Sybille Mazurek · Maria Shoshan
Editors

Tumor Cell Metabolism

Pathways, Regulation and Biology

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Preface

Almost all papers and reviews on the altered energy metabolism of tumor cells use the nearly century-old discovery of the Warburg effect as a starting point. One practical implementation of the Warburg effect, i.e., FDG-PET scan based on the increased glucose uptake of tumor cells, has already had an enormous impact on clinical routines and decisions. By contrast, the implications and consequences of the Warburg effect for the understanding of tumor biology and hence also for future treatment strategies are still in a state of development, intense research, and excitement.

About a decade ago, when interest in the Warburg effect was clearly rekindled and growing, there was considerable focus on the roles of energy and ATP metabolism in tumor biology. This has now changed to the understanding that tumor cell metabolism is fundamentally altered—indeed, to the point of being named a hallmark of cancer (Hanahan and Weinberg 2011)—and that these alterations develop in order to support not only energy production but also macromolecule synthesis required for rapid proliferation (Ward and Thompson 2012). A complementing understanding is that these alterations continue to develop with, and to influence, tumor progression. A key illustration of the progressive alterations is the adaptability of tumors to cellular stress (in particular, nutrient and oxygen restriction, and chemo- and radiation therapy). These responses involve a remarkable metabolic flexibility which in turn can involve schoolbook biochemistry as well as newly discovered metabolic pathways and complex processes such as glutaminolysis, novel signal transduction cascades, and autophagy.

Mitochondria have already since Warburg's time been in focus; however, while they were long believed to be "damaged" in tumor cells, it is now understood that, yes, their various functions may be altered, but these changes make mitochondria efficient contributors to the metabolic plasticity of the tumor cell. The rerouting, or alternative uses, in tumor cells of classical biochemical pathways—from the tricarboxylic acid cycle to nucleotide synthesis—is therefore now under scrutiny, as is the regulation of cellular mitochondrial content. The small but complex mitochondrial genome

(mtDNA) may harbor possibly cancer-specific mutations, the significance of which needs to be examined.

That cellular metabolism is an important determinant of tumor cell phenotype as well as tumor biology thus implies an extremely complex network of possible signaling pathways, enzymes, and metabolites. In addition, while some pathways are likely common to most cancer cells, many others are not always required or present in a given cancer cell. In short, research in this field seeks to answer such diverse questions as which oncogenes initiate altered metabolism, and how; moreover, what are the connections between metabolism and resistance to therapy, and between metabolism, differentiation states, and metastasis. It is also of interest to determine which pathways are most commonly altered, and conversely, which ones might be of diagnostic or predictive value or might be targeted in individualized therapy.

It is of course impossible for one single book to cover all aspects of the influence of cellular metabolism on tumor biology and tumor cell phenotypes, and we are more than regretfully aware that the present book leaves big gaps in terms of topics. What we do present here includes on the one hand specifics such as chapters on the roles and possible clinical value of specific oncogenes, enzymes and pathways, and an example of the use of metabolic tracers, and on the other hand also more general overviews of hypoxia, autophagy, and the microenvironment, and not least, overviews of metabolic wiring and the troubling flexibility of tumor cells.

Due to the sophistication and heterogeneity of the metabolic wiring and flexibility of tumors, research in this field is still in a state of mapping and charting. We hope that this book will contribute both to a general understanding of the complexity and to further mapping and interest in these intriguing questions. To then decipher what the metabolic profiles of tumors—whether in terms of proteomes, metabolomes, kinomes, mitochondrial functions, etc.—actually imply in terms of therapeutic targets, tumor progression, and prognosis is a major task for the future.

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Erratum to Chapter 2: Tumor Cell Complexity and Metabolic Flexibility in Tumorigenesis and Metastasis

Michael V. Berridge and Patries M. Herst

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In a previous version of this chapter an incorrect figure was shown for Fig. 2.1. The correct Fig. 2.1 is shown below. The Publisher would like to apologize for this mistake. The original version of the chapter has been corrected accordingly online and in print (see DOI 10.1007/978-3-7091-1824-5_2).

