From gametogenesis and stem cells to cancer: common metabolic themes

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TABLE OF CONTENTS

- A Brief Introduction to Energy Metabolism and Bioenergetics
  - Mitochondrial structure and function in bioenergetics
  - Fueling the mitochondria: it all starts with glucose
- Methods
- Metabolic Shifts During Gametogenesis and Early Embryogenesis
  - Spermatogenesis
  - Oogenesis and early development
- Energy Metabolism in Pluripotent Stem Cells
  - Mitochondrial function in ESCs
  - Mitochondrial function in induced pluripotent stem cells
- Metabolism: Where Stem Cells Meet Their Cancer Counterparts
  - How metabolism defines the identity of cancer and stem cells
  - The significance of enhanced glycolysis for proliferating cells
  - Metabolic pathways conferring anabolic advantages to the Warburg effect
  - Shared molecular basis of the Warburg effect
  - When metabolism takes over
  - Metabolic adaptations in reproductive cancers
  - Possible metabolic strategies in cancer treatment
- Conclusions and Future Directions

BACKGROUND: Both pluripotent stem cells (PSCs) and cancer cells have been described as having similar metabolic pathways, most notably a penchant for favoring glycolysis even under aerobicosis, suggesting common themes that might be explored for both stem cell differentiation and anti-oncogenic purposes.

METHODS: A search of the scientific literature available in the PubMed/Medline was conducted for studies on metabolism and mitochondrial function related to gametogenesis, early development, stem cells and cancers in the reproductive system, notably breast, prostate, ovarian and testicular cancers.

RESULTS: Both PSCs and some types of cancer cells, particularly reproductive cancers, were found to obtain energy mostly by glycolysis, often reducing mitochondrial activity and oxidative phosphorylation. This strategy links proliferating cells, allowing for the biosynthesis reactions necessary for cell division. Interventions that affect metabolic pathways, and force cells to change their preferences, can lead to shifts in cell status,
increasing either pluripotency or differentiation of stem cells, and causing cancer cells to become more or less aggressive. Interestingly metabolic changes in many cases seemed to lead to cell transformation, not necessarily follow it, suggesting a direct role of metabolic choices in influencing the (epi)genetic program of different cell types.

**CONCLUSIONS:** There are uncanny similarities between PSCs and cancer cells at the metabolic level. Furthermore, metabolism may also play a direct role in cell status and targeting metabolic pathways could therefore be a promising strategy for both the control of cancer cell proliferation and the regulation of stem cell physiology, in terms of manipulating stem cells toward relevant phenotypes that may be important for tissue engineering, or making cancer cells become less tumorigenic.

**Key words:** reproductive system / metabolism / mitochondria / stem cells / cancer

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**A Brief Introduction to Energy Metabolism and Bioenergetics**

**Mitochondrial structure and function in bioenergetics**

Energy metabolism in somatic cells is highly dependent on mitochondrial function. Mitochondria are dynamic organelles that can display different morphological and functional arrangements within a cell, according to its energy demands. Therefore, they can appear as small individual ovoid structures or as an extensive tubular network, and this morphological arrangement can be altered by constant fission and fusion events that, in conjunction with cytoskeleton-dependent organelle trafficking, alter the shape and distribution of mitochondria within a cell (Brookes, 2005). Structurally four distinct mitochondrial regions can be distinguished: the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), which define two other compartments: the intermembrane space and the mitochondrial matrix (Fig. 1A–C). These membranes are significantly different from each other. The OMM defines the perimeter and presents a high number of proteins called porins (the voltage-dependent anion channel—VDAC, for example) involved in the assembly of transmembrane aqueous channels that make the OMM permeable to ions and other small molecules. In contrast, the IMM constitutes a very effective impermeable barrier and presents a high number of invaginations known as cristae. Most of its weight (76%) is due to proteins, and it is extremely rich in cardiolipin, a lipid that reduces proton permeability and confers fluidity and protein stability. Unsaturated lipids in the IMM appear to be required for optimal function (Fariss et al., 2005). A main feature of the IMM is the presence of multi-protein complexes of the electron transport chain (ETC) that are linked to the generation of adenosine triphosphate (ATP). Recent studies have shown that cristae organization is more flexible and complex than originally thought, and is directly correlated with cellular ATP demands, meaning that if a cell requires more ATP, their mitochondria will acquire a larger total area of cristae, and vice-versa (Chance and Williams, 1956; Jezek and Hlavata, 2005; Zick et al., 2009).

