucleic acid biosynthesis (41). PPP flux via glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PD) is also redox-coupled to reduced glutathione (GSH) generation required to support glutathione peroxidase (GPx)-mediated detoxification of both organic and inorganic peroxides (23,42). Catalase can also detoxify inorganic peroxides, but not organic peroxides. As such, GSH and GPx activity assume predominant roles in cellular responses to chronic oxidant stress involving lipid peroxidation. Interestingly, PPP flux is also directly coupled to caspase inhibition and the antagonism of apoptogenic signaling (23,43,44).

Hexosamine biosynthesis from Glc is increased in cancer and is a prerequisite for glycoprotein, glycosaminoglycan and glycosphingolipid generation (45–47). Associated O-linked protein glycosylation also contributes to several cardinal features of cancer, including increased proliferation, apoptotic resistance and enhanced invasive potential (48,49). Hexosamine flux also activates trophic factor signaling coupled to glutamine (Gln)

uptake, providing a specific mechanism for coordinating Glc and Gln metabolism in cancer (45).

Gluconeogenesis is not a major feature of most cell types, including cancers, but both glycolysis and glycyeroneogenesis share common enzymatic steps with gluconeogenesis that are relevant to cancer (50,51). Steps shared with glycolysis are sequentially and directionally reversed, and gluconeogenesis requires separate enzymes to bypass irreversible rate-controlling glycolytic reactions catalyzed by HK, phosphofructokinase (PFK) and pyruvate kinase (PK). As such, glycolysis and gluconeogenesis are reciprocally regulated and spatiotemporally segregated in different cell types and intracellular compartments. Although glycolysis is the principal source of 3-phosphoglycerate (3-PG) for glycerol and triacylglycerol (TAG) synthesis, glyceroneogenesis can also generate 3-PG to support lipogenesis, serine (Ser) biogenesis and one-carbon metabolism essential for cancer progression and growth (50,51).

Lipid metabolism in cancer

Although most early attention to cancer metabolism focused on dysregulated glycolysis, alterations in lipid metabolism are also widely recognized (6,21,52,53). In fact, increased lipogenesis is considered a hallmark of many aggressive cancers (54,55), with *de novo* fatty acid (FA) synthesis supporting membrane biogenesis, as well as the energetic demands of proliferation, even if extracellular lipid is available (21,54–56). Lipogenesis also increases membrane lipid saturation, thereby reducing susceptibility to direct peroxidation and cellular damage (55).

Acetyl-coA is required for *de novo* FA synthesis (57) and is largely generated from pyruvate by intramitochondrial PDH, which irreversibly directs glycolytic flux into the TCA cycle (Figure 2) (50). Cataplerotic citrate derived from this cycle is then converted back to acetyl-coA in the cytosol by adenosine triphosphate (ATP)–citrate lyase (ACL) (58) before conversion to malonyl-coA by acetyl-coA carboxylase (ACC). Fatty acid synthase (FASN) then catalyzes the condensation of malonyl-coA and acetyl-coA to form long-chain FA. Both ACC and FASN are rate controlling and are overexpressed in cancer (54). Interestingly, ACC also contributes to epigenetic regulation by directly competing with histone acetylation for available acetyl-coA (59). Elevated Glc utilization supports lipogenesis at multiple levels (54,58). In addition to generating pyruvate for acetyl-coA production, increased glycolytic flux supplies 3-PG for glyceroneogenesis, and parallel branched pathway flux via the PPP provides reducing power in the form of NADPH for lipid biosynthesis. The TCA cycle is carbon-neutral, so cataplerotic citrate carbon losses for lipogenic acetyl-coA formation must be offset by anaplerotic carbon input for the cycle to proceed (50). Although Glc-derived pyruvate is most important in this regard, other anaplerotic inputs such as Gln-derived α-ketoglutarate (αKG) also help balance these losses in support of *de novo* lipid biosynthesis. For example, reductive synthesis of acetyl-coA from Gln-derived αKG can occur under hypoxic conditions (57,60,61) or when HK2 cannot properly direct Glc flux into anabolic fates (38).

Lipolytic metabolism of both endogenous and exogenous lipids is also observed in cancer (6,40,53). Monoacylglycerol lipase (MAGL; Figure 2) is overexpressed in cancer and mediates FA retrieval from neutral intracellular lipids (62), whereas stearoyl-coA desaturase (SCD) mediates FA retrieval from exogenously scavenged lysophospholipids (60). In addition to these intracellular lipases, cancer cells express extracellular lipases, and co-expression of cell surface lipoprotein lipase (LPL) with CD36, which mediates FA uptake, permits the uptake and utilization of FA derived from extracellular TAG de-esterification (Figure 2) (53,63,64).

Both lipogenic and lipolytic phenotypes can co-exist in cancer (6,40,53), where FA are channeled into biosynthesis of both structural and signaling lipids (65). Lipophagy is also increasingly recognized as a regulated mechanism for intracellular lipid recycling to meet catabolic and anabolic demands (66–68). The existence of multiple FA-generating mechanisms to meet cellular needs (53,69) suggests an expanded metabolic repertoire well suited for adaptative flexibility to respond to changing substrate availability that could provide important selection advantages for cancer.

Protein metabolism in cancer

Cancer cells conserve endogenous proteins and their constituent amino acids more avidly than normal cells (70). They also scavenge systemic nitrogen and maintain positive nitrogen balance, serving as 'nitrogen sinks' that contribute to cancer cachexia (6,70). Warburg and his contemporaries observed ammoniagenesis in cancers that was increased in the absence of exogenous substrate and reduced in the presence of Glc (5,10,14), suggesting both a capacity to utilize endogenous proteins and protein-sparing effects of Glc. Since cancer cells lack intracellular storage forms of protein, endogenous recycling of functional and structural proteins is likely, although selectivity in targeting specific proteins for proteolysis remains to be directly addressed. The anabolic or catabolic benefits of such recycling have historically been viewed as by-products of other primary cellular processes, rather than their *raison d'etre*. Autophagy plays important roles in recycling excess or damaged intracellular components for internal consumption (68,71,72) and likely represents one contributor to these processes.

Amino acid biosynthesis supports cellular needs that cannot be met by substrate abstraction from the environment. These processes are intimately intertwined with Glc metabolism and require anabolic input from glycolysis or the TCA cycle. Ser biosynthesis, in particular, is upregulated in cancer (38,51,73,74), providing methylene groups for one-carbon reactions important for nucleotide synthesis involving the folate pathway and homocysteine methylation to yield methionine in the methionine cycle (51,74). Both Ser and homocysteine serve as important substrates for the biosynthesis of other amino acids (51), including cysteine, which is a substrate for GSH generation important for the maintenance of cellular redox status. The methionine cycle also supports methyltransferase reactions important for histone modification and other post-translational changes of epigenetic relevance (74,75). Ser biosynthesis is initiated by phosphoglycerate dehydrogenase, which is strongly induced by protein restriction and employs glyceroneogenic 3-PG as a substrate (51). In principle, phosphoglycerate dehydrogenase competes with glycolytic GAPDH for required NAD^+ cofactors, which could favor the use of glyceroneogenic 3-PG derived from malate and the TCA cycle (51). As much as half of all anapleurotic Gln flux in cancer cells may be linked to Ser biosynthesis (73). Cancer cells avidly abstract exogenous Gln from their environment and are also capable of Gln biosynthesis, which plays key roles in solid tumor adaptation to nutrient deprivation and/or hypoxia (76).

Gln also plays other important roles in cancer metabolism (77,78). Gln supports transamination reactions important for purine and pyrimidine biosynthesis, and Gln-derived αKG supports reductive biosynthesis of acetyl-coA for lipogenesis under hypoxic conditions (57,61), suggesting additional metabolic flexibility to adapt to variations in substrate availability and environmental conditions. It is also of considerable interest that

only a fraction of available Gln is oxidized or otherwise diverted for anabolic purposes (79). High rates of metabolic flux support sustained proliferation (79), but the rate of glutaminolysis—like that of glycolysis—still greatly exceeds the catabolic and anabolic needs of cancer cells (8,13,80,81). These high rates of major pathway flux have important metabolic control implications for anabolic branched pathways (13).

The gestalt of intermediary metabolism in cancer

Altered cellular metabolism crucially supports the increased anabolic and catabolic demands of rapidly proliferating cancer cells (21). These demands can vary widely in both magnitude and direction in different anatomic locations and across diverse cell populations (4,12,15). Endergonic and exergonic processes, however, cannot operate independently of one another and must be coupled. Energy metabolism is closely coupled to anabolic activity and other energy-requiring processes like active transport (Figure 3, *left panel*) (6,8). The fundamental balance between ATP generation and its hydrolysis has been recognized for decades (8,80–83), but the importance of this coupling is still widely underappreciated. Cells cannot function at an energy deficit, and the potential for cellular energy generation uniformly exceeds its utilization in intact cells (8,25,80,81). ATP conservation is central to metabolic regulation, and consumption is a key driver of ATP generation (8,12,84). Recognition of these fundamental relationships originally led to the concept of cellular adenylate energy charge (AEC) as a major controlling factor in metabolic regulation (82,85), Low AEC values correspond to elevated adenosine monophosphate (AMP) levels and favor catabolic processes, whereas high AEC values correspond to increased ATP abundance and favor anabolic processes. These counterbalancing effects serve to assure that dynamic cellular demands can be met by appropriate diversion of cellular resources.

The metabolic changes associated with cancer are highly integrated—just as they are in normal cells (6,8,86) —and cannot be properly considered outside the context of the cellular gestalt (12). As such, a holistic understanding of how myriad cancer-associated changes interact with one another is essential. Examination of individual enzymes or pathways in isolation risks overlooking crucial organizational and control principles in intact cells (87,88). Consideration of cancer metabolism as a system will require multiple complementary experimental approaches drawn from classical biochemistry, as well as molecular biology. Metabolic flux and control analysis is crucial to understanding such changes, insofar as alterations in substrate or product abundances alone give limited information regarding metabolic flux (13). Similarly, if metabolic capacity is not limiting and exceeds cellular demands, then changes in individual enzyme or transporter abundances may not accurately or fully reflect either cellular needs or metabolic flux. Even where increased metabolic capacity can be demonstrated, it does not necessarily follow that cancer cells always—or ever—operate at maximum capacity (8,13,25,80).

Intermediary metabolism is a complex interconnected series of processes that can individually drive, augment or counterbalance each other (Figures 1 and 2). As such, secondary, compensatory or coupled responses may be of greater pathophysiological importance to carcinogenesis than primary initiating direct changes (Figure 2). Metabolic flux through one pathway may promote pathology development, whereas flux via another path may have the opposite effect. As such, relative counterbalancing or augmenting contributions may be more important than the absolute magnitude of individual processes (Figure 4). As an example, oxidative metabolism represents a major source of reactive oxygen species (ROS) (89), whereas PPP flux is a major driver of counteracting antioxidant quenching mechanisms (41,42). The end products of glycolysis, pyruvate and lactate, may also directly detoxify ROS (90–95).

All metabolic flux occurs under non-equilibrium conditions, and for individual enzymatic reactions, displacement from equilibrium represents a major determinant of the magnitude and direction of associated flux (96). All steps within a pathway exert some level of control over flux (79,96), but under steady-state conditions, reactions that reside farthest from equilibrium are best positioned to restrict flux and exert control (96). In open systems like cancer cells, substrate and cofactor availability, as well as downstream product removal and metabolic feedback, also dynamically contribute to flux control (96). These factors are of particular importance to metabolic phenotype development in cancer cells, which must depend upon *de novo* synthesis or macromolecular recycling for substrates that are unreliably or only intermittently available from extracellular sources. Cancer cells demonstrating the ability to utilize multiple substrates exhibit hierarchical preferences, with Glc generally favored over other substrates (6). Such preferences probably serve to conserve endogenous lipids and proteins when alternate exogenous substrates are available.

Catabolic and anabolic support of cancer growth

Both glycolysis and the TCA cycle are amphibolic pathways that support the anabolic, as well as catabolic, needs of rapidly proliferating cancer cells (21,23,50,51). Catabolic support roles have historically garnered the most attention, but the importance of anabolic support for the proliferative cancer phenotype is also now widely recognized (21,23). All rapidly proliferating cells require increased nucleic acid biosynthesis, membrane biogenesis and protein synthesis to increase biomass (24). Newly synthesized proteins also require post-translational modifications for proper targeting and function (51,97–99). These biosynthetic processes and asymmetric secondary active transport of exogenous substrates and ions are both supported, in turn, by cellular energy derived from both glycolysis and oxidative metabolism. Specific requirements for TCA cycle carbon balance (50) and specific cofactor coupling arrangements (Figure 3) serve to help coordinate these catabolic and anabolic contributions.