The online version of the original chapter can be found under
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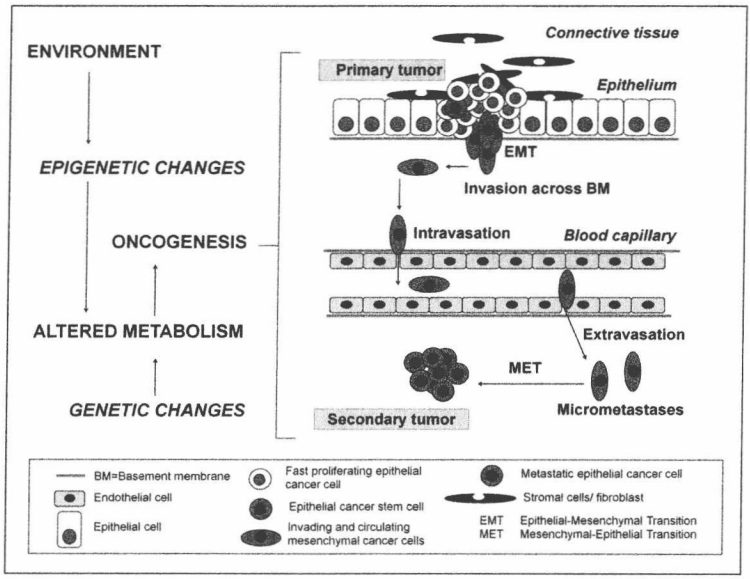

Fig. 2.1 Overview of oncogenesis of epithelial tumors. Oncogenesis takes place in context of the tumor microenvironment that includes oxygen tension, pH, nutrient supply, and interactions with other cell types in close proximity of the tumor, such as stromal cells and immune cells. Initiation begins with oncogenic mutations or epigenetic changes in expression of tumor suppressor genes and oncogenes of an epithelial cell, leading to alterations in cancer cell metabolism. Altered metabolism drives the establishment of a primary epithelial tumor which is initially contained within its tissue of origin. Some cancer stem cells undergo EMT, which makes them less sticky and more aggressive, breaking the basal membrane barrier and invading underlying tissues. Some of these mesenchymal tumor cells enter blood vessels (intravasation) and travel in the blood stream as circulating tumor cells. Once they leave the blood stream (extravasation), they form micrometastases in new tissues and organs. Once they undergo MET, they grow into macrometastases or secondary tumors of epithelial origin

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Part I
On the Roles of Altered Tumor Cell Metabolism in Tumor Biology

Chapter 1
Metabolic Remodeling in Bioenergetic Disorders and Cancer

Emilie Obre and Rodrigue Rossignol

Contents

1.1 Energy Metabolism, Control, and Regulation

The basic principles of energy metabolism regulation were deciphered in the late 1950s with the work of Warburg, Lenhinger, Krebs, Chance, Petersen, Weinhouse, and Vaupel among several others (Scheffler 1999; Weinhouse 1956). The regulation of controlling enzymes belonging to glycolysis, PDH complex, and Krebs cycle, all involved in ATP synthesis, mostly occurs by metabolic intermediates as

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ATP itself, citrate, F1, 6BP, and Pi. Another level of regulation of mitochondrial energy fluxes, as ATP synthesis or respiration was identified by Chance and Williams in the 1950s with the so-called respiratory control by ADP (Chance and Williams 1956; Cogliati et al. 2013). Thereafter, a large number of additional molecular regulations of oxidative phosphorylation (OXPHOS) were identified, as the recently discovered OPA1-dependent stabilization of the respiratory supercomplexes (Cogliati et al. 2013), the ATP synthase-dependent assembly of complex III (Ostojić et al. 2013), and the energy state-dependent RHEB-induced control of mitochondrial turnover (Melser et al. 2013). Consideration of the numerous means to regulate ATP transduction in the cell led to the notion of a "multistep control" of energy metabolism (Benard et al. 2010). More recently, the regulation of energy metabolism was closely linked, in a mutual way, with the control of cell growth and division. For instance, a signaling pathway central to cell biology and governed by the HiF1α transcription factor was shown to mediate a shift in a subunit of respiratory chain complex IV (Fukuda et al. 2007). Conversely, succinate accumulation in the cytosol is capable of inhibiting HiF1α degradation and to promote its stabilization (Pollard et al. 2005). Likewise, the AMP-activated protein kinase (AMPK) pathway stimulates the expression of several OXPHOS proteins when ATP needs are increased, as testified by a higher ADP/ATP ratio in the cytosol (Hardie et al. 2003). Another central pathway is the control of energy metabolism is the PGC1α pathway, a transcription co-activator, which participates in the stimulation of oxidative phosphorylation in cooperation with ERR-α or to the induction of gluconeogenesis in cooperation with HNFAα (Lustig et al. 2011). The RAS protein, involved in the control of cell mitogenic activities, also controls oxidative phosphorylation, both in cancer and noncancer tissues (Wei et al. 2012; Palorini et al. 2013; Gough et al. 2009; Telang et al. 2007). A role in modulation of OXPHOS capacity was also discovered for MYC and for p53, both of which play central roles in the control of cell growth and division, leading to the emerging concept of oncobioenergetics (Jose and Rossignol 2013). Central to this review article, a new layer of upper- or meta-regulation of energy metabolism was identified with the discovery of rewiring of metabolic circuits, governed by genetic determinants connected or not with changes in cell microenvironment. In particular, this upper level of bioenergetic control makes the link between catabolism and anabolism, thereby providing a more integrated view of cell metabolism plasticity. Prior to discussing the molecular bases and the physiology of metabolic remodeling, we provide below a rapid overview on cellular bioenergetics.