Although they are wholly integrated in the cell, mitochondria present some genetic and metabolic autonomy. This genetic autonomy stems from the fact that they have their own genome, mitochondrial DNA (mtDNA) which is maternally inherited and usually amplified through oocyte maturation (Barritt et al., 2000). Human mtDNA is a double-stranded circular molecule that encodes 13 polypeptides for crucial subunits for the ETC complexes and 22 tRNAs and 2 rRNAs necessary for the translation of these 13 polypeptides (Fig. 1D). Interestingly, the replication and gene expression of the mtDNA is regulated by proteins encoded by nuclear DNA (Ramilho-Santos et al., 2009; St John et al., 2010). Despite being functionally recognized as the ‘power houses’ of the cell, given that they provide the majority of cellular ATP, a growing number of different roles have similarly been attributed to mitochondria. For instance, mitochondria are involved in the β-oxidation of fatty acids, heme biosynthesis, the metabolism of amino acids, the synthesis of iron/sulfur clusters, thermogenesis and apoptosis (Ernster and Schatz, 1981; Rouset et al., 2004; Zick et al., 2009).

Mitochondrial ATP is synthesized through a series of electron transfer processes linked with oxidative phosphorylation (OXPHOS). The cell can up or down-regulate this process according to its needs, so it is logical to postulate that each tissue could have its own mitochondrial bioenergetic pattern (Collins et al., 2002; Boneh, 2006). Complexes I–IV are responsible for electron transfer and compose the mitochondrial respiratory chain. The last element is the ATP synthase (often noted as Complex V; Fig. 1E). Briefly, Complex I (NADH-ubiquinone oxidoreductase) and Complex II (succinate-ubiquinone oxidoreductase) accept electrons from nicotinamide adenine dinucleotide (NADH) and from succinate dehydrogenase-associated FADH₂ (reduced flavine adenine dinucleotide), originating from glycolysis and the Krebs cycle and from the Krebs cycle, respectively. Afterwards, the electrons will be delivered to membrane-embedded ubiquinone. The reduced form of this ubiquinone molecule transfers the electrons to Complex III (ubiquinone-cytochrome c oxidoreductase). A second mobile carrier, cytochrome c, then transfers electrons from Complex III to Complex IV (cytochrome c-oxygen oxidoreductase), where four electrons will reduce molecular oxygen to water (Brookes, 2005; Mazat et al., 2013) (Fig. 1E). As will be discussed ahead, electron transfer can also result in the formation of reactive oxygen species (ROS), possibly resulting in oxidative stress.

The energy transfer involved in this electron transfer pathway allows Complexes I, III and IV to pump protons throughout the IMM to the intermembrane space leading to the generation of a proton gradient. This proton gradient forms the proton motive force (∆p) that is composed by two distinct components; one electric (mitochondrial membrane potential: ΔΨrm) that results from the charge separation between the two sides of the membrane and one proton concentration-dependent (∆pH). The proton gradient is then used by the ATP synthase to form ATP (Collins et al., 2002). There are other important mitochondrial proteins involved in ATP production, such as the phosphate carrier (phosphate/H⁺ sinport) and the adenine nucleotide translocator. This last protein is responsible for
the exchange of ADP from the cytosol for ATP (Boyer, 1993; Scheffler, 2001; Jezek and Hlavata, 2005; Mazat et al., 2013).