Metabolic cancer cell phenotypes can reflect primary changes in metabolic control, as well as capacity (12,22,79,82,83), and both substrate availability and cellular catabolic and anabolic demands represent major phenotypic determinants. A direct relationship exists between cellular adenosine triphosphatase (ATPase) activity and ATP generation (8,80,83), and in the setting of non-limiting substrate availability, cellular energy production largely changes in response to demand, not *vice versa*. This well-described, albeit underappreciated, relationship is an important driver of metabolism in normal cells and cancer cells alike.

Metabolic contributions to—or antagonism of—cellular stress

Cellular stress is a net function of the balance between the magnitude and nature of all incident stressors and the corresponding adequacy of intrinsic cellular coping strategies (Figure 4A). There is considerable heterogeneity in both stress responses and outcomes associated with different cell types or tissues, even under

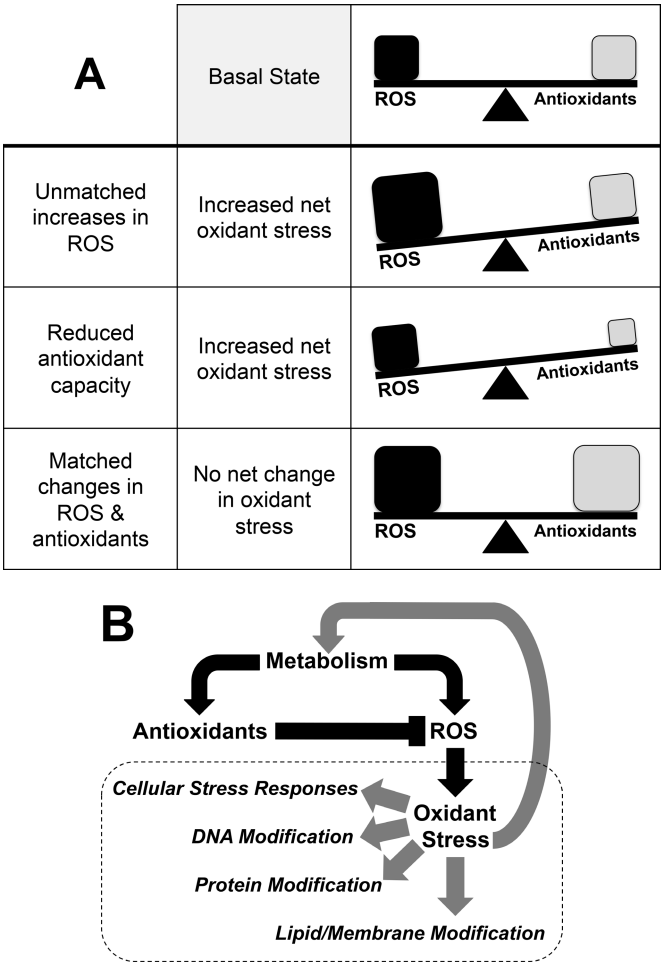

Figure 4. (**A**) Oxidant stress reflects the dynamic balance between oxidant stressors (e.g. ROS) and antioxidant coping mechanisms. As such, unmatched primary increases in ROS or primary decreases in antioxidant capacity—or both—may lead to phenotypically indistinguishable increases in net oxidant stress. (**B**) Intermediary metabolism contributes to both ROS generation and opposing antioxidant coping mechanisms. Imbalances resulting in net oxidant stress can lead to oxidative modification of macromolecules, organelles and cellular effectors with functional consequences that directly or indirectly contribute to cancer development (highlighted area). Net oxidant stress can also feedback to influence metabolic flux and thereby attenuate or intensify these contributions.

identical conditions. In principle, metabolic reprogramming can contribute to both the propensity for cancer development and cancer cell selection via either metabolic promotion or alleviation of stress. An expanded metabolic repertoire may enhance the inherent flexibility of cancer cells (12,73,100,101), thereby enabling them to thrive under highly variable conditions and to favorably adapt to changing microenvironments and the myriad associated stresses encountered by rapidly proliferating cancer cells. Metabolic stress, including oxidant stress, has been associated with carcinogenesis, although the ability of metabolism to antagonize, as well as promote, such stress suggests both direct and indirect mechanisms whereby metabolism can contribute to cancer genesis, progression, selection and control. Several forms of stress relevant to cancer are briefly considered below.

Oxidative stress

By definition, cellular oxidative stress reflects the net effects of both oxidant stressors and intrinsic antioxidant coping mechanisms (102). As such, oxidant stress may mechanistically arise from increased oxidant stressors, reduced antioxidant coping capacity or both (Figure 4A). Oxidant stress can also represent either a cause or a consequence of metabolic alterations (103) that serve to antagonize or promote oxidant stress—or both. The antioxidant coping strategies of cancer cells ostensibly mimic those of normal cells and are intimately intertwined with metabolism, which can both generate and detoxify oxidant species (Figure 4B). Direct non-enzymatic oxidant quenching has historically received less attention than redox-coupled antioxidant mechanisms. Several metabolic intermediates of the major amphibolic pathways, however, possess known antioxidant properties that complement their canonical catabolic and anabolic roles. For example, α-ketoacids such as pyruvate and αKG are potent antioxidants (90,91,93,104), and α-hydroxyacids such as lactate exert similar protective effects (92,94). These observations suggest intrinsic mechanisms for buffering any pro-oxidant effects of metabolism and the possibility of specific antioxidant roles for glycolysis and the TCA cycle that are in addition to those traditionally ascribed to PPP flux and glutathione reductase activity.

Both inorganic and organic peroxides contribute to endogenous oxidant stress, although organic peroxides, particularly lipid peroxides, are of greater potential biological importance. Catalase detoxifies inorganic but not organic peroxides, whereas GPx is capable of detoxifying both. Glc flux via the PPP plays a major role in this process through $NADP^+$/NADPH redox coupling with glutathione reductase, and primary increases in HK

activity, which gates entry into this pathway, increases PPP flux and protects against oxidative stress (23). It also bears noting that ROS can transduce mitogenic signals at low levels where oxidant stress and macromolecular damage may be less of a consideration (105,106), suggesting additional mechanisms whereby metabolism interacts with realistic environmental exposures.

Hypoxic stress

Cells in rapidly growing tumors are subject to widely varying O_2 tensions (61,107). Cancer-associated adaptations to hypoxic stress are well described, but the specific roles played by hypoxia in the earliest origins of cancer are still incompletely defined. Hypoxic signal transduction plays established roles in regulating gene expression associated with both cancer development and metabolism (61), suggesting causal contributions. Warburg hypothesized that repeated exposures to sublethal concentrations of respiratory poisons (so-called chemical hypoxia) was sufficient to induce cancer formation due to associated primary structural and functional changes in mitochondria (11). Although a primary role for mitochondrial damage in cancer genesis is now widely discounted (6,12,23,108), the reported ability of chronic intermittent hypoxia to promote the carcinogenic transformation of cultured myocardial fibroblasts (109) is consistent with the notion that chronic hypoxia or hypoxia-associated changes may directly or indirectly contribute to metabolic reprogramming and cancer development. However, these findings have not been independently validated during the course of the intervening half-century, and hypoxia *per se* has not been shown to unambiguously increase either spontaneous or inducible cancer development *in vivo* (6). Nonetheless, the ability to tolerate widely varying O_2 tensions has profound implications for cancer cell survival and selection during tumor growth, tissue invasion and metastasis. As such, the contributions of hypoxia to metabolic reprogramming are probably necessary, if not sufficient, prerequisites for cancer development and progression.

Nutritional stress

Cancer cells, particularly metastatic cells, are exposed to highly variable nutrient concentrations (6). Given the increased anabolic and catabolic demands placed on these cells by rapid and uncontrolled proliferative growth, nutrient variability poses major challenges for both carcinogenesis and cancer progression that may help explain metabolic reprogramming requirements in cancer. This can also serve as a basis for selection when individual cells compete for limited available resources.

Physical stress

Cancer cells are also subject to highly variable physical forces during both tumor growth and metastasis. Rapidly growing tumors are subject to intrinsic and extrinsic compression associated with increased tumor biomass, heterogeneous tissue densities and altered extracellular matrix composition. Hydrostatic and oncotic pressure changes also contribute to elevated interstitial fluid pressure within solid tumors (110,111). In addition to shear stresses associated with cellular migration through interstitial and vascular compartments, cancer cells are exposed to varying hydrostatic and oncotic pressures during metastasis. Deforming stresses play a major role in metastatic selection (112), and malignant cancer cells exhibit increased resistance to shear stress (113). Since intermediary metabolism influences membrane composition and fluidity and also powers membrane repair functions (114,115), it is reasonable to speculate that these differences have metabolic determinants.

Other forms of cellular stress

As a consequence of systemic homeostasis and the constancy of the *milieu intérieur* (116), most normal cells are not exposed to significant physicochemical stresses under physiological conditions. In contrast, the structural and functional changes associated with rapidly growing tumors subject cancer cells to stresses that differ qualitatively and quantitatively from their normal counterparts. As such, other potential forms of stress capable of influencing or selecting for cellular metabolism also warrant brief consideration. These conditions can have a primary metabolic basis or induce metabolic adaptive responses—or both. For example, tumors exhibit lower pH than normal tissues (6,107). Glycolytic metabolism's ability to influence microenvironmental pH is well described, and extracellular pH measurements are frequently used interchangeably to monitor glycolytic responses. However, traditional attributions of extracellular acidification to associated lactate production ignore the fact that the pKa of 3.87 for lactate strongly disfavors acid formation under broad physiological conditions (117). Microenvironmental pH changes in tumors thus reflect oxidative CO_2 elaboration (118) and the variable contributions of metabolic H^+ generation coupled to extracellular extrusion via secondary active Na^+/H^+ antiporters and monocarboxylate cotransporters (61,119). H^+ extrusion, accompanied by the export of monocarboxylates such as lactate, helps explain the fidelity of lactate as a marker of extracellular acidification. Both intratumoral pO_2 and pH are spatially heterogeneous and poorly correlated with each other (120), and a corresponding lack of concordance between extracellular pH and lactate accumulation also exists (121,122). The ability of glycolysis-deficient Ras-transformed cells to acidify their extracellular environment like their glycolysis-competent counterparts is also compatible with such a contention (118). Nonetheless, just as cellular metabolism can influence environmental pH, the converse is also probably true.

Relationships between dysregulated metabolism and other hallmarks of cancer

It is unlikely that dysregulated metabolism is functionally independent of other cancer hallmarks given the number of known shared regulatory factors involved (21,38,123–126) and the fundamental anabolic and catabolic demands placed on cancer cells by core hallmarks such as sustained proliferation (6,21). Metabolism probably plays critical deterministic and supporting roles in cancer development, just as it does in normal development. Not surprisingly, a number of metabolic parallels, including similar glycolytic phenotypes, have been drawn between normal developing tissue and cancer (6,30). The phenotypic heterogeneity and unrestrained proliferative behavior of cancer may ultimately limit the generalizability of such comparisons to specific cancer types or stages, but dysregulated metabolism remains well positioned to serve as a fundamental enabler of other cancer hallmarks (3,127).

Metabolic dysregulation and reprogramming are strongly associated with cancer development (21), but there is limited evidence to support primary oncogenic roles for these changes. There is also a general tendency to discuss carcinogenesis and cancer progression interchangeably, as if they share a common metabolic basis. Although plausible, this inference has not been

experimentally validated or characterized. Similar roles are assumed, but the specific underlying changes and precise role(s) played by dysregulated metabolism in cancer genesis need not be identical to those associated with cancer progression. An understanding of the specific temporal and mechanistic relationships between exposures, altered metabolism, carcinogenesis and the development of other cancer hallmarks—along with an assessment of the persistence and potential reversibility of individual changes along the cancer continuum (Figure 5)—is needed to provide important mechanistic insights into fundamental cancer biology that can ultimately be exploited for therapeutic benefit or cancer prevention.