In most human tissues, mitochondria provide the energy necessary for cell growth and biological activities. It has been estimated that about 90 % of mammalian oxygen consumption is mitochondrial, which primarily serves to synthesize ATP, although in variable levels according to the tissue considered and the organism's activity status. Mitochondria intervene in the ultimate phase of cellular catabolism, following the enzymatic reactions of intermediate metabolism that degrade carbohydrates, fats, and proteins into smaller molecules such as pyruvate, fatty acids, and amino acids, respectively (Fig. 1.1). Mitochondria further transform these energetic elements into NADH and/or $FADH_2$, through β-oxidation and the

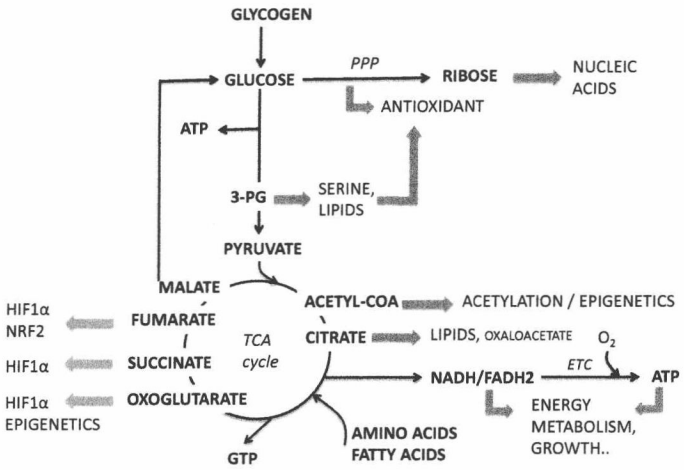

Fig. 1.1 Overview of energy metabolism pathways. In this chapter we refer to the production of biological energy in the form of adenosine triphosphate or ATP. This process occurs primarily through glycolysis, the end product of which is pyruvate and through subsequent oxidative phosphorylation. In most tissues, the pyruvate enters the mitochondrion and generates acetyl-CoA which is further oxidized at the level of the Krebs cycle to produce ATP, NADH, and $FADH_2$. The latter reduced equivalents are further oxidized by the respiratory chain to generate ATP via chemiosmosis, at the level of the F_1F_0 ATP synthase. This second mechanism of ATP production is referred to as oxidative phosphorylation. The Krebs cycle can also process alpha-ketoglutarate formed from glutamine, via glutaminolysis, or acetyl-CoA generated from fatty acids beta-oxidation. Those anaplerotic pathways are of particular importance in cancer cells. The citrate produced in the Krebs cycle can also escape this cycle (truncated Krebs cycle) and serve for lipid synthesis. As discussed in this chapter, this canonic description of energy metabolism does not apply to several cancers where the pathways are truncated (glycolysis and Krebs), rewired (anaplerotic entries from canonical or noncanonical glutaminolysis), and branched (lipid or serine synthesis from glycolysis). Therefore, while the pentose phosphate pathway and the Krebs cycle generate both reducing equivalents (NADH, NADPH, FADH2), ATP and GTP used for energy needs, these pathways also produce intermediates such as 3PG used for biosynthesis. Some metabolites such as fumarate can also modulate transcription factors as NRF2 while oxoglutarate can serve as substrate for HIF1α degradation and acetyl-CoA for histone acetylation. This figure illustrates the close link between catabolism, anabolism, and genetic/epigenetic regulations. *3-PG* 3-phosphoglyceric acid; *PPP* pentose phosphate pathway; *TCA* tricarboxylic acid cycle, i.e., Krebs cycle; *ETC* electron transport chain