**Fueling the mitochondria: it all starts with glucose**

In order for OXPHOS to take place, mitochondria require NADH and succinate, but before these substrates become available to mitochondrial complexes an extensive number of reactions must take place in different cell compartments. The breakdown of glucose via glycolysis in the cytoplasm involves ten cytoplasmic reactions yielding two molecules of pyruvate, ATP and NADH. Glycolysis occurs in both aerobic and anaerobic conditions, with two distinct phases. The preparatory phase involves ATP expenditure, while the second phase, called the ‘payoff’ phase, results in net ATP formation. The rate of glycolysis is controlled by several factors, namely the availability of intermediate metabolites and a complex balance between ATP consumption, NADH levels and by the regulation of several key enzymes, including Hexokinase, which will be addressed in more detail ahead.

There are distinct possible routes by which pyruvate can be further metabolized. Under aerobic conditions, pyruvate is transported into the mitochondria where it is oxidized to yield acetyl-co-enzyme A (Acetyl-CoA), which will enter the TCA cycle. The second route, the cytosolic reduction of pyruvate to lactate via lactic acid fermentation, is usually related with anaerobic situations although this reaction also occurs in aerobic conditions in erythrocytes or in some types of tumors, as will be discussed (Berridge et al., 2010). Importantly, pyruvate can also have an anaerobic fate, providing the carbon skeleton for the synthesis of the amino acid alanine (Costello and Franklin, 2005).

The oxidative decarboxylation of pyruvate into acetyl-CoA is mediated by the pyruvate dehydrogenase complex (PDH), a highly regulated multienzyme complex located in the mitochondria. Acetyl-CoA will then enter the Krebs cycle by condensing with oxaloacetate and producing citrate. Citrate goes through a series of redox reactions that conventionally results in the production of ATP and GTP, CO₂ and more importantly in the production of reducing equivalents (NADH and FADH₂/succinate) that will provide the mitochondrial ETC with electron donors for OXPHOS. Simultaneously, this set of reactions also incur the regeneration of important metabolic intermediates. Importantly, shifts in these metabolic processes define distinct changes in cell status, as exemplified by the events taking place during mammalian gametogenesis.

**Metabolic Shifts During Gametogenesis and Early Embryogenesis**

Sexually reproducing organisms rely on germ cells to deliver the genetic and epigenetic information needed to create a new organism. Primordial germ cells (PGCs) are the founder cells of the germline lineage (Sundan, 2007). They are generated in the pluripotent epiblast and migrate from the posterior primitive streak to the genital ridge (Anderson et al., 2000; Yang and Oatley, 2014) where chemical stimuli from the surrounding cells signal PGCs to either adopt a male germ cell fate or commit to a female germ cell lineage, giving rise to the sexually dimorphic germ stem cells (GSC) (Soder, 2007; Gilbert, 2013; Stukenborg et al., 2013).

**Spermatogenesis**

Male PGCs differentiate into gonocytes and remain mitotic until they enter a period of latency (Vergouwen et al., 1991; Western et al., 2008). PGCs, however, can give rise to pluripotent carcinoma in situ (CIS) cells, the precursors of all types of testicular germ cell tumors (TGCT) that account for nearly 96% of all testicular tumors (Richardson et al., 2008; Bustamante-Marin et al., 2013). Interestingly it has been suggested that testis formation requires a higher mitochondrial activity than the female gonad (Mittwoch, 2004; Matoba et al., 2008).