Interactions between metabolism and apoptotic resistance

Growth factor signaling antagonizes apoptogenic stimuli and regulates intermediary metabolism (23,44). These dual intersecting functions may have a conserved evolutionary basis (33). PI3K–Akt–mTOR signaling, in particular, plays important roles in coordinating metabolism and promoting cell survival, and the specific contributions of Akt hyperactivation to oncogenesis have been attributed to fundamental roles in cellular energy metabolism that combine to inhibit apoptosis, increase cell proliferation and accelerate oncogenic mutation rates (34). The Glc dependence of anti-apoptotic growth factor and Akt signaling contrasts markedly with the Glc independence of the corresponding effects of anti-apoptotic Bcl-2 family members (33). In fact, it was the recognition of this fundamental difference in metabolic requirements that initially led to the identification of the novel anti-apoptotic and pro-survival roles played by mitochondrial HK1 and HK2 (33). These high-affinity HK isoforms physically and functionally interact with mitochondria at outer membrane contact sites where both pro- and anti-apoptotic signals are known to converge (23,33). They mediate the anti-apoptotic functions of growth factors by specifically promoting mitochondrial metabolite exchange that directly couples intra- and extramitochondrial metabolism and via direct antagonism of pro-apoptotic Bcl-2 protein interactions with mitochondria (23,33). Similar integrated roles for other mitochondria-coupled ATPases (e.g. glycerol kinases) have been suggested but not yet demonstrated (23).

Interactions between metabolism and genomic instability

Mutagenic carcinogens may act either directly or indirectly to produce genotoxic effects. Indirect effects on genomic stability can also be mediated through primary effects on intermediary metabolism and the cellular environment. Mechanisms contributing to such changes include—but are not restricted to—oxidant stress. There is evidence to support the notion that chronic oxidative stress is a major contributor to nuclear genomic instability via secondary genotoxicity, although the magnitude and relevance of these effects have been questioned in the absence of accompanying DNA repair mechanism defects. Chronic oxidative stress is strongly associated with cancer development (128,129) and correlates with DNA structural changes that predate the appearance of overt histopathological changes or typical features of cancer (130,131). Functional mutational changes may involve either coding or *cis*-acting regulatory regions of genes encoding either the primary metabolic machinery or its upstream regulators (Figure 6). Similarly, mitochondrial genomic instability due to metabolism-associated oxidant stress is commonly invoked as an explanation for observed mutations in cancer-derived mitochondrial DNA, although this has not been directly demonstrated. A recent report of reduced, rather than increased, mitochondrial genomic instability in cancer tissue (132) is therefore of considerable interest. Intriguingly, these findings, which still remain to be validated, could challenge conventional dogma by suggesting that the mitochondrial genome is somehow stabilized in cancer, possibly via metabolic alterations that serve to reduce the accumulation of mitochondrial mutations that normally contribute to aging (132,133). It remains for future studies to address this apparent discrepancy between mitochondrial and nuclear genomic stability and its relevance to cancer and dysregulated metabolism.

Interactions between metabolism and replicative immortality

Cancer cells overexpress telomerase (134). In addition to its roles in maintaining chromosomal length, telomerase expression has been associated with increased Glc utilization, lactate accumulation and glycolytic enzyme expression (135). Interestingly, telomerase can also be imported into mitochondria where it

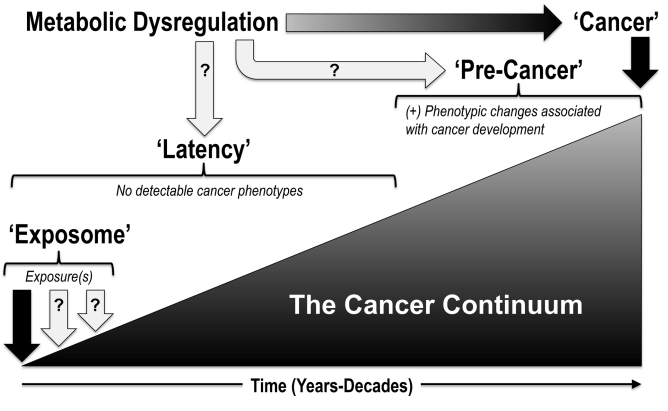

Figure 5. The metabolic phenotypes associated with carcinogenesis and during latency—and their specific relationship(s) to both parental cell phenotypes and the metabolic hallmarks of established cancer—represent key knowledge gaps. Carcinogenic exposure(s) may not result in characteristic cancer phenotypes for years or even decades. It is not presently known, however, whether the classical hallmarks of metabolic reprogramming and dysregulated metabolism precede or follow development of other recognizable cancer phenotypes. Little is known about the metabolic phenotype(s) of cells or tissues destined to produce cancer during periods of latency between exposure and the development of overt histopathological changes. Where metabolic changes occur in this disease continuum remain to be established, and their direction, magnitude, reversibility and relationships to established cancer phenotypes will require careful characterization. Once delineated, it will be incumbent on future studies to establish whether or not such changes are binary and whether they are necessary and/or sufficient for cancer development.

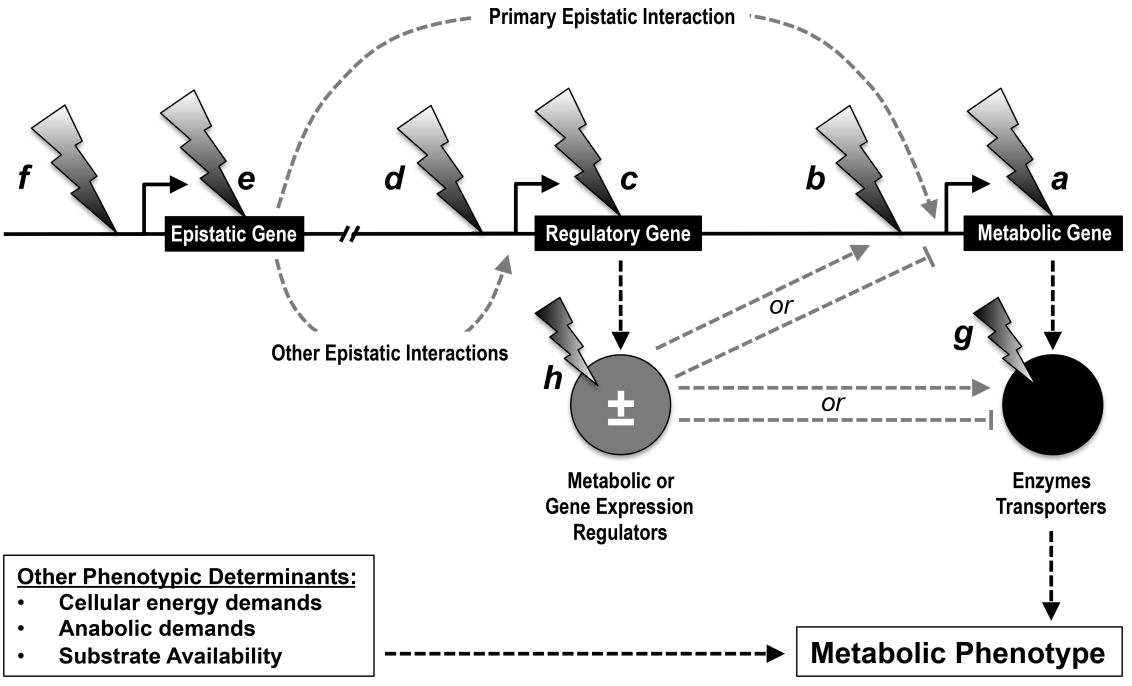

Figure 6. Direct and indirect genotoxic and non-genotoxic contributions to metabolic dysregulation. Genotoxicity may directly influence metabolism by mutagenic disruption of either metabolic gene product function (*a*) or *cis*-acting elements important for expression (*b*). By extension, genotoxicity may indirectly influence the same processes via disruption of upstream regulatory gene product function (*c*) or expression (*d*). Alternatively, genotoxic effects (*e*,*f*) may disrupt important epistatic interactions between distant genetic loci. Non-genotoxic effects (*g*,*h*) may also contribute to metabolic phenotype development. By definition, both direct and indirect genotoxic effects, as well as non-genotoxic effects, must interact with other dynamic drivers of metabolism to determine the ultimate metabolic phenotype. As a consequence, this phenotype may not always be fixed

can protect mitochondrial function and cellular growth (136). The mechanisms underlying these effects and their specificity for—and relevance to—cancer have not been delineated, but the ability of ROS to activate telomerase suggests bidirectional mechanisms for adaptive or maladaptive interactions between metabolism and telomere maintenance important to both replicative viability and survival.

Interactions between metabolism, tumor-promoting inflammation and immune system evasion

Inflammation promotes the development and progression of many cancers and enjoys an interactive, cyclical relationship with metabolism. Glc and lipid metabolism directly influence immune cell function (137–139), and specific metabolic dependencies of innate and adaptive immune cells can promote direct competition with cancer cells for limited intratumoral resources—including O_2 and nutrients—thereby promoting immune evasion (140). Altered microenvironmental pH or redox changes can also affect immune cell function and local cancer surveillance (138,140–142).

In addition, immune cells directly interact with cancer cells via bidirectional proinflammatory signals mediated by a variety of factors, including cytokines and extracellular metabolites. For example, extracellular adenine nucleotides, succinate, NAD^+ and urate can serve as proinflammatory metabolic signals promoting immune responsiveness (139,143), suggesting specific mechanisms whereby metabolism may help drive inflammation. The reciprocal ability of proinflammatory cytokines to influence metabolism in diverse cell types (140,144–147) suggests that trophic cytokines can directly couple inflammation to metabolism, providing a potential basis for vicious cycle development between inflammation and cancer metabolism.

Interactions between metabolism and sustained proliferative signaling

Cellular transformation by oncogenic viruses or cellular oncogenes is characterized by altered metabolism (6,8,107,148–150) and increased proliferative growth (151). Tumor suppressor inactivation, like oncogene activation, is also linked to metabolic dysregulation. Specific changes vary by cancer type and individual oncogenic effector involvement, but alterations in both Glc and Gln metabolism are common (107,152).

Many oncogenes and most proteins with known cancer-associated somatic mutations are tyrosine kinases capable of mediating proliferative and trophic signals (24,153). Alterations in receptor and non-receptor tyrosine kinase signaling can have metabolic, as well as trophic, proliferative and anti-apoptotic consequences (44,154). As such, exposures that activate oncogenes or mimic their trophic actions can contribute to metabolic reprogramming and dysregulation. For example, oncogenic Ras promotes the development of multiple cancer hallmarks, including metabolic reprogramming (3) and proliferative signaling pathway activation (86). It promotes glycolysis, reduces oxidative TCA cycle metabolism and enhances both Glc and Gln channeling into anabolic pathways (46,107,149,155). Oncogenic Ras also decouples Glc and Gln metabolism in support of cancer cell growth (156), and Ras-induced cancers characteristically exhibit heightened Glc dependence (157). Akt hyperactivation is also commonly observed in cancer and contributes to multiple cancer hallmarks, including proliferation and dysregulated metabolism. Akt also mediates the anti-apoptotic effects of growth factors—phosphorylatable hexose-dependent effects that involve the interaction between HK and mitochondria (23,29,34,52). The ability of Akt to regulate metabolism is phylogenetically more conserved than its anti-apoptotic functions,

which correlate with the appearance of apoptogenic mitochondrial functions, suggesting an evolutionary basis for these interactions (23).

Transcriptional regulators represent another important class of cellular oncogenes, and cancer-associated somatic mutations in *trans*-acting factors are second only to protein tyrosine kinase mutants (153). For example, Myc upregulation is capable of promoting the development of multiple cancer hallmarks (3) via transcriptional coordination of gene expression promoting proliferation and metabolism (124). Myc-overexpressing cells exhibit both increased glycolysis and glycolytic gene expression (158).

The tumor suppressor p53, is activated by DNA damage, cellular stress and oncogenic signal transduction (151) and exhibits pleiotropic anti-proliferative and metabolic effects that include metabolic cell cycle arrest (52,159). p53 also induces factors involved in DNA repair and maintenance of cellular redox homeostasis (150,151,160). Among these factors, Tp53-induced glycolysis and apoptosis regulator (TIGAR) redirects Glc flux from glycolysis into the PPP, thereby augmenting NADPH-dependent GPx activity and enhancing antioxidant capacity (161). Based on sequence homologies, TIGAR was originally classified as a fructose bisphosphatase capable of directionally opposing the actions of PFK (161). Recent biochemical characterizations of this enzyme have suggested alternate metabolic substrates and have called this primary classification into question (162). Nonetheless, TIGAR still provides an important mechanistic link between p53 and its pleiotropic effects on metabolism. Interestingly, TIGAR also interacts with anti-apoptotic mitochondrial HK2 (163), although the functional implications of this interaction are incompletely delineated. Other p53 effects on metabolism include the promotion of oxidative Glc and lipid metabolism and reduced lipogenesis (125,150,164). Effects on FA oxidation are observed even in the presence of physiological Glc concentrations (164). The ability of p53 to regulate autophagy (165) also has catabolic implications, particularly in the setting of nutritional stress, and suggests additional potential influences on metabolic phenotype development (71).

