

PREFACE: ONCOMETABOLISM: A NEW FIELD OF RESEARCH WITH PROFOUND THERAPEUTIC IMPLICATIONS

In 1924, the German physiologist Otto Heinrich Warburg was the first to report the propensity of neoplastic cells to metabolize glucose via aerobic glycolysis rather than via the citric acid cycle (also known as Krebs cycle) as a fuel for mitochondrial respiration (Koppenol, Bounds, & Dang, 2011; Warburg, 1924). For a long time since then, however, much greater attention has been attracted by the discovery of the genetic and epigenetic alterations that characterize cancer cells than by their metabolic profile. Such a tendency was so pronounced that, at the end of the twentieth century, several chemotherapeutic agents specifically targeting oncogene addiction, i.e., the process whereby cancer cells rely for their survival and growth on the constitutive activation of oncogenic signaling pathways and/or on the permanent inactivation of oncosuppressive mechanisms, were licensed for use in cancer patients (Luo, Solimini, & Elledge, 2009). Conversely, no chemotherapeutic agent specifically devised to target the metabolism of cancer cells was available then, nor it is now, although (1) several widely employed and effective chemotherapeutics including methotrexate, 5-fluorouracil, gemcitabine, and many others (which are cumulatively known as antimetabolites), *de facto* exert antineoplastic effects by operating as metabolic inhibitors (but were discovered and developed based on empirical, as opposed to mechanistic, grounds); and (2) the safety and therapeutic profile of many of these agents are being evaluated in a growing number of clinical trials (Chabner & Roberts, 2005; Galluzzi, Kepp, Vander Heiden, & Kroemer, 2013). Indeed, it is only over the past decade that the complexity and prominent therapeutic implications of oncometabolism, which can be defined as the ensemble of metabolic rearrangements that accompany oncogenesis and tumor progression, have been fully recognized as a central aspect of malignant transformation (Hanahan & Weinberg, 2011). As a result of such a refocus in the interest of researchers and clinicians, the metabolic rewiring of cancer cells is now viewed as a rich source of targets for the development of novel chemotherapeutic agents, and an intense wave of investigation currently explores this possibility (Galluzzi et al., 2013; Vander Heiden, 2011).

At odds with long-standing beliefs, it is now clear that the so-called Warburg effect represents only the tip of the iceberg of metabolic alterations

associated with oncogenesis, which also encompass an increased flux through the pentose phosphate pathway, elevated rates of lipid biosynthesis, intense glutamine consumption, an improved control of redox homeostasis, and (at least in the initial steps of malignant transformation) decreased levels of macroautophagy (Schulze & Harris, 2012; Vander Heiden, Cantley, & Thompson, 2009; White, 2012). A few other common misconceptions about oncometabolism are in the process of being reconsidered based on preclinical and clinical findings from several laboratories worldwide.

First, the metabolic rewiring of neoplastic cells should not be considered as a self-standing hallmark of malignancy, but rather as a phenomenon that intimately accompanies, allows for and cannot be mechanistically separated from many, if not all, aspects of oncogenesis (Galluzzi et al., 2013; Locasale & Cantley, 2011; Wellen & Thompson, 2012). Accumulating evidence indicates indeed that (1) several metabolic intermediates such as ATP, acetyl-CoA, α -ketoglutarate, and reactive oxygen species play a major role in cell-intrinsic as well as cell-extrinsic signaling pathways (Galluzzi, Kepp, & Kroemer, 2012; Locasale & Cantley, 2011; Wellen & Thompson, 2012); (2) multiple proteins with prominent metabolic functions such as cytochrome *c* (which operates as an electron shuttle in the mitochondrial respiratory chain) and the M2 isoform of pyruvate kinase (PKM2, which catalyzes the last step glycolysis) participate in signal transduction (Galluzzi, Kepp, & Kroemer, 2012; Galluzzi, Kepp, Trojel-Hansen, & Kroemer, 2012; Gao, Wang, Yang, Liu, & Liu, 2012; Luo et al., 2011; Yang et al., 2011); and (3) several proteins initially viewed as "pure" signal transducers including (but not limited to) the antiapoptotic Bcl-2 family members BCL-X_L and MCL1 also impact on metabolic functions such as the handling of Ca²⁺ ions at the endoplasmic reticulum and the enzymatic activity of the F₁F₀ ATP synthase (Alavian et al., 2011; Perciavalle et al., 2012; Rong & Distelhorst, 2008).

Second, the metabolic changes linked to malignant transformation should not be considered as a general property shared by all types of cancer. It has indeed been clearly demonstrated that several variables including tissue type and oncogenic driver (and presumably many others) determine the metabolic profile of developing tumors (Yuneva et al., 2012). This has obvious implications for the use of metabolic inhibitors in cancer therapy.

Third, it should be kept in mind that the metabolic profile of neoplastic cells is far less specific than previously thought, but (with some exceptions) resemble that of highly proliferating nontransformed cells (Altman & Dang, 2012; Michalek & Rathmell, 2010). This notion is corroborated by the fact

that the most severe side effects of antimetabolites involve highly proliferating normal tissues, such as the intestinal epithelium and bone marrow (Chabner & Roberts, 2005). Nonetheless, the clinical success of these widely employed chemotherapeutic agents points to the existence of a therapeutic window for the use of metabolic inhibitors in cancer patients (Galluzzi et al., 2013; Vander Heiden, 2011). As it stands, multiple facets of oncometabolism can be considered as forms of "nononcogene addiction," a term referring to the fact that the survival of malignant cells relies not only on the constitutive activation of oncogenes and/or the permanent inactivation of oncosuppressive mechanisms but also on a wide array of genes and functions that are not inherently tumorigenic (Luo et al., 2009).

Finally, tumors (in particular solid neoplasms but also hematological malignancies) should no longer be considered as homogenous entities predominantly composed of malignant cells. Indeed, it is now clear that neoplastic lesions contain a large amount of nontransformed cells, including endothelial, stromal, and immune cells, and that oncogenesis takes place in the context of a complex network of physical and functional interactions among the malignant and nonmalignant components of the tumor microenvironment (Nagaraj & Gabrilovich, 2010; Pietras & Ostman, 2010). These interactions, part of which have direct metabolic implications (Nieman et al., 2011; Sonveaux et al., 2008; Whitaker-Menezes et al., 2011), are also attracting attention as targets for the development of novel antineoplastic agents (Galluzzi, Senovilla, Zitvogel, & Kroemer, 2012; Zitvogel, Galluzzi, Smyth, & Kroemer, 2013).

In *OncoMetabolism*, a thematic collection covering two volumes of the successful *Methods in Enzymology* series, leading researchers summarize the current state of the field from both a conceptual and methodological standpoint. The first volume, entitled "*Conceptual background and bioenergetic/mitochondrial aspects of oncometabolism*," provides a robust theoretical background on cancer-associated metabolic alterations, discussing how these relate to other aspects of oncogenesis such as the relentless proliferation and resistance to death exhibited by neoplastic cells. Thereafter, this volume offers a collection of techniques that can be employed to study the major bioenergetic and mitochondrial aspects of oncometabolism, including (but not limited to) alterations in glycolysis and oxidative phosphorylation. The second volume, entitled "*Cell-wide metabolic alterations associated with malignancy*," proposes a series of methods for the investigation of global facets of oncometabolism, including (but not limited to) deregulations in Ca^{2+} fluxes and autophagy, as well as (malignant) cell- or tissue-wide metabolomic alterations.

OncoMetabolism is expected to provide a comprehensive and reliable methodological guide to beginners and experts in this exciting and rapidly expanding area of cancer research.

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