Spermatogonial stem cells (SSCs) are the male GSCs and represent a minor fraction of the undifferentiated spermatogonial pool that provides for continuous spermatogenesis due to their ability to self-renew and maintain a pool from which progenitors arise before committing to differentiation (Tegelenbosch and de Rooij, 1993; Oatley and Brinster, 2008). Morphological studies have classified spermatogonia into type A, intermediate, and type B. Type A spermatogonia are classified as Asingle (As), Apaired (Apr), or Aaligned (Aal) and SSCs are thought to be part of the As spermatogonia pool whose frequency is 0.02–0.03% of all germ cells (Tegelenbosch and de Rooij, 1993; Kanatsu-Shinohara and Shinohara, 2013). In primates, As is further categorized in Ad (dark) or Ap (pale), based on distinct levels of chromatin condensation. Type Ad are considered the reserve stem cells, while Ap divide symmetrically to produce either new Ap or differentiate in type B spermatogonia (Martin and Seandal, 2013; Yang and Oatley, 2014).

Spermatogenesis takes place in the seminiferous tubules and is a highly dynamic and metabolically active process in which an interdependent population of germ cells goes through gradual transformations in order to produce functional haploid spermatozoa. The fully functional testis includes both somatic cells (Sertoli and Leydig cells) as well as germ line cells (spermatogonia, spermatocytes, spermatids and sperm cells). These cells are thought to have distinct metabolic preferences and mitochondrial morphology/activity (De Martino et al., 1979; Robinson and Fritz, 1981; Grootegoed et al., 1984; Nakamura et al., 1984; Bajpai et al., 1998; Meinhardt et al., 1999). In fact we can group cells according to their substrate preferences: preferentially glycolytic (spermatogonia, mature sperm and the somatic Sertoli cells) and more reliant on mitochondrial OXPHOS (spermatocytes and spermatids) (Fig. 2) (Robinson and Fritz, 1981; Grootegoed et al., 1984; Nakamura et al., 1984; Bajpai et al., 1998; Meinhardt et al., 1999). This pattern of metabolic preference could be a reflection of a matter of opportunity since seminiferous tubule fluid is rich in lactate and poor in glucose (Bajpai et al., 1998).

**Methods**

A computerized literature search was conducted using Medline and Web of Knowledge for metabolic pathways prevalent in the testis and ovary (focusing on oogonia and spermatogonia), sperm, oocytes, early embryos, embryonic stem cells (ESCs), induced pluripotent cells, and reproductive system cancers. Metabolic pathways involved in cell energy homoeostasis, and several aspects of mitochondrial function (such as ATP synthesis and components of the Krebs cycle) were the aspects searched for. Relevant literature was selected to determine common themes throughout the reproductive system, with a special focus on detecting and discussing the common metabolic themes, with functional implications, such as those that might lead to the development of therapeutic targets, or tools that could influence the efficiency of stem cell differentiation.
However, substrate availability is clearly not the only factor, as it cannot explain why, for example, the slow dividing spermatogonia that reside on the basement membrane of the seminiferous tubule with full access to O₂ due to neighboring blood vessels, still prefer to remain mostly glycolytic with low mitochondrial activity, a feature shared with other stem cells and some cancer cells, as will be discussed (Ramalho-Santos et al., 2009; St John, 2012). Similarly, and as a reverse example, spermatocytes have limited access to O₂ and still use enhanced glycolysis. It is also interesting to note that compared with mitochondria of other tissues, testis mitochondria consume less oxygen although they reach approximately the same electric potential (Amaral et al., 2009; Amaral and Ramalho-Santos, 2009; Mota et al., 2009).

**Oogenesis and early development**

After lineage commitment, the female germ cell immediately initiates meiosis which then is arrested at the diplotene stage of meiosis I (Evron and Blumenfeld, 2013; Kerr et al., 2013). Mitochondria are the most abundant organelles in the early embryo (Motta et al., 2000; Sathananthan and Trounson, 2000) and, in normal situations, are transmitted exclusively from the oocyte (Cummins, 2001). Mitochondrial function is crucial for functional oocytes, as mitochondrial dysfunction and compromised cell respiration have been correlated with infertility as well as development problems in human preimplantation embryos (Fissore et al., 2002; Thouas et al., 2003; Ramalho-Santos et al., 2004).

In the human oocyte, there are around 10⁵ mitochondria per gamete (Chen et al., 1995; Jansen and de Boer, 1998), propagated from a restricted founder population present in PGCs (Jansen, 2000; Cummins, 2001), thus guaranteeing mitochondrial homogeneity in the mature oocyte, and therefore in the embryo. Throughout oogenesis, the increase in mitochondria number parallels the increase in oocyte volume, accompanied by ultrastructural changes (Wassarman and Josefowicz, 1978; Motta et al., 2000; Au et al., 2005) (Fig. 2). In the mature oocyte, each mitochondrion possesses a single copy of mtDNA (reviewed in Facucho-Oliveira and St John (2009), Jansen and de Boer (1998) and St John et al. (2010)).

A combination of metabolic pathways takes place in the developing follicle. The oocyte and granulosa cells establish functional connections, both via signaling pathways and gap junctions. A subpopulation of granulosa cells that accompanies the oocyte upon ovulation (and which is known at that stage as the cumulus cells) is responsible for maintaining these connections. Primordial follicles use both pyruvate and glucose, suggesting that both OXPHOS and glycolysis are active (Biggers et al., 1967; Boland et al., 1993; Wycherley et al., 2005). Interestingly, cumulus cells will consume glucose and produce pyruvate that can be used by the oocyte (Jansen and Burton, 2004). In fact, pyruvate constitutes a central metabolite for meiotic maturation as well as for the first cleavage division (Biggers et al., 1967), evidenced by the increase in pyruvate uptake accompanied by a boost in O₂ consumption during oocyte maturation (Harris et al., 2009). The mature oocyte has a high ATP turnover, supplied by mitochondrial respiration (Dumollard et al., 2004) and by pyruvate uptake (Leese, 1995), and zygotes also use this metabolite as a main energy substrate (Biggers et al., 1967; Leese and Barton, 1984).

During early embryo development, OXPHOS substrates are metabolized from the zygote to the morula stage, and ATP levels and O₂ consumption remain basically constant (Slott et al., 1990; Van Blerkom et al., 1995). Interestingly, in later stages, the pattern changes and glycolysis becomes the predominant metabolic pathway, although both glycolysis and OXPHOS may coexist (Dumollard et al., 2007). Although changes in metabolism could again reflect adjustments to the substrates available in distinct region of the female reproductive tract (Jansen and Burton, 2004), blastocysts with a significantly higher glucose uptake prior to transfer are more likely to implant and develop rather than fail to develop (Gardner and Leese, 1987), and embryo vitality seems to be associated with a low ‘quiet’ metabolism with limited mitochondrial activity (Lane and Gardner, 1996; Leese, 2012). Some authors claim that mitochondria in mouse and human blastocysts are homogenous and elongated elements (Sathananthan and Trounson, 2000), while other postulate the existence of two types of mitochondria in the mouse blastocyst: spherical mitochondria in the inner cell mass (ICM; which will give rise to the embryo, and, if removed from context, to pluripotent ESCs) and elongated mitochondria in the trophectoderm (TE), which will contribute to the placenta (Barnett et al., 1996; Van Blerkom, 2008). However, the while the ICM cells have low...
mitochondrial membrane potential, the TE cells are highly polarized and very metabolically active, producing more ATP and consuming more oxygen (Barnett et al., 1996; Houghton, 2006; Van Blerkom et al., 2006), suggesting that, as is the case with spermatogonia, cells with high development potential remain more glycolytic.

Energy Metabolism in Pluripotent Stem Cells

Mitochondrial function in ESCs

Pluripotent ESCs that can differentiate into any cell type in the adult organism are isolated from the blastocyst ICM, grow in colonies, and can be propagated indefinitely in culture. Moreover they recapitulate metabolically some of the blastocyst characteristics noted above. Although there are lineage-specific differences, it has been shown that, similarly to ICM cells, undifferentiated human ESC (hESC) have few ovoid mitochondria arranged in small perinuclear clusters, with immature morphology evidenced by the presence of few cristae and low electron lucid matrix (Fig. 2) (Oh et al., 2005; St John et al., 2005; Cho et al., 2006).