Cell cycle-associated changes in metabolism are also recognized (166) but poorly understood. A metabolic cell cycle checkpoint requiring adenosine monophosphate (AMP)-activated protein kinase (AMPK)-induced p53 activation normally couples cell cycle to nutritional status (159) and other interactions between AMPK, p53 and PI3K–Akt–mTOR signaling are known (125). Collectively, they may serve to coordinate energy metabolism with both trophic and stress-induced cellular responses.

Interactions between metabolism and angiogenesis

Many of the same factors and conditions favoring angiogenesis also modulate metabolism (107), suggesting coordinated regulation. Angiogenesis also places catabolic and anabolic demands on poorly vascularized tissues with restricted access to O_2 and metabolic substrates. Intermediary metabolism in resource-constrained environments thus plays crucial catabolic and anabolic support roles in rapidly growing angiogenic tumors. Hypoxia, in particular, represents an important stimulus for both angiogenesis and metabolic change, with hypoxia-inducible factor (HIF) serving as a master integrator for many of these responses that, in aggregate, advantage cancer cells subjected to hypoxic stress (61,107). Mitochondria-derived ROS also play important roles in HIFα stabilization and hypoxic signaling (167). There is a bidirectional relationship between hypoxic signaling and metabolism, with αKG serving as an important metabolic substrate for prolyl hydroxylases regulating HIFα turnover (61).

Interactions between metabolism, tissue invasion and metastasis

Of all the cancer hallmarks identified by Hanahan and Weinberg (3,127), the capacity for tissue invasiveness and metastasis is arguably the most specific for cancer (15). Other hallmarks can be individually shared with many normal and benign tumor cells (15), and associated gene expression patterns vary considerably across intratumoral cell populations (168). As such, delineating the specific relationships between dysregulated metabolism and successfully invasive or metastatic cancer phenotypes are of paramount importance to understanding the contributions of metabolism. Metastasis is a highly selective and inefficient process (112,169). Studies comparing metastatic cells to parental tumor cells have confirmed significant heterogeneity in metastatic potential and are consistent with the notion that metastatic success is determined by selection (149,168). The ability to successfully invade tissue or metastasize is therefore probably a function of the intrinsic characteristics of the cell, as well as the environment (168). By definition, both local tissue invasion and distant metastasis involve cell migration through heterogeneous environments (168). So adaptations that equip cells to tolerate and survive environmental transitions are likely candidates for selection. Given the inherent variability in environmental conditions, including O_2 and nutrient availability, metabolism seems ideally suited to fulfill this criterion (149).

Cancer cells are bidirectionally interactive with the local tumor microenvironment, which is both shaped by—and selects for—altered metabolism (149,170). This relationship is not fixed for cancer cells within rapidly growing tumors or during local tissue invasion or metastasis, a fact that probably contributes to cancer heterogeneity (4,120). From a selection perspective, it can be argued that environmentally restrictive or inflexible metabolic phenotypes could be potentially maladaptive for cells exposed to the widely varying conditions anticipated within rapidly growing tumors and during invasion or metastasis (12).

The ability of cancer cells to influence their local microenvironment can also directly enhance their invasive and/or metastatic potential. For example, microenvironmental reducing conditions activate matrix metalloproteinases via direct effects on redox-sensitive cysteine residues that can promote both extracellular matrix remodeling and local tumor invasiveness (171).

Interactions between metabolism and epigenetic regulation relevant to multiple hallmarks

Epigenetic changes play important roles in carcinogenesis and have been associated with the development of multiple cancer hallmarks. Many of these changes can also be transgenerationally retained, like mutational changes (76,154,172,173). Intermediary metabolism has been linked to epigenetic gene regulation via a number of non-exclusive mechanisms (173). First, AMPK directly phosphorylates histones and mediates stress-induced changes in gene transcription (174), suggesting specific mechanisms whereby cellular energy status can be coupled to transcriptional stress responses. In addition, ACC catalyzes the initial rate-controlling step of *de novo* FA synthesis—the carboxylation of acetyl-coA to yield malonyl-coA—and globally competes with protein acetylation for available acetyl-coA (59). Given the central importance of histone acetylation in chromatin remodeling (175) and established roles for acetylation in the

regulation of core elements of the transcriptional machinery (99), this represents another potentially important link between intermediary metabolism and epigenetic transcriptional regulation. Inhibition of histone deacetylases by lactate accumulation (176) also suggests additional coupling mechanisms.

Mitochondrial ROS overproduction activates hexosamine pathway activation and O-linked transcription factor glycosylation and activation (177). This plays myriad roles in gene regulation that are relevant to both proliferation and metabolism. Reciprocal relationships between O-linked glycosylation and phosphorylation of transcription factors have also been reported (97,177). Interestingly, AMPK regulates histone O-linked glycosylation and *vice versa* (178), suggesting additional mechanisms coupling gene regulation to nutrient and energy status. Lastly, ornithine decarboxylase is essential for cell growth and proliferation (179) and directly couples metabolism to gene regulation by catalyzing the synthesis of cationic polyamines, which interact with anionic DNA and influence both DNA structure and the ability *of trans*-acting nuclear regulatory factors to bind their cognate *cis*-acting DNA binding sites.

Potential metabolic targets for environmental exposures

Against this important biological backdrop, major metabolic pathways (e.g. glycolysis, lipogenesis, the PPP and the TCA cycle) and signaling pathways associated with metabolic regulation were considered as potential metabolic targets, and selected prototypical targets were examined for evidence of cross-talk with other cancer hallmarks in the published literature. Corresponding evidence for pro-carcinogenic environmental exposures capable of promoting metabolic reprogramming and dysregulation was then considered and used to identify prototypical exposures with the potential to act on these targets. Both lists, merely intended to provide representative examples of potential starting points for future directed study, are subject to a number of caveats related to both underlying assumptions and gaps in our present understanding of the metabolic features of exposure-associated carcinogenesis that are addressed below. Limitations in the ability of existing risk assessment frameworks to inform our understanding of the underpinnings and specific contributions of cancer metabolism are also considered.

Conceptual overview of potential metabolic targets

Pro-carcinogenic exposures can target cellular metabolism at a number of different levels via both direct and indirect mechanisms. In principle, multiple contributing mechanisms can also combine in different manners to yield the same phenotype (Supplementary Figure S2, available at *Carcinogenesis* Online), and changes in a given metabolic pathway can engender reciprocal or complementary changes in other competing or coupled pathways. Distinguishing between primary and secondary metabolic alterations is thus crucial to understanding the relationships between specific exposures and associated pro-carcinogenic and metabolic changes, particularly following prolonged latent periods accompanying exposure-associated cancer development. Durable cancer-specific effects must also be distinguished from similar short-term toxic or adaptive responses. In general, exposures can directly target discrete gene products responsible for (i) key metabolic reactions, (ii) cellular transport or (iii) regulatory factors responsible for the coordination, control or integration of sequential metabolic steps. The possibility must also be entertained that pro-carcinogenic effects may be indirectly mediated by changes in substrate or cofactor availability, allosteric feedback or environmental alterations that physicochemically favor or disfavor pro-carcinogenic events (Figures 2 and 3). Exposures may also target metabolism at the cellular organizational level by perturbing supramolecular complex formation important for cellular structure or function or by disrupting metabolic compartmentalization important for metabolic channeling or its control.

Identification of potential targets for metabolic dysregulation

Selected metabolic processes with established functional importance or regulatory differences in cancer are depicted in Figure 2, and key associated metabolic or regulatory factors are listed in Table 1. Given their established biological importance, any of these factors could potentially serve as direct or indirect targets for metabolic dysregulation. To focus the search for such targets, a more limited set of prototypic targets amenable to modulation by environmentally relevant exposures were also selected (Table 2; Supplementary Table S1, available at *Carcinogenesis* Online), and iterative cross-hallmark comparisons were made to identify possible interactions between specific dysregulated metabolic features and other cancer hallmarks as described in both the Introduction and the accompanying capstone article (20). A major limitation of these searches involved the unexpected paucity of unambiguous evidence for direct causal relationships between dysregulated metabolism and carcinogenesis. In general, the published literature was found to be highly biased by associative and descriptive studies that were neither designed nor intended to directly address specific metabolic contributions to carcinogenesis. In Table 2 and Supplementary Table S1, available at *Carcinogenesis* Online, changes in selected prototypic targets were classified as having the potential to promote or antagonize development of non-metabolic hallmarks based on directional responses to common exposures. In some cases, evidence of both promotion and antagonism was identified. Exposure and/or model differences, and dissimilar endpoints could account for some of these observations, although it bears noting that dysregulated metabolism is not a singular entity, so multiple directionally divergent relationships between 'metabolism' (broadly defined) and individual hallmarks are not only possible but expected.

Potential metabolic targets generally fall into several broad functional categories listed in Table 1. For potential targets with multiple molecular forms, targeting may be restricted to specific isoforms. The central amphibolic roles played by glycolysis and the TCA cycle make these pathways particularly attractive targets for primary or secondary dysregulation. By virtue of its essential involvement in every aspect of intermediary metabolism and as a major determinant of flux through both anabolic branched pathways and the TCA cycle, glycolysis has naturally garnered the greatest attention. Other metabolic pathways may also constitute primary targets, but they would, of necessity, involve accompanying changes in amphibolic flux via glycolysis and the TCA cycle to fully support the anabolic and catabolic needs of rapidly proliferating cancer cells. As such, this list is not intended to be either comprehensive or definitive. Rather, it provides biologically plausible examples of primary metabolic or regulatory targets suitable for additional study that are derived from our knowledge of the types of metabolic changes associated with cancer, our understanding of their underlying biochemical mechanisms and their regulatory characteristics.

Table 1. Selected metabolic pathway targets with established importance in cellular metabolism

Individual pathway targets	Metabolic importance
Glycolysis (amphibolic)	
HK	• Catalyzes the first committed step of Glc metabolism, which represents the entry point to all major physiologic pathways of Glc utilization (23) • High-affinity HK1 and HK2 isoforms physically and functionally interact with mitochondria and directly couple intra- and extramitochondrial metabolism; major mediators of the anti-apoptotic functions of trophic factors (23,34) • The inducible HK2 isoform is overexpressed in cancer and favors anabolic metabolism, whereas the constitutive HK1 isoform favors catabolic Glc flux (35,37,38)
PFK	• Major irreversible rate-controlling step of glycolysis (180,181) • PFK1 regulated by AEC, as well as PFK2; PFK2 activated by AMPK
GAPDH	• Mediates critical binary NAD^+/NADH coupling with either mitochondria or LDH to maintain glycolytic flux in the presence or absence of O_2, respectively
PK	• Major irreversible rate-controlling step of glycolysis • The low affinity PKM2 isoform is strongly expressed in cancers and may serve to redirect glycolytic flux into anabolic pathways supporting lipid, nucleotide and Ser biosynthesis (182–186)
LDH	• Catalyzes the reversible NAD^+/NADH-dependent interconversion of pyruvate and lactate • Important source for NAD^+ required for glycolytic flux via GAPDH in the absence of O_2 (187,188)
PDH complex	• Mediates the critical step committing the products of glycolysis to an oxidative fate via the TCA cycle, namely irreversible pyruvate decarboxylation to yield intramitochondrial acetyl-coA
PPP	
Glucose-6-phosphate dehydrogenase	• Rate-controlling PPP enzyme and, along with the downstream PPP enzyme 6-phosphogluconate dehydrogenase, represents the principal source of NADPH for both reductive lipid biosynthesis and the antioxidant activity of GSH–Px (189,190)
TCA cycle (amphibolic)	
IDH	• Cancer-associated mutations in both IDH1 and IDH2 promote oncometabolite formation (57,100,191–194) • Contributes to reductive synthesis of acetyl-coA from Gln-derived αKG under hypoxic conditions (57)
Fumarate hydratase	• Cancer-associated mutations; loss of activity can result in fumarate accumulation and disruptive non-enzymatic succination of cysteine residues in other proteins (191)
SDH	• Shared component of both the TCA cycle and the ETC (Complex II) (195) • Oxidizes succinate to form fumarate and reduced flavin adenine dinucleotide, thereby mediating e^- transfer to ubiquinone in the ETC • Cancer-associated mutations (191)
Lipogenesis	
ATP–citrate lyase	• Generates acetyl-coA for lipogenesis and regulatory protein acetylation from cataplerotic citrate • Upregulated in cancers (22)
ACC	• Catalyzes the first rate-controlling step in *de novo* lipogenesis • Demonstrated roles in epigenetic regulation (59)
FASN	• Important rate-controlling step in lipogenesis • Upregulated in cancers (196,197)
Lipolysis	
LPL	• Mediates extracellular FA retrieval from TAGs for uptake and utilization (53,196–198)
MAGL	• Mediates intracellular FA retrieval from TAG stores (62)
SCD	• Mediates FA scavenging from lysophospholipids under hypoxic conditions (60)
Amino acid biosynthesis	
Phosphoglycerate dehydrogenase	• Major role in Ser biosynthesis (51,73,183,199) • Commonly amplified in cancer (195)
Mitochondrial electron transport chain assembly and function	
Complex I (NADH–ubiquinone oxidoreductase)	• Catalyzes electron transfer from NADH to ubiquinone with associated membrane proton translocation(200,201)
Complex II (SDH)	• Only membrane-bound member of the TCA cycle • See SDH above
Complex III (ubiquinol–cytochrome c oxidoreductase)	• Catalyzes electron transfer from ubiquinol to cytochrome c with associated membrane proton translocation • The Q_o site serves as a cellular O_2 sensor and serves to transduce a hypoxic signal and stabilize HIFα stabilization via ROS release (167)
Complex IV (cytochrome c oxidase)	• Only irreversible component of the respiratory chain • Catalyzes the oxidation of cytochrome c • Binds—and inhibited by—CO, NO, cyanide and azide; physiological NO decreases affinity for O_2 (202)