Krebs cycle. Those reduced equivalents are then degraded by the mitochondrial respiratory chain in a global energy converting process called oxidative phosphorylation (OXPHOS) where the electrons liberated by the oxidation of NADH and $FADH_2$ are passed along a series of carriers regrouped under the name of "respiratory chain" or "electron transport chain" (ETC) and ultimately transferred to molecular oxygen (Fig. 1.2). ETC is located in the mitochondrial inner membrane, with an enrichment in the cristae. ETC consists of four enzyme complexes (complexes I–IV) and two mobile electron carriers (coenzyme Q and cytochrome c). These complexes are composed of numerous subunits encoded by both nuclear

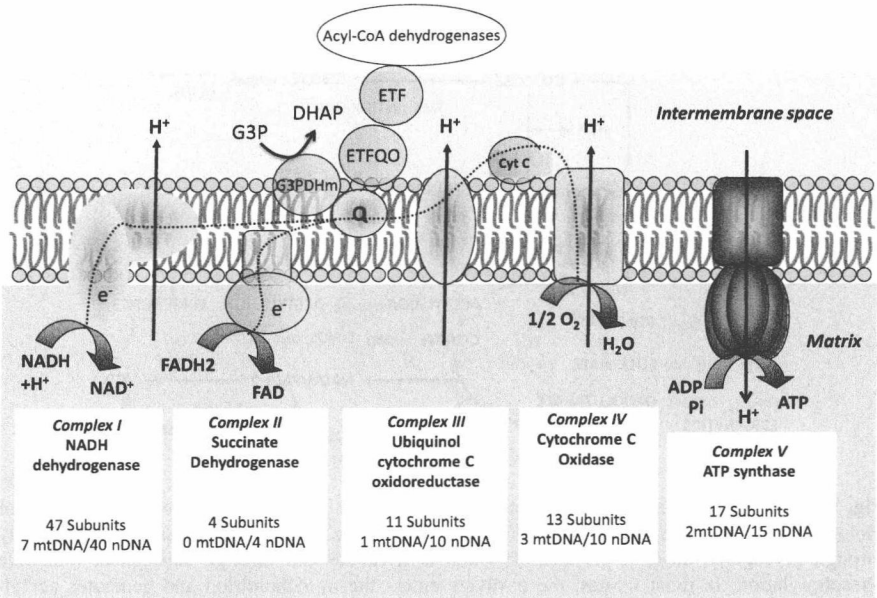

Fig. 1.2 The respiratory chain. For mammals, the respiratory chain consists of four enzyme complexes (complexes I–IV) and two intermediary substrates (coenzyme Q and cytochrome c). The NADH, H^+, and $FADH_2$ produced by the intermediate metabolism are oxidized further by the mitochondrial respiratory chain to establish an electrochemical gradient of protons, which is finally used by the F_1F_0-ATP synthase (complex V) to produce ATP, the only form of energy used by the cell. In this simple representation of the respiratory chain, the supramolecular organization (supercomplexes, dimers) is not shown. Of importance for this chapter, electrons can also be delivered to the respiration chain at the level of coenzyme Q by the ETF system or by the glycerol 3 phosphate dehydrogenase system. The respiratory chain can generate reactive oxygen species, and current research in the field of cancer metabolism indicates that such feature plays a role in metabolic remodeling, notably in metastasis. Uncoupling proteins can be expressed in the inner mitochondrial membrane to modulate ROS production. Also, different isoforms of complex IV subunits were found in cancer cells (COX4-1 and COX-2), depending on HIF1α stabilization. Lastly, mutations in mtDNA, impacting respiratory chain complexes activity, were found in a large number of tumors. *ETF* electron-transferring-flavoprotein dehydrogenase, *mtDNA* mitochondrial DNA, *nDNA* nuclear DNA

genes and mitochondrial DNA, with the exception of complex II (nuclear only). It was demonstrated that these complexes assemble into supramolecular assemblies called "supercomplexes" or respirasome (Schägger and Pfeiffer 2000; Schagger 2001). It is still debated whether some complexes, as complex I, can be found alone or if all are embedded in supercomplexes. In addition to the classic ETC components, other proteins are involved in the oxidation of nutrient-derived reduced equivalents and the subsequent reduction of coenzyme Q, used for ultimate ATP synthesis. This is the case for the electron-flavoprotein system, composed of the ETF and the ETF-QO, which connect fatty acid oxidation and coenzyme Q

reduction. The glycerol-3-phosphate dehydrogenase, which oxidizes cytosolic NADH to reduce mitochondrial FAD, also supports oxidative phosphorylation and participates to REDOX homeostasis. Lastly, the NADH-shuttling system, as the malate-aspartate shuttle, also supports OXPHOS and REDOX homeostasis via the delivery of cytosolic NADH to the mitochondrial matrix. The oxidation of NADH or $FADH_2$ by complex I or complex II, respectively, triggers the transfer of electrons from complex I (or II) to complex IV and mediates the extrusion of protons from the matrix to the intermembrane space, thus generating an electrochemical gradient of protons ($\Delta\hat{u}_H{}^+$) which is finally used by the F_1F_0 ATP synthase (i.e., complex V) to produce adenosine triphosphate (ATP), the main energetic currency of the cell. This gradient has two components: an electric potential ($\Delta\Psi$) and a chemical potential ($\Delta\mu_H{}^+$) that can also be expressed as a pH gradient (ΔpH). According to the chemiosmotic theory proposed by Peter Mitchell (1961), $\Delta\hat{u}_H{}^+ = \Delta\Psi - Z\Delta\text{pH}$, with $Z = -2.303$ RT/F.

Under physiological conditions, mitochondrial energy production can alternate between two energy steady states: basically, at state 4 (also denominated the "leak respiration state"), respiration is slow and ATP is not produced ($\Delta\Psi$ is high), while during state 3, respiration is faster and ATP is largely produced ($\Delta\Psi$ is lower). In particular conditions, such as mitochondrial inner membrane permeabilization or the use of a chemical uncoupler, $\Delta\Psi$ can be totally dispersed. As a consequence, respiration is accelerated and ATP production annihilated. The inhibition of respiratory chain complexes also generally decreases $\Delta\Psi$. Under physiological conditions, it is considered that mitochondria produce ATP in an intermediate state lying between state 3 and state 4. As shown by E. Gnaiger, respiration strongly depends on the availability of energy substrates which are multiple and can cooperate at the level of the Q-junction, thereby determining the value of the apparent maximal (uncoupled) respiration (Gnaiger 2009). ATP is the only form of energy used by the cell, and when produced in the mitochondrion, it is exported to the cytosol by the adenine nucleotide translocators (ANT1-4) in exchange for cytosolic ADP. Generally, the transport of energy metabolites, nucleotides, and cofactors in and out of the mitochondrial matrix is performed by specific transporters located in the inner membrane (Palmieri and Pierri 2010). These carriers can consume the membrane electrochemical gradient or not, depending on their mechanism of transport (electroneutral or electrogenic). A large part of OXPHOS regulation occurs at the level of these carriers, as shown by the control of the glutamate-aspartate shuttle (SLC25A12 also named Aralar or AGC1) by calcium (Fig. 1.3) and the "Gas pedal" model proposed by Frank Gellerich (Gellerich et al. 2013). Studies of metabolic control also showed that a large part of the control of mitochondrial respiration is located at the level of substrate carriers (Rossignol et al. 2000).

Therefore, the regulation of mitochondrial energy production at the level of ETC is concerted and multisite (Fig. 1.4) since modulations have been described at the level of the individual complexes, membrane leak, respirasome cohesion, or carrier activity. One should add to this molecular description the numerous signaling pathways that modulate OXPHOS properties (AMPK, HIF1α, PGC1α, RAS,