In general, differentiation involves a shift from small individual oval mitochondria to dynamic tubular networks, with an increase in the number of mitochondrial cristae, suggesting higher OXPHOS activity (Cho et al., 2006; St John et al., 2010). It has also been shown that hypoxia may facilitate cell growth and pluripotency (Ezashi et al., 2005; Kondoh et al., 2007). The rationale for using low O2 tension in ESC culture is related to the conditions found in the female reproductive tract, thus mimicking the physiological environment for ICM cells. However, this is not a straightforward issue, because, although O2 may vary throughout the tract, it also seems sufficient to maintain active OXPHOS (see Ramalho-Santos et al. (2009) for review).

Several studies have differentiated ESCs in vitro and observed changes in mitochondrial dynamics during differentiation (St John et al., 2005; Facucho-Oliveira et al., 2007). When ESCs differentiate the number of mitochondria increases and changes in morphology can also be observed.
(Cho et al., 2006; Kondoh et al., 2007; St John et al., 2010). Concomitantly with an increase of mitochondrial number during ESC differentiation, the rates of O₂ consumption and ATP production in the cell increase as well, while lactate production decreases (Chung et al., 2007) suggesting that a switch in energy metabolism from glycolysis to oxidative phosphorylation is required for proper cell differentiation (Chung et al., 2007). The increase in the number of mitochondria and OXPHOS in differentiated cells also leads to an increase in ROS production (Cho et al., 2006; Saretzki et al., 2008), which is important for the differentiation of hESCs into cardiomyocytes (Crespo et al., 2010). Several authors have reported similar characteristics in adult stem cell differentiation (Carrière et al., 2004; Allameh et al., 2014; Kim et al., 2014).

In addition mitochondrial-based apoptosis may also contribute to cell differentiation (see Ramalho-Santos et al. (2009)), and mtDNA could also play an important role, given the deficient neuronal differentiation in ESC carrying mtDNA mutations that results in severe biochemical deficiency (Luoma et al., 2004).

**Mitochondrial function in induced pluripotent stem cells**

Induced pluripotent stem cells (iPSCs) are somatic cells that have been reprogrammed to pluripotency by using defined factors, such as Oct3/4, Sox2, Klf4 and c-myc and can be formed by distinct methodologies (Folmes et al., 2011b). Importantly, recent data point to the same profile of mitochondrial activity in pluripotent iPSCs that has been described for ESCs (Fig. 2). This includes similar morphological, metabolic and transcriptional profiles in terms of mitochondrial-related events and the management of oxidative stress (Armstrong et al., 2009; Prigione et al., 2010). There is also evidence that in iPSCs mitochondrial mass, mtDNA copy number, and the expression levels of genes associated with mitochondrial biogenesis are comparable to hESCs. The same can be stated regarding mitochondrial properties and energy metabolism. However, there is also some information suggesting that inadequate/incomplete reprogramming of somatic cells to a pluripotent phenotype may also be reflected in terms of mitochondrial properties (Armstrong et al., 2009; Prigione et al., 2010, 2011; Varum et al., 2011). Therefore it appears that both pluripotent and adult stem cells such as spermatogonia share a glycolytic profile, and tend to maintain low mitochondrial activity particularly as it pertains to OXPHOS. Interestingly this profile is shared with many types of cancer cells.

**How metabolism defines the identity of cancer and stem cells**

It is now well established that a preferentially glycolytic metabolism is instrumental for rapid proliferation. Indeed, the induced overexpression of specific glycolytic enzymes is sufficient to bypass senescence in mouse embryonic fibroblasts, while depletion of such enzymes has the reverse effect, shortening cellular life span (Kondoh et al., 2005a, b). Moreover, it has been shown that the proliferative capacity of mouse ESCs (mESC) is similarly strongly correlated with glycolytic flux and low OXPHOS (Kondoh et al., 2007).

The regulation of pluripotency maintenance versus cell differentiation involves adaptations in the cellular metabolic infrastructure, ultimately leading to a metabolic shift from a preferentially glycolytic energetic profile in undifferentiated cells to a more oxidative metabolism in differentiating cells. These adaptations will not only empower the differentiation process by conveying energy and metabolite demands for the differentiating cells, but, as we now know, also contribute to the maintenance of pluripotency in stem cells. In fact, different studies have shown that strategies to up-regulate the glycolytic pathway in detriment of OXPHOS, either by growing cells in hypoxic environments or by directly targeting metabolic processes, are beneficial to maintain stem cell...
pluripotency (Spitkovsky et al., 2004; Ezashi et al., 2005; Varum et al., 2009; Mandal et al., 2011).

Similarly, the application of these strategies has proven successful to improve the reprogramming efficiency of iPSC (Yoshida et al., 2009; Esteban et al., 2010; Folmes et al., 2011a, b; Blaschke et al., 2013). For example, stimulating glycolysis with 0-fructose-6-phosphate or by pyruvate dehydrogenase kinase (PDK1) activation also enhances iPSCs reprogramming efficiency (Folmes et al., 2011a; Panopoulos et al., 2012).

Importantly, metabolic reprogramming precedes genetic reprogramming (Folmes et al., 2011a) and hence, the former might be an active player influencing the overall success of the cellular reprogramming process. On the contrary, glycolysis inhibition using 2-deoxy-o-glucose (ZDG), a competitive glucose analog presenting a hydrogen atom substituting the hydroxyl group at carbon two, 3-bromopyruvate (3BrPA), an inhibitor of hexokinase-2, and dichloroacetate (DCA), an inhibitor of PDK1 that restores pyruvate dehydrogenase complex activity and OXPHOS, also reduce reprogramming efficiency (Zhu et al., 2010; Folmes et al., 2011a; Panopoulos et al., 2012).

The relationship between pluripotency and metabolism in stem cells was further stressed when it was discovered that the core pluripotency circuitry, including the pluripotency genes, Oct4, Sox2, and Nanog and their associated networks, share points of convergence with signal transduction and activator of transcription 3 (STAT3), a master metabolic regulator controlling the oxidative to glycolytic switch (Wegrzyn et al., 2009).

Furthermore, the stemness factor Oct4 has other targets associated with energy metabolism, which may impact the balance between glycolysis and OXPHOS (Jaenisch and Young, 2008; Kang et al., 2009). Chromatin modifiers, such as polycomb repressor complexes, which promote pluripotency, also target metabolic enzymes within their active gene sets (Brookes et al., 2012).

Confirming the link between pluripotency and enhanced glycolysis, the inhibition of mitochondrial respiratory chain Complexes I and III, by Rotenone and Antimycin A, respectively, reduces cardiomyocyte differentiation, due to an impairment of oxidative phosphorylation and possible effects in calcium signaling (Spitkovsky et al., 2004; Chung et al., 2007). It has also been recently shown that the application of Antimycin A is capable of inhibiting the neuronal differentiation of mouse ESCs, maintaining them in a pluripotent state even when grown under stringent differentiation-promoting conditions (Pereira et al., 2013). A correlation between mitochondrial membrane potential, metabolic rate and the differentiation of mESCs has also been described, with cells with low or high MMP showing differences in efficiency of mesodermal differentiation and ability to form tumors, although both populations were indistinguishable in terms of pluripotency markers (Schieke et al., 2008).

The significance of enhanced glycolysis for proliferating cells

When considering the peculiar metabolism of proliferating cells, the choice of what appears to be an energetically unfavorable option might seem odd. Indeed, whereas the glycolytic process yields two ATP molecules per glucose consumed, the complete oxidation of a single glucose molecule through glycