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Review

The metabolic cooperation between cells in solid cancer tumors

Philippe Icard [a,b,*], Perrine Kafara [b,c,d], Jean-Marc Steyaert [a], Laurent Schwartz [a], Hubert Lincet [b,c,d]

[a] Ecole Polytechnique, Laboratoire d'Informatique, Palaiseau, France
[b] Biologie et Thérapies Innovantes des Cancers Localement Agressifs (BioTICLA) EA 4656, F-14032 Caen, France
[c] Normandie Univ France, France
[d] Centre François-Baclesse, Caen, France

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ABSTRACT

Cancer cells cooperate with stromal cells and use their environment to promote tumor growth. Energy production depends on nutrient availability and O_2 concentration. Well-oxygenated cells are highly proliferative and reorient the glucose metabolism towards biosynthesis, whereas glutamine oxidation replenishes the TCA cycle coupled with OXPHOS-ATP production. Glucose, glutamine and alanine transformations sustain nucleotide and fatty acid synthesis. In contrast, hypoxic cells slow down their proliferation, enhance glycolysis to produce ATP and reject lactate which is recycled as fuel by normoxic cells. Thus, glucose is spared for biosynthesis and/or for hypoxic cell function. Environmental cells, such as fibroblasts and adipocytes, serve as food donors for cancer cells, which reject waste products (CO_2, H^+, ammoniac, polyamines…) promoting EMT, invasion, angiogenesis and proliferation. This metabolic-coupling can be considered as a form of commensalism whereby non-malignant cells support the growth of cancer cells. Understanding these cellular cooperations within tumors may be a source of inspiration to develop new anti-cancer agents.

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1. Introduction

Cancer tumors are composed of an ecosystem of evolving clones competing and cooperating with each other and with other cells within their microenvironment. They include a mixture of cancerous and non-cancerous cells such as stromal cells, adipocytes and macrophages, all cooperating towards promoting division, invasion and dissemination. Life results from symbiotic cooperation: animal cells are heterotrophs and absorb sugars and oxygen, whereas they emit CO_2 and water; plant cells are autotrophs, absorb CO_2, emit oxygen and form sugars. Thus, cells that breathe need cells that photosynthesize and vice versa. In this review, we have endeavored to clarify the cooperation that seems to take place in tumors, emphasizing the fact that cancer cells

* Corresponding author at: BioTICLA EA 4656, Centre de Lutte Contre le Cancer François Baclesse, Avenue du Général Harris, BP5026, 14076 Caen Cedex 05, France. Tel.: +33 2 31 45 52 08.
E-mail address: philippe.icard@hotmail.fr (P. Icard).

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optimize the resources present in their environment to proliferate. We demonstrate that environmental cells, such as adipocytes and fibroblasts, may serve as food donors for cancer cells which reject waste products (CO_2, H^+, ammoniac, polyamines…), promoting proliferation and dissemination. The rate of proliferation would be dependent on the dynamic reconstitution of ATP and cofactors (NAD^+, NADPH, H^+), which is controlled by the HIF-1/HIF 2 ratio, which decreases gradually with O_2 concentration [1–4]. The signaling pathways (activation of oncogenes such as c-Myc, inhibition of suppressor genes such as P53 and PTEN) that support tumor proliferation are not presented in this review and readers are referred to several other reviews [5–11]. In our paper we focus on understanding the biochemical pathways that support this type of commensalism, which may be a source of inspiration for developing new anti-cancer treatment options.

2. Cancer cells adapt their proliferation in relation to O_2 concentration

Cancer cells demonstrate extraordinary plasticity to adapt to variations in the conditions of their microenvironment. Cancer cell proliferation is dependent on O_2. Well-oxygenated cells, which are located close to blood vessels, proliferate at a higher rate, because they are supplied by nutrients, growth factors and O_2 in abundance. Their biosynthesis is efficiently supported by ATP, which is produced by mitochondrial oxidative phosphorylation (OXPHOS). In contrast, less oxygenated cells rely more on glycolysis [1,12–16], which may become the main, if not the unique cause of ATP generation. Hypoxia forces cells to develop strategies to survive and proliferate, but at a lower rate [1,3,17–19]. Thus, it is not surprising that these "robust" cells correspond to higher malignancy grades [20,21] and chemoresistant cells [22,23].

2.1. Normoxic cancer cells divide rapidly

The capability of well-oxygenated cells to produce ATP is maximal when OXPHOS–ATP is functioning well. Various growth factors, such as angiogenic factors, are secreted by cancer cells, stimulating the formation of new blood vessels. The angiogenesis phenomenon provides more oxygen and nutrients [24] and favors cell division in a positive feed-back loop. Glucose, alanine (Ala) and glutamine (Gln) serve as essential nutrients and as elementary "bricks" for building new cells [25–30]. To divide, cells need to reorient the glucose metabolism towards synthesis. Thus, other sources, such as glucose, are needed to produce pyruvate, such as Ala, oxaloacetate (OAA) and lactate. These molecules sustain the tricarboxylic cycle (TCA), coupled with the production of ATP by oxidative phosphorylation (OXPHOS). Glucose transformation towards anabolic pathways is favored by the re-expression of the M2 embryonic form of pyruvate kinase (PKM2). The switch from the inactive (dimeric) isoform of PKM2 to the active (tetrameric) form seems to be an oscillating process, controlled by allosteric regulation implying the concentration of fructose 1,6 diphosphate (F1,6P) [31,32]. When the dephosphorylated tetrameric form of PKM2 is activated, glucose transformation leads to ATP and lactic acid production [32–37] (Fig. 1). In contrast, dimeric PKM2 preponderance leads to an accumulation of intermediate molecules upstream of PKM2, which are diverted towards the pentose phosphate pathway (PPP) and towards the formation of metabolites such as glycerol and serine. PPP produces ribose and NADPH,H^+, whereas glycerol serves lipid synthesis and serine, the latter molecule sustaining tetrahydrofolate and nucleotide synthesis. Thus, the major share of the pyruvate entering the TCA cycle in cancer cells may derive from the transformation of Ala and Gln, which originate from muscle proteolysis, participating in cachexia [25,38]. Note that dimeric PKM2 could have a nuclear kinase activity, phosphorylating the STAT3 transcription factor, which activates several oncogenic genes such as *c-myc*, *bcl-x_L* [33,39,40] and *mek5* [41].

Warburg observed in the 1920s [42] that oxygenated cancer cells could produce lactate. This aerobic glycolysis contrasts with the regulation which takes place in normal cells, where lactate formation is stopped in the presence of O_2 (Pasteur effect). This regulation is due to the mitochondrial production of citrate and ATP, which exercise a negative feedback control on phosphofructokinase1 (PFK1), arresting glycolysis [43]. The switch from glycolysis to TCA cycle activation is under the control of HIFα, which is inactivated by prolyl hydroxylase (PHD) in the presence of oxygen [44]. Cancer cells lose this regulation because HIF 1α remains activated, even in normoxia, due to inactivation of PHD by various mechanisms, such as accumulation of intermediates of the TCA cycle (succinate, fumarate, α-ketoglutarate (α-keto)) [45,46]. Lactate production should be explained principally as the activation of lactate dehydrogenase-5 (LDH-5) associated with the inhibition of pyruvate dehydrogenase (PDH), which is inactivated by pyruvate dehydrogenase kinase 1 (PDK1). The latter enzyme is activated by HIF-1 [17]. To explain the loss of Pasteur effect regulation, we hypothesized that citrate was at low concentration in proliferative cancer cells and did not exercise a negative feed-back on phosphofructokinase (PFK). This hypothesis was recently confirmed in clinical studies showing that citrate concentration is drastically reduced in prostate cancer cells and correlated with aggressiveness [47]. We demonstrated that citrate administration arrests cancer cell proliferation and renders cells sensitive to chemotherapy [48–50].

2.1.1. Glutaminolysis sustains OXPHOS–ATP production, nucleotides and lipid biosynthesis

In normoxia, the TCA of cancer cells would be preferentially sustained by Gln oxidation due to PDH inactivation. Gln is the preferential mode of blood nitrogen transportation (NH_4^+), since 90% of the body's glutamine is stored in muscle. Gln is transported at the plasma membrane by ASCT2 (SLC1A5), a Na^+-dependent amino acid transporter which is essential for glutamine uptake by fast epithelial growing tumor cells [51,52]. By cytosolic glutaminase (GLS), Gln provides glutamate (Glu) and/or amine groups for nucleotides, amino acids (e.g. serine), tetrahydrofolate and glutathione synthesis. Gln is also converted by the mitochondrial GLS into Glu, a reaction that produces ATP. Glu is transformed by glutamate dehydrogenase (GDH) into α-keto which replenishes the TCA, leading to the production of several intermediates such as oxaloacetate (OAA) [53,54] (Fig. 1). OAA is at very low concentration in mitochondria and must be regenerated at each cycle. In the case of lack of glutamine, cells might obtain OAA through pyruvate carboxylation, by activating pyruvate carboxylase (PC) [55,56]. Then, citrate synthase (CS) condenses OAA and acetyl-CoA into citrate which is exported in the cytosol by a transporter [57]. This exportation could be due to aconitase inhibition in response to diffusible products of nitric oxide (NO) [2,58–60] probably produced by cells from a hypoxic environment, in particular macrophages [61]. ATP-citrate lyase (ACLY) over-expression has been reported in numerous cancer cells for review [62,63]. ACLY reforms acetyl-CoA and OAA in the cytosol. Acetyl-CoA sustains de novo lipogenesis which is one of the major metabolic pathways required by cancer cells to divide [64,65], and also serves the acetylation of proteins such as histones. The regulation that orients acetyl-CoA towards one pathway rather than another remains to be investigated (Fig. 2). Modifications in pH, described later, could influence enzymatic activities such as histone acetyl transferase (HAT) [18]. Histone deacetylations are thought to support proliferation by inactivation of suppressors and activation of oncogenic genes. The decrease in HAT activity and/or in citrate concentration (and thus in acetyl-CoA) could play a major role in acetylation processes associated with genetic and epigenetic modifications. As such, a decrease in citrate could be associated with mutations, since it has been reported in glucose deprivation, which was found to be linked with K-Ras mutations [66].

In contrast, OAA derived from citrate transformation by ACLY is converted into aspartate by aspartate amino transferase (ASAT) or into malate by malate dehydrogenase (MDH) (Fig. 2). As a result, glutamine catabolism sustains the transaminase reactions producing pyruvate and aspartate by alanine amino transferase (ALAT) and ASAT, respectively.