Table 1. *Continued*

Individual pathway targets	Metabolic importance
Hexosamine biosynthesis	
Glutamine:fructose-6-phosphate amidotransferase	• First committed step of hexosamine biosynthesis which provides substrate for O-GlcNAc modification of proteins • Hexosamine biosynthetic pathway flux is required to support trophic signaling and maintain Gln uptake needed for both growth and survival (45)
Cellular transport mechanisms	
Facilitated hexose transporters (GLUT)	• Mediates cellular Glc uptake • GLUT1 overexpression associated with cancer progression and poor prognosis (203)
CD36	• Mediates cellular lipid uptake (53,198)
Monocarboxylate transporters	• Mediate the coupled extracellular extrusion of protons and monocarboxylates such as lactate (61)
VDAC	• Outer mitochondrial membrane channel that partners with the adenine nucleotide translocator in the inner mitochondrial membrane to form anionic metabolite exchange conduits at contact sites • Implicated in mitochondrial permeability transition pore formation and apoptogenic cytochrome c release following pro-apoptotic Bcl-2 protein binding • Molecular target of GSK3β signaling and mitochondrial HK binding responsible for regulating anion exchange and antagonizing apoptogenic signals above
Others	
TIGAR	• Promotes Glc entry into the PPP in cancer cells to enhance nucleotide biosynthesis and antioxidant activity (163); originally classified as a low affinity fructose bisphosphatase, this biochemical identity has recently been called into question (162,204) • Relationship to p53 incompletely delineated (163) • Interacts directly with mitochondrial HK (163)
AMPK	• Energy-sensing enzyme • Contributes to Pasteur effect via direct phosphorylation and activation of PFK2 • Inactivates key biosynthetic enzymes (85,205)
Sirtuins	• NAD^+-dependent deacylases that regulate post-translational acylation (i.e. acetylation, succinylation and malonylation) of diverse target proteins, including histones (206,207)

Prototypic targets selected for cross-hallmark comparison based on current available evidence are also listed in Table 2.

Major rate-controlling steps in essential metabolic pathways are obvious potential targets for metabolic reprogramming, insofar as they represent important nodes for the integration and control of both major and branched pathway flux. In principle, however, any essential step in a series of non-redundant reactions can be targeted to alter metabolism and/or its control. The overall metabolic impact of individual changes are likely to be dictated by a number of considerations, including the presence or absence of multiple functionally redundant isoforms, the presence or absence of major kinetic barriers to alternate paths of flux and relative cellular dependence on the affected pathway(s).

Glycolysis

In glycolysis, HK, PFK and PK are logical targets by virtue of established roles in controlling glycolytic flux (Figure 2). GAPDH also warrants consideration due to the fact that flux at this step is dependent upon either mitochondria- or LDH-derived NAD^+ to proceed in the presence or absence of O_2, respectively (23). In normal cells, this coupling is typically binary and reciprocal (23,122,187,188), whereas both couplings appear simultaneously permissible in cancer. Specific isoforms of HK and PK have particular relevance to cancer. For example, HK2 is overexpressed in cancer and promotes both anabolic metabolism and cell survival (23,38). Cancer cells also strongly express a highly regulated and less active form of PK (PKM2) that promotes diversion of Glc flux into anabolic pathways such as the PPP and Ser biosynthesis (182,183). PKM2 interacts with a number of cellular regulatory factors (208) and has multiple pleiotropic actions, including novel moonlighting functions (209) as a transcriptional coactivator and a protein tyrosine kinase (184,210,211). Major moonlighting functions described for other glycolytic enzymes, including HK1, HK2 and GAPDH, suggest the possibility that metabolic enzymes may contribute to carcinogenesis via mechanisms distinct from their canonical enzymatic functions (209).

Lipogenesis, lipolysis and the PPP

Key enzymatic targets in both *de novo* FA synthesis (e.g. ATP–citrate lyase [ACL], ACC and FASN) and lipolysis (e.g. LPL, MAGL and SCD) and their control have already been implicated in cancer development (52–54) and warrant additional scrutiny, both individually and in combination (Figure 2). Given the essential support roles played by PPP flux in lipogenesis, nucleic acid biosynthesis and resistance to oxidative stress (41), glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PD) also represent major candidate targets meriting additional study (Figure 2).

TCA cycle

Within the TCA cycle, heritable cancer-associated mutations have been identified in both succinate dehydrogenase (SDH; ETC complex II) and fumarate hydratase (89,191). ROS generation and mitochondrial mutagenesis have been implicated in cancer pathogenesis associated with these mutations (89). Mitochondrial NAD^+-dependent isocitrate dehydrogenase (IDH3) irreversibly catalyzes ETC-linked isocitrate oxidation, whereas mitochondrial (IDH2) and cytosolic (IDH1) $NADP^+$-dependent isoforms can mediate bidirectional isocitrate–αKG interconversion

Table 2. Cross-hallmark effects for selected metabolic targets

Dysregulated metabolism—potential targets	Evasion of anti-growth signaling	Angiogenesis	Genetic instability	Resistance to cell death	Immune system evasion	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor-promoting inflammation	Tumor microenvironment
Glycolysis	–	+	Unknown	+	+	+	+	+	+	+
Pyruvate dehydrogenase (PDH)	–	Unknown	Unknown	Unknown	Unknown	–	–	–	+	–
TCA cycle	–	+	+	–	Unknown	–	–	+	+	+
Electron transport chain	Unknown	Unknown	Unknown	–	Unknown	–	+	Unknown	+	+
Lipid metabolism	–	+	+	Unknown	+	±	+	–	+	+
Increased ROS	±	+	+	+	+	±	+	±	+	±
mTOR signaling	–	+	Unknown	+	+	±	+	+	+	+
Gluconeogenesis	–	+	+	–	Unknown	–	+	Unknown	+	+

Interactions between selected targets listed in the left-hand column and individual cancer hallmarks were classified as having the potential to promote (+, *green cells*) or antagonize (–, *red cells*) development of individual cancer hallmarks based on directional changes associated with—or the demonstrated ability to promote or antagonize—development of cancer-associated phenotypes. Where indicated, evidence for the potential to both promote *and* antagonize cancer-associated phenotype development was found (±, *yellow cells*) or insufficient evidence was found in the literature to warrant such speculation (Unknown, *black cells*). For specific references used to construct this table, see Supplementary Table S1 and associated references in Supplementary Information, available at *Carcinogenesis* Online.

(192). The latter reaction can directly couple with lipogenesis and epigenetic acetylation via reductive acetyl-coA formation by ACC (57,59). Cancer-associated mutations in both IDH1 and IDH2 occur early in carcinogenesis (212) and lead to NADPH-dependent generation of the novel oncometabolite 2-hydroxyglutarate (2HG) which inhibits αKG-dependent enzymes important for hypoxic gene regulation and competes with biosynthetic reactions and GSH generation for available NADPH, thereby affecting lipogenesis, antioxidant protection, signal transduction, and epigenetic regulation (57,191–193,212–214).

Organizational or compartmental targets

The specific intracellular locations where metabolic events occur can help determine both the ultimate fate and functional importance of individual metabolic reaction products. Widespread metabolic compartmentalization (37,87,88,215) and the archetypal example of mitochondria–HK coupling (23,35,37) are both compatible with this notion. As such, some abnormalities observed in cancer could relate to altered compartmentalization that redirects metabolic channeling and/or favors specific physical and functional interactions that promote cancer cell growth and survival (23,33,216).

In principle, pro-carcinogenic exposures can also affect intermolecular interactions required for the formation and function of complex organizational structures, including cell membranes, organelles, chromatin, and supramolecular metabolic enzyme complexes such as metabolons (217,218) or ETC supercomplexes (219). Such targeting can be considered in both structural and functional terms and can involve both individual components and higher order integrated complexes. For example, fundamental contributions by mitochondrial ETC activity to carcinogenesis are widely accepted and can reflect both functional and structural mitochondrial changes (5). All respiratory complexes except complex II (SDH) can physically and functionally associate in dynamic supercomplexes such as the complex I-, III-, and IV-containing respirasome (219,220). Formation of these complexes influences both overall ETC function and individual respiratory complex turnover (219), suggesting mechanisms whereby ETC function may be targeted at the level of supercomplex assembly rather than at the level of individual respiratory complex components. As such, both individual ETC complex activities and supercomplex assembly represent potentially attractive targets for carcinogenic disruption (200,219,221). Mitochondrial targeting could also involve altered ETC functional coupling with transmembrane metabolite exchange and/or redox-driven extramitochondrial processes. In addition to their fundamental catabolic and anabolic roles, mitochondria also serve as major ROS generators (102,171). If not counterbalanced by intrinsic antioxidant coping mechanisms (102), ROS accumulation can lead to oxidant stress, activation of oncogenic signaling and promotion of genomic instability. Mitochondria also importantly buffer cytosolic calcium concentrations (171) and initiate and control apoptosis via permeability transition pore formation and apoptogenic cytochrome c release (33,171).

Other organellar targets include the endoplasmic reticulum and the plasma membrane, the latter incorporating both cell surface trophic factor receptors and specific transport mechanisms for transmembrane metabolite exchange (Figure 2). In addition to direct targeting of transport or signal transduction (addressed below), membrane organization and function can also be targeted through changes in membrane composition or structure that influence cellular function by altering membrane integrity or fluidity or via generation of cell surface clearance signals that alter cellular lifespan. Importantly, not

all intracellular compartmentalization is bounded by cellular membranes, so exposures that alter the normal establishment of non-organellar compartments or intracellular chemical gradients (e.g. involving H^+, Ca^{++}, adenine nucleotides, or nicotinamide adenine nucleotides) could also contribute to metabolic dysregulation.

Metabolite transport mechanisms

Specific cellular uptake mechanisms are required for internalization of exogenous substrates, including hexoses (e.g. GLUT [facilitated Glc transporters]), lipids (e.g. CD36), amino acids (222,223) and monocarboxylates such as lactate and pyruvate (218) (Figure 2). As such, transport mechanisms represent an important general class of potential carcinogenic targets. Mitochondrial and plasmalemmal ATPase activity coupled to transmembrane ion translocation critical for electrochemical gradient maintenance needed to support asymmetric metabolite partitioning is also intimately coupled to cellular energy metabolism (8).

Mitochondrial HK also promote cell survival, in part, via direct coupling with mitochondrial metabolite exchange (33). The voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane and the adenine nucleotide translocator in the inner mitochondrial membrane partner to allow movement of anionic metabolites such as adenine nucleotides, inorganic phosphate, pyruvate and succinate into—and out of—mitochondria. ATP–ADP exchange via this conduit directly couples intramitochondrial ATP generation with extramitochondrial ATP hydrolysis (Figure 3) (33) and is controlled by HK binding through mechanisms involving supramolecular complex assembly at mitochondrial contact sites (23,33,224). It is therefore of considerable interest that VDAC and the adenine nucleotide translocator have also been implicated in mitochondrial permeability transition pore formation. Competition between HK and pro-apoptotic signals converging at VDAC-enriched mitochondrial contact sites is thought to directly couple metabolism to the antagonism of apoptogenic stimuli (23). As noted previously, these coupling mechanisms may also directly contribute to the Crabtree effect and the coordination of metabolism in different intracellular compartments (23).

Signal transduction targets

Numerous signaling effectors can transduce trophic, stress and energy status signals within cells. Although not metabolism-specific, they frequently serve to couple metabolism with proliferative and cell survival functions crucial for all cells. These pathways frequently overlap or intersect with oncogenic signaling mechanisms and can assume particular importance in cancer. Trophic signal transduction pathways constitute particularly attractive targets for metabolic reprogramming and dysregulated metabolism (21,34,123). Hypoxic regulation of metabolism is also highly integrated with cellular signaling cascades involved in proliferation and stress responsiveness. As such, metabolism can be indirectly targeted via a variety of factors capable of modulating signal transduction pathways or associated coupling mechanisms that are capable of exerting metabolic control.

AMPK is a major sensor and regulator of cellular energy balance that may mediate the tumor suppressor effects of (LKB1) (225). LKB1 activates AMPK under appropriate conditions, and its loss is common in cancer (225). AMPK is stimulated by AMP levels and low corresponding AEC values, and its activation promotes a shift from anabolic to catabolic processes (226). Direct metabolic effects attributed to AMPK include increased Glc utilization and FA oxidation with corresponding reductions in lipogenesis and protein synthesis, which can be partly attributed to altered activation of key biosynthetic enzymes (205). These changes partly underlie the rationale for using pharmacologic activators of AMPK (e.g. metformin and salicylates) to treat selected cancers (225,227). The relationships between metabolism and energy signals are not fixed, and both metabolism and its regulation by LB1/AMPK/mTOR signaling are highly contextual in nature (228). Similar relationships exist between metabolism and trophic factor signaling.

Sirtuins are NAD^+-dependent deacylases with established roles in intermediary metabolism, cellular stress responsiveness and DNA maintenance and repair (206,207). They influence genomic stability via primary effects on Glc and lipid metabolism and secondary effects on oxidant stress resistance and epigenetic histone acylation (206,229). In addition to effects in cancer cells, sirtuins can indirectly influence cancer cell survival and growth via immunomodulatory effects in activated immune cells (139,230).

Metabolic pathways importantly transduce cellular signals in addition to their conventional enzymatic and metabolic functions (Supplementary Figure S1, available at *Carcinogenesis* Online) (231). As such, metabolic disruption may have profound extra-metabolic consequences not fully reflected in conventional metabolic profiles or assays. The metabolic effects of altered flux through a given pathway may also be mediated by exhaustion of—or competition for—limited quantities of shared cofactors that alter normal metabolic coupling mechanisms (e.g. disruption of oxidoreductase coupling via development of redox sinks) (Figure 3). Signal transduction pathways responsible for metabolic niche signaling or capable of influencing cancer dormancy or reactivation are also attractive candidates for study (232).

Given its contextual and dynamic nature, efforts to better understand cancer metabolism must obligatorily consider the complexity and heterogeneity of cancer cells, their environment and their interactions. Cancer biology can vary considerably over dimensions of both time and space (4) and may be amplified by deterministic considerations such as anabolic and catabolic demands imposed by proliferation or cellular stress. As such, variations in substrate or O_2 availability or extracellular pH may provide logical platforms for investigation, but the corresponding importance of individual molecular targets may vary in parallel.

Evidence for pro-carcinogenic environmental exposures capable of promoting metabolic reprogramming and dysregulation

Toxicological data, available for many suspected or known environmental carcinogens, frequently lack mechanistic and functional information regarding their specific roles as determinants of metabolic hallmark development. Effects of agents examined in isolation also cannot be simply extrapolated to complex mixtures, particularly at low concentrations (1,17–19). The fundamental contributions of—and requirements for—metabolic restructuring in carcinogenesis are still incompletely delineated and, in many cases, have not been directly examined. Thus, neither a sufficient understanding of the potential pro-carcinogenic effects of realistic everyday exposures nor their potential metabolic targets is available. As such, more rigorous experimental attention to fundamental underlying perturbations in cellular

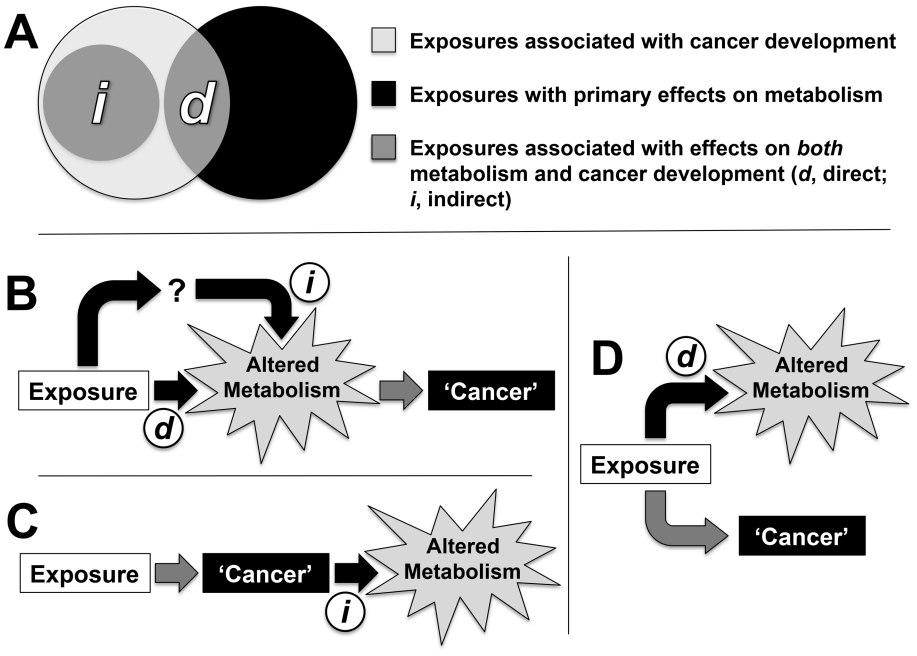

Figure 7. Possible hierarchical relationships between environmental exposures, carcinogenesis and metabolism. (**A**) Metabolic changes may be either a direct (*d*) or indirect (*i*) consequences of environmental exposure. Only those subsets of exposure associated with *both* carcinogenesis and dysregulated metabolism (*i* and *d*) are considered above. The metabolic hallmarks of cancer may represent either a cause (**B**) or a consequence (**C**) of cancer development. (**D**) In principle, associated metabolic changes could also represent epiphenomena arising in parallel but bearing no direct causal relationship to cancer development *per se*. The absence of such a direct causal relationship does not preclude important roles for adaptive metabolic selection advantages. Most experimental approaches to the study of metabolic reprogramming and dysregulated metabolism in cancer have not been designed to distinguish between these scenarios.

metabolism by both individual exposures and the exposome (233) is clearly needed.

In principle, pro-carcinogenic exposures may be directly genotoxic, indirectly genotoxic or non-genotoxic (234,235). Exposures that are not directly genotoxic may be indirectly genotoxic via mechanisms involving cellular metabolism (Figure 6), which can represent either a cause or a consequence of genotoxicity (Figures 1 and 7). For example, exposures with primary effects on oxidant stress or its amelioration can indirectly promote genotoxic injury. Both direct and indirect genotoxic or mutagenic stresses affect the mitochondrial genome, as well as the nuclear genome. They may also reflect the induction or repair of nuclear or mitochondrial DNA leading to reactive changes that may involve altered metabolism. Many toxicants are capable of damaging mitochondria (236), but toxicant-induced mitochondrial dysregulation with the potential to incur metabolic shifts to a pro-oncogenic state has been poorly studied, and not every toxic reaction resulting in changes mimicking cancer hallmarks is necessarily carcinogenic. Ultimately, rigorous validation is still needed to ensure that environmentally realistic exposures, including mixtures, are unequivocally linked to the development of both cancer and accompanying phenotypic hallmarks such as dysregulated metabolism. Ubiquitous agents present the most obvious opportunities for widespread continuous exposure, but there is nothing to preclude substantive contributions by more environmentally restricted or discontinuous exposures as well. Even universal exposures may vary in degree and need not be fixed to be pertinent to cancer development. These complex interactional possibilities, coupled with the fact that low-dose combinatorial effects on metabolism-supported and/or-limited cancer development and progression have not been rigorously or comprehensively addressed, speak to major gaps in our understanding of environmental cancer risk and the specific roles played by metabolism in associated cancer development.

Selected prototypical exposures with the potential to act on metabolic targets

A cross-hallmark search analogous to that employed for molecular target selection was used to identify prototypical exposures with the potential to promote metabolic reprogramming or dysregulation. Exposure classes identified as candidates for further scrutiny included organophosphates (e.g. diazinon and malathion), pyrethroids (e.g. cypermethrin), heavy metals (e.g. Fe, Cu, Ni and Cd), ETC poisons (e.g. rotenone) and reactive aldehydes (e.g. acrolein) (Table 3; Supplementary Table S2, available at *Carcinogenesis* Online). Agents were selected for further study based on perceived environmental ubiquity and evidence of the ability to either directly or indirectly promote cancer hallmark-like effects and are intended as representative examples only.

Organophosphates

Low dose exposures to organophosphate insecticides such as diazinon and malathion are common and have been associated with increased cancer risk (237–241). Members of this chemically diverse group of agents share the common ability to irreversibly inactivate cholinesterases and other Ser hydrolases via covalent modification of catalytically active Ser residues (242). Organophosphates are also known endocrine-disrupting chemicals (17,241), which makes them ideal candidates for the study of low-dose metabolic effects given the intrinsic sensitivity of the endocrine system (17) and established endocrine actions relevant to many of the hallmarks

Table 3. Cross-hallmark effects for selected chemical exposures

Dysregulated metabolism—prototypical disruptor candidates	Evasion of anti-growth signaling	Angiogenesis	Genetic instability	Immune system evasion	Resistance to cell death	Replicative immortality	Sustained proliferative signaling	Tissue invasion and metastasis	Tumor-promoting inflammation	Tumor microenvironment
Cypermethrin	Unknown	Unknown	+	Unknown	+	Unknown	+	+	+	Unknown
Acrolein	±	–	+	Unknown	±	±	–	+	+	–
Rotenone	–	Unknown	+	Unknown	–	±	–	–	–	+
Metals (e.g. cadmium, chromium, copper, iron and nickel)	±	±	+	+	±	±	±	+	±	+
Hexythiazox	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Organophosphates (e.g. diazinon and malathion)	+	+	+	Unknown	–	–	±	Unknown	+	Unknown

Exposures listed in the left-hand column, chosen for their potential to act on selected metabolic targets, were broadly classified as promoters (*+, green cells*) or antagonists (*–, red cells*) for the development of other listed cancer hallmarks based on associations with and/or demonstrated experimental ability to promote or antagonize cancer-associated phenotype development. Where indicated, evidence for the potential to both promote and antagonize cancer-associated phenotype development was found (*±, yellow cells*), or insufficient evidence was found in the literature to warrant such speculation (Unknown, *black cells*). For specific references used to construct this table, see Supplementary Table S2 and associated references in Supplementary Information, available at *Carcinogenesis* Online.

of cancer, including effects on metabolism, apoptotic susceptibility and proliferation (17,154). Although direct cholinergic contributions to cancer development have been suggested, organophosphate-induced oxidant stress and associated genotoxicity are thought to play more important etiologic roles (242). Interestingly, low level exposures during development have been associated with persistent postnatal abnormalities in both Glc and lipid homeostasis in rodents (243). The ability of organophosphates to covalently modify and inhibit cellular lipases, which are Ser hydrolases like acetylcholinesterases (244), suggests a least one mechanism whereby these agents may directly influence intermediary metabolism and promote compensatory reprogramming. Other direct metabolic effects are not well delineated.

Pyrethroids

Environmental exposures to pyrethroids, such as cypermethrin, are also common (245) and have been associated with oxidant stress (242,246) and alterations in both carbohydrate and lipid metabolism (247,248). Although the molecular underpinnings of these metabolic changes have been incompletely defined, pyrethroids are classified as EDC (17,154) and directly influence ion transport (246,249,250), suggesting several potential mechanisms for interaction with metabolism.

Reactive aldehydes.

Reactive aldehydes, such as acrolein, are ubiquitous in the environment and possess demonstrated carcinogenic potential in animals (251). Acrolein, in particular, directly forms DNA adducts and inhibits DNA repair mechanisms that can amplify the toxicity of other agents. Mitochondrial DNA is particularly susceptible to such mutagenic damage due to absent nucleotide excision repair mechanisms (252). Acrolein and other reactive aldehydes like hydroxynonenal and oxynonenal are also produced endogenously by lipid peroxidation (251), suggesting both endogenous and exogenous sources of exposure and a specific basis for mechanistic interactions with other classes of agents that promote oxidant stress. Interestingly, these compounds are detoxified by the promiscuous metabolic enzyme aldose reductase, which has much greater affinity for these agents than for Glc (253) and is overexpressed in cancers (254).

Metals

Metals are ubiquitous in both biological systems and the environment (245,255–257). Their biocatalytic importance is underscored by the fact that roughly half of all enzymes are metalloproteins (255,258). It is therefore not surprising that disruption of metal homeostasis can have profound pathophysiological consequences. Carcinogenic roles for both organic and inorganic forms of heavy metals are well-established (245,257). Unliganded metal ions such as iron (Fe), cadmium (Cd), copper (Cu), cobalt (Co), chromium (Cr) and vanadium (V) are capable of disrupting normal biocatalytic functions and generating ROS via either Haber–Weiss or Fenton-type reactions (256). Arsenic (As) and Cr are also capable of direct free radical generation (256). Metal ions thus represent important exogenous sources of ROS, and metal-induced oxidant stress and lipid peroxidation have been implicated in carcinogenesis (242,256). Although selective enzyme inactivation via covalent modification of thiols and other metal-reactive groups are well described (259), low-dose As exposure has been reported to augment metabolism in a manner reminiscent of cancer, possibly via induction of hypoxic signaling (259a, 259b, 259c). Metalloestrogenic contributions to hormone-responsive cancers have also been reported (260). As a

class of agents, metals have been identified as potentially capable of promoting the development of multiple cancer hallmarks (Table 3) and are thus attractive candidate effectors in both carcinogenesis and cancer hallmark development. Broad low level environmental exposures to barium (Ba), molybdenum (Mo), cesium (Cs), thorium (Th), tungsten (W) and uranium (U) are also well documented (245), although their relative pro-carcinogenic importance and metabolic effects are incompletely understood.

Specific caveats in cross-hallmark comparisons to prototypic pro-carcinogenic exposures

Prototypic exposure selection biases

Only previously studied exposures found in the published literature are included in the list of prototypic exposures selected for cross-hallmark comparison (Table 3). By definition, important unstudied or understudied exposures will be underrepresented in such a list. As a consequence, this list is incomplete and reflects fundamental literature biases that require special consideration when planning or conducting experiments addressing pro-carcinogenic responses to environmental exposures. The listed prototypic exposures are merely intended as possible starting points for future studies addressing these deficiencies.

Implicit assumptions in cross-hallmark comparisons

Assessment of the ability of prototypic exposures to influence multiple cancer hallmarks warrants brief discussion. The very notion that an exposure can monolithically either promote or oppose the development of a given phenotype belies the dichotomous nature of metabolism and presumes singular contributions and common underlying mechanisms, as well as similar time courses of action and directional congruence across models. Since no single model is sufficient for the study of cancer metabolism, all such studies should ideally be experimentally validated in diverse cancer-relevant models under non-monotonic conditions (18). Selected comparisons were largely between monotonic exposures and the development of individual hallmarks with no set requirements for evidence of either cancer specificity or the concomitant or sequential development of multiple hallmarks in a common model under identical—preferably environmentally relevant—conditions. These may not be trivial considerations given the intrinsic heterogeneity of cancer cells (4,29,30,120,168,261) and the fact that the various hallmarks examined are neither fixed nor specific for cancer (4,15,262). A disproportionate focus of the current literature on the effects of industrial chemicals may also overlook many important exposures to natural carcinogens, radiant energy and infectious agents (1,263). Given the paucity of relevant functionally validated data and known publication biases against low dose non-monotonic responses (17), it is likely that many important environmentally relevant exposures were not captured by these searches. Other promising exposures identified during the course of this review, but not captured by the prototypic exposure search, were not included due to space constraints or prior classification as known or probable carcinogens. Of these, benzo[*a*]pyrene probably warrants brief mention as one of the few known agents capable of inducing sustained metabolic alterations *in vivo* following a single systemic exposure (264).

Selectivity requirements for prototypic pro-carcinogenic exposures

Although an attempt was made to identify exposures with the potential to selectively modulate metabolism, not all pro-carcinogenic exposures need to selectively affect metabolism to contribute to cancer development. Recognizing that multiple simultaneous or sequential insults or defects may be required for carcinogenesis (1,127,265), it is conceivable that any mechanistic selectivity required for cancer development may be provided by a subset, rather than all, of the required promotional insults, whether simultaneous or sequential. Non-selective exposures may combine with more selective insults to yield selective derangements. For example, if oxidant stress is an important determinant of disease development, the nature of the stress—including its magnitude, duration, location and physicochemical basis—may be more important than its source(s). In principle, a non-selective agent could simply lower the susceptibility threshold for other, more selective agents or *vice versa*. Underlying comorbid disease states and genetic susceptibilities also play important roles in the establishment of predisposing or permissive conditions conducive to cancer development. The roles for multiple simultaneous, sequential or cumulative effects may also differ between targets, effectors and individual hosts. Metabolism itself may serve as an enabler of other carcinogenic contributors. For example, general permissive effects on cell metabolism could indirectly support cancer development by supporting associated proliferation and growth and/or by providing selection advantages via the flexibility to utilize alternate substrates to adapt to varying environmental conditions.

Implicit assumptions and corresponding knowledge gaps related to the metabolic features associated with early carcinogenesis and latency

It is reasonable to assume that metabolic phenotypes associated with early carcinogenesis share at least some features with established cancers, although this has not been firmly established. The temporal relationships between environmental exposures and cancer development are frequently extended (so-called latency; Figure 5), which increases experimental complexity due to the sheer number of potential intermediate effectors and the extended timeframes over which direct and indirect effects may evolve. As such, there is a need for early surrogate markers of cancer development. Cancers arise from phenotypically diverse tissues and retain core parental cell gene expression patterns (22), suggesting alternate paths to common shared phenotypes that can differ both qualitatively and quantitatively during cancer development (Figure 5). For example, a highly glycolytic cancer phenotype arising from a glycolysis-dependent parental tissue such as brain would presumably develop via fundamentally different mechanisms than a similarly glycolytic cancer arising from tissues with a lower dependence on glycolysis such as liver or the endocrine pancreas. Since the metabolic phenotype of cancer is neither fixed nor specific for cancer (4), it is plausible to assume that changes associated with carcinogenesis may vary similarly. As such, there is a compelling need to both define and better understand the changes associated with both early carcinogenesis and established cancer. The persistence and reversibility of effects associated with the entire spectrum of cancer development and their identity with fully established cancer phenotypes warrant particular attention (Figure 5). The ability of discontinuous exposures to mimic continuous exposures and cumulative effects also require careful scrutiny.

Assessment of pro-carcinogenic potential in complex environmentally relevant mixtures

Implicit in the concept of exposome-specific effects (233) are notions of additive and synergistic contributions to the aggregate carcinogenicity of complex low concentration chemical

mixtures (266,267). As such, compounds or classes of chemicals already considered—or suspected as—isolated carcinogens in the classical sense may contribute to cancer genesis and progression in complex mixtures at concentrations not traditionally deemed carcinogenic. These compounds thus warrant reconsideration as well. It is not practical to assume that individual contributions to the effects of complex mixtures can be simply deduced from aggregate responses. It is also perhaps not practical to assume that common mechanisms of action are always a given for agents within classes (250), nor can it be confidently assumed that agents from different classes have different mechanisms or modes of action. Not every pro-carcinogenic compound in a low-dose chemical mixture need act with the same mechanism of action, on the same cells, or even at the same time, so spatiotemporal considerations may be as important as specific mechanisms of action. For these reasons, conventional approaches for study, such as those specified within the World Health Organization/International Programme on Chemical Safety framework (268), may miss meaningful low dose interactions in promoting metabolic changes, the development of other phenotypic hallmarks and cancer development. Future studies must be specifically designed to address these issues.

Acutely toxic versus long-term pro-carcinogenic effects

Another major experimental difficulty encountered in the selection and study of exposures with the potential to reprogram metabolism involves the fact that candidate exposures frequently exhibit acute toxicity or elicit acute cellular responses that can be qualitatively or quantitatively indistinguishable from changes associated with true long-term carcinogenic effects. As such, it can be inherently difficult to distinguish acute toxic effects from cellular responses mimicking known cancer hallmarks if unambiguous relevance to cancer development is not demonstrated. There is, however, no established requirement that pro-carcinogenic agents must be acutely toxic nor that toxicity obligatorily leads to carcinogenicity. In fact, it can be argued that many, if not most, pertinent environmental exposures need not be demonstrably toxic.

Limitations of current toxicology screening approaches and future directions

Experimental approaches to carcinogenesis have historically focused on high level exposures associated with robust short-term effects. Given the practical limitations and expense of *in vivo* testing for carcinogenic potential (19), increasing emphasis has been placed on probabilistic *in vitro* high throughput screening (HTS) approaches that rely on surrogate *in vitro* 'single point' pathway activation testing in a standard cell model (235). Much of the focus has also shifted to the establishment of 'safe' single agent exposure thresholds in these models (19). In this regard, conventional toxicological assays and current HTS methods alone are ill-suited to define or focus the specific role(s) of dysregulated metabolism in carcinogenesis. Many screening platforms rely on the ability to discern 'toxicity signatures' and may provide associative information with limited specificity for—or mechanistic insights into—cancer metabolism *per se*. Given the highly contextual nature of metabolism, both assay conditions and the biochemical appropriateness of specific metabolic changes may be as important as their fundamental nature or direction. Alterations in control may also be as important as alterations in capacity (12,13) and may be missed in screens specifically targeting gene expression changes. Additional testing, including metabolic flux analysis, is thus needed to establish metabolic relevance, provide associated mechanistic insights and identify specific pro-carcinogenic inputs. Specificity for individual cancer types and the generalizability of results obtained in single models must also be assessed. Promiscuous assays are likely to identify non-specific agents or effects. Newer systems biology approaches to toxicological screening and evidence-based toxicology bring numerous strengths to the table and, in theory, have the power to markedly expand chemical testing capabilities. Unfortunately, they are also uniquely limited in their ability to address dysregulated metabolism. For example, the United States Environmental Protection (EPA) Agency Toxicology Forecaster (ToxCast) and associated multiagency Toxicology in the 21st Century Program (Tox21) screening platforms address toxicity or toxic response pathway activation, but they do not yield cancer-specific results.

The ToxCast platform is a heterogeneous collection of *in vitro* HTS assays used to identify agents capable of promoting gene expression changes that mimic toxicity or disease development *in vivo*. None of these assays directly assess metabolism, and their monotonic single-point nature limits their ability to provide important spatiotemporal and functional information needed to delineate specific metabolic contributions, address the reversibility of observed changes or distinguish between acute toxicity and more sustained carcinogenic effects involving common effectors. They also do not recapitulate the complexity and heterogeneity of *in vivo* biological responses to the exposome (233). For example, *trans*-activation by the Myc oncogene has been associated with alterations in both Glc and Gln metabolism (152), and numerous metabolic gene transcripts have been identified in the Myc-induced transcriptome. The *MYC* gene has also been mapped to the hallmark of 'energy metabolism' by an EPA literature review process (235). It is somewhat disconcerting, however, that ToxNet screening using a standard *MYC* reporter gene assay has not validated this association (235). This negative result may have any number of potential explanations, none of which exclude Myc involvement in metabolic changes associated with cancer. This assay presumes a unitary mode of *trans*-activation and employs a single hepatocellular carcinoma cell line stably transfected with a chimeric reporter gene construct driven by a canonical *cis*-acting Myc-binding motif fused in a non-native context to a minimal heterologous promoter sequence (269,270). Positive results thus require validation of endogenous target gene transcript changes in representative cancer models, and negative results can be completely uninformative. The Tox21 program will seek to expand the reach of ToxCast by pooling the combined HTS resources of multiple United States federal agencies (270a). The emphasis of these HTS platforms, however, is still firmly on new monotonic *in vitro* assays not designed nor equipped to specifically address metabolism *per se*. As such, they have limited direct utility in the detection or characterization of metabolic changes associated with cancer development.

No universal metabolic gene expression changes have yet been identified in cancer, and cellular origin strongly impacts overall metabolic gene expression patterns (22). Approaches designed to detect large gene expression changes assume that changes in capacity are sufficient to account for metabolic phenotype development and do not address the dynamic controlling influences of substrate availability, allosteric feedback or cellular energy demands in intact cells (Supplementary Figure S1, available at *Carcinogenesis* Online). As such, they may fail to detect crucial determinants of dysregulated metabolism. The routine use of fixed

non-physiological culture conditions for HTS assays also represents a methodological cause for concern, as the nutrient largesse associated with standard culture conditions fail to recapitulate pertinent *in vivo* growth and selection conditions and may strongly influence results.

Genomic sequencing initiatives launched to identify somatic mutations associated with cancer development (271,272) have been driven, in part, by identification of specific mutations associated with trophic signaling and oncometabolite generation (191,273). The metabolic consequences of such mutations—which may occur on the background of germline or somatic mutations in susceptibility genes important for DNA repair and maintenance (153)—require empiric determination via conventional biochemical methods for which few experimental shortcuts exist. Given the predominance of non-coding mutations (273,274) and the increasingly recognized importance of nonlinear epistatic gene interactions and epigenomic *cis*-acting regulatory element modifications in disease development (Figure 6) (274), more comprehensive systems-based approaches incorporating such biological knowledge into genotype analysis and interpretation are also needed (274).

Despite their conceptual appeal, unitary toxicological modes of action are not always predictable (255) and must be empirically validated, especially for dynamic and interactive processes such as intermediary metabolism. These considerations assume even greater importance in carcinogenesis, which is a complex, multistage process where no universal mechanistic requirements have yet been identified. Given the inherent limitations of existing systems biology frameworks and platforms, novel or complementary approaches are needed to address the metabolic consequences of environmental exposures and their specific contributions to carcinogenesis and associated hallmark development. Genomic, transcriptomic, proteomic and metabolomic approaches (Supplementary Figure S1, available at *Carcinogenesis* Online) provide powerful opportunities to identify specific patterns of gene expression and/or metabolite accumulation that distinguish cancer cells and help focus additional targeted study, albeit with the caveat that metabolomic data, in its simplest form, provides static information in the form of contextual snapshots of highly dynamic metabolic processes (86,275). Multiple distinct pathways may share individual metabolic intermediates (96), so conventional metabolic flux analysis under biologically relevant conditions is still needed to fully interpret this information. By definition, the experimental relationships between the exposome and the metabolome are not fixed (Supplementary Figure S1, available at *Carcinogenesis* Online), so such studies need to be carefully designed and standardized, as the type and magnitude of metabolic flux within cells will dynamically reflect a variety of intrinsic and extrinsic experimental variables, including substrate availability, cell cycle stage, environmental conditions and extant energy demands. As such, perturbational profiling strategies (155,188) may enhance or complement conventional transcriptomic, proteomic, metabolomic and functional screening approaches to the identification of mechanistic determinants of metabolic change.

Finally, no single model is probably sufficient to address the complex and heterogeneous metabolic changes that support cancer development and progression, and common cellular phenotypes—such as proliferation—can exhibit diverse underlying mechanistic bases and metabolic dependencies (16). However, a better understanding of the fundamental metabolic requirements and associated molecular prerequisites for cancer development is likely to accelerate progress in the field. Recent advances in targeted genomic modification and the availability of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9-based genome-wide mutational screening libraries makes phenotypic screening for obligatory metabolic gene requirements in cancer hallmark development and selection feasible (276–278). As such, this represents a promising new screening platform for addressing the underlying requirements of functional alterations not currently amenable to study via HTS approaches. The ability to screen for specific metabolic phenotypes and selective growth or survival advantages, without *a priori* assumptions, should facilitate the identification of specific gene expression requirements for (i) metabolic phenotype development or loss, (ii) changes in metabolic control or (iii) the development of tolerance or flexibility to respond to altered growth conditions or stresses. In theory, screens can be specifically devised to mimic microenvironmental conditions to identify genetic requirements for the ability to thrive under nutrient-limited, hypoxic, oxidative, acidotic or other stressful physicochemical conditions, both individually and in combination. In principle, they can also be designed to select for co-development of other cancer hallmarks or to identify specific genetic requirements for carcinogenic susceptibility.

Discussion

Metabolic reprogramming and dysregulation are widely recognized correlates of—if not absolute prerequisites for—both cancer genesis and progression. If and where metabolic changes constitute obligatory steps on the path of carcinogenesis, however, remain incompletely delineated (Figure 5). Most work in the field has focused on the hallmarks of established cancer, but the metabolic features associated with cancer genesis could fundamentally differ in nature, magnitude or direction from those associated with established cancer or its progression. As such, there is a compelling need for additional basic research to understand the timing of appearance and subsequent natural history of characteristic metabolic changes, as well as their mechanistic underpinnings and specific functional contributions to cancer development and progression. In their seminal 1981 report to Congress, Doll and Peto (1) argued that both ‘mechanistic’ and ‘black box’ approaches to the study of cancer were needed to reduce avoidable environmental risks. Now, over three decades later, this assessment is still valid. It can be argued, however, that our mechanistic understanding of carcinogenesis has failed to keep pace with our ability to identify risk. In the specific case of cancer metabolism, current HTS strategies for risk assessment have the potential to widen this gap if not obligatorily coupled to rigorous functional analysis under biologically relevant conditions.

Warburg’s proposed primary role for fixed mitochondrial defects in cancer development (5,11,279) has now been largely discounted (6,7,23,280). Nonetheless, it does not follow that mitochondria cannot—or do not—contribute to cancer genesis and progression (6,281), albeit perhaps not in the manner that Warburg originally envisioned. Given their vital amphibolic roles, fundamental involvement seems likely, if not obligatory (171). Consistent with this notion, most cancer cells have unimpaired or increased capacities for oxidative metabolism (6,7,23), and the cataplerotic and catabolic support roles played by mitochondria in anabolic cancer metabolism are increasingly recognized. As such, simple characterizations of cancer metabolism as reflecting a discrete shift from one type of metabolism to another are probably invalid (12,23) and owe more to Warburg’s original

hypotheses than his data or the subsequent literature (5,6,8,12). While it is reasonable to speculate that metabolic changes associated with cancer are necessary but insufficient for carcinogenesis, additional basic research is needed to address the specific roles played by such changes in cancer susceptibility, genesis and progression, as well as their timing, interrelationships and importance relative to other fundamental hallmarks of cancer. It remains to be seen whether dysregulated metabolism is a cause or a consequence of cancer development—or both (Figures 1 and 7). Given their ubiquity, it seems highly unlikely that metabolic changes associated with cancer are simply non-deterministic by-products of cancer development. The robust catabolic and anabolic requirements of rapidly proliferating cancer cells and the associated stresses that accompany rapid cell growth make it more likely that dysregulated metabolism provides an expanded metabolic repertoire serving to remove or minimize constraints limiting cancer development, growth or selection.

Cellular metabolism is inherently complex and dynamically responsive to intrinsic and extrinsic factors relevant to cancer development and its progression (16). These factors are neither necessarily fixed nor specific for cancer and include ambient growth conditions, intrinsic and extrinsic trophic signals, substrate availability, proliferative state and associated catabolic and anabolic cellular demands. These complex interrelated variables may differ both quantitatively and qualitatively within or between cells and may fluctuate in direction, duration and intensity. Accordingly, metabolic phenotypes may vary widely between cancer cells at different intratumoral locations and at sites of metastasis (16,168). They may also reflect changes in intrinsic substrate preferences independent of—or in addition to—substrate availability or metabolic capacity. These factors and the reversibility of associated phenotypic changes must be rigorously interrogated when comparing cancer cells with their normal counterparts or parental precursors. The capacity for cellular energy generation greatly exceeds its utilization (8,25,80), and only a fraction of the potential energy available to cells is ultimately required for their survival (12,81). As such, metabolic control is probably a greater phenotypic determinant than metabolic capacity (12,13). Conventional biochemical analysis and flux studies are thus still needed to complement epidemiological and genetic approaches to the problem. Strictly statistical or 'gene's eye' views (282) of carcinogenesis and cancer metabolism are unlikely to fully address these issues.

Experimental approaches to carcinogenesis have typically been designed to address the simplest and most robust responses and interactions—the so-called low hanging fruit in cancer development. Although justifiable on practical grounds, these approaches frequently involve untested or unproven fundamental assumptions regarding the functional or environmental relevance of demonstrable changes—or their absence. Foremost among these considerations is the common tendency to assume that the largest changes are biologically most important and the converse inference that a lack of demonstrable change betokens an absence of biological effects. The latter can be particularly problematic in studying intermediary metabolism, insofar as (i) changes in metabolic flux need not be accompanied by steady-state changes in the absolute abundance of metabolic intermediates and (ii) very small changes in the direction or magnitude of flux may have profound functional consequences and a disproportionately large phenotypic impact.

In addition to addressing common misconceptions, this review has attempted to broadly outline key unmet needs and unresolved issues in the field, in part, to provide a conceptual framework for future efforts focused on the mechanistic understanding of metabolism's roles in exposure-associated cancer development. A number of major questions and experimental challenges remain. For example, the reversibility of identifiable determinants of metabolic change associated with cancer development needs to be addressed. The relationships between short-term actions of candidate effectors and persistent metabolic changes also require mechanistic interrogation to identify key transitional events and critical coupling mechanisms linking metabolism to cancer development. The ability of discontinuous exposures to mimic continuous exposures also needs to be addressed. To effectively prognosticate, treat and ultimately prevent cancer, a fundamental understanding of its underlying biology—particularly its mechanistic origins, its spatiotemporal evolution and its fundamental phenotypic determinants—will ultimately be required. Environmental exposures do not occur *in vacuo*, however, and associated metabolic changes will, by definition, occur against the backdrop of complex interactions with other environmental, genetic and epigenetic factors associated with cancer development and progression. Associations between some cancers and exposures incurred during embryonic development suggest specific developmental context requirements (283,284) and are illustrative of this concept.

Our fundamental understanding of cancer metabolism, its underlying mechanistic determinants, its control, its limits of capacity and its causal relationships with the development of both cancer and its accompanying hallmarks would be best served by the following general recommendations in designing follow-on research:

1. Both known and suspected carcinogens should be systematically examined for metabolic effects at environmentally relevant concentrations and exposures. Metabolism should also be interrogated as both a potential cause and consequence of carcinogenesis (Figures 1 and 7), with the caveats that cancer is heterogeneous and relationships between metabolism and cancer development may differ according to both cellular origin and stage of progression (4). Given the long latent periods associated with cancer development following implicated exposures (Figure 5) (285–287), a better understanding of the temporal and causal relationships between carcinogenic exposures and the intermediate effectors linking them to their ultimate targets is required (Figures 1 and 7). Early surrogate markers of carcinogenesis or carcinogenic commitment are also needed to facilitate these efforts (288).
2. Rather than examining individual exposure-related outcomes in isolation, the field would also be well served by more integrated approaches to the study of cancer biology that remain firmly anchored to unambiguous cancer-specific endpoints. The integration of multidisciplinary examination of environmentally relevant complex exposures into existing experimental frameworks should be a research priority for policy makers, and systems biology approaches to the study of carcinogenesis should fully incorporate current biological and biochemical knowledge. In addition, correlative high throughput data should be viewed as critical translational research platforms for the generation of specific mechanistic hypotheses that can be taken back to the laboratory for refinement and definitive testing.
3. Metabolic studies of exposure-associated cancer development should obligatorily be conducted under environmentally and biologically relevant conditions, with special attention to dynamic controlling factors such as substrate availability, metabolic feedback, environmental conditions and extrinsic trophic signals. Studies should also be

designed to explore non-monotonic relationships, as well as the sequence and natural evolution of individual phenotypic characteristics. The assumption of linear-no threshold models provides some rationale (albeit controversial) for studying high dose exposures, but there is no theoretical support for the idea that results of high-dose chemical perturbations can be simply extrapolated to low dose scenarios.
4. Finally, better triangulation and causal interrogation of the specific spatiotemporal and mechanistic relationships between environmental exposures, carcinogenesis and cancer hallmark development—particularly for dysregulated metabolism—is needed.

These recommendations directly address crucial gaps in our present understanding of the metabolic contributions to environmental carcinogenesis. They are intended to extend or complement, but not supplant, existing efforts to identify, target and characterize mechanistic contributions to carcinogenesis.

The lifetime exposome, cancer and intermediary metabolism are all inherently complex and pleiomorphic entities, and their study, both individually and in combination, is subject to numerous caveats and experimental limitations. Simple solutions to important complex problems are always desirable, but inherent complexity also sometimes demands intricate approaches and answers. There are few viable shortcuts in the study of metabolism, and individual changes must always be considered in the context of the cellular gestalt. With this in mind, a pair of quotes pertinent to both metabolic complexity and its study—and used by Efraim Racker to close his now-classic tome on bioenergetics (8)—are reproduced as an epilogue below:

> I have yet to see a problem however complicated that, when you look at it the right way, does not become more complicated.—Paul Adleston
>
> Everything should be made as simple as possible but not simpler.—Albert Einstein

Supplementary material

Supplementary Figures S1 and S2, Tables S1 and S2 and other Supplementary Information can be found at http://carcin.oxfordjournals.org/.

Note Added in Proof

Space requirements precluded specific review of many important aspects of normal system-wide metabolic homeostasis (e.g. the Cori and Randle cycles), as well as detailed treatment of tumor-host relationships. It is therefore important to emphasize in closing that cancer metabolism, in all its forms, is ultimately an open system engaged in metabolic exchange with the host, a fact that must be taken into account in both experimental and therapeutic approaches to cancer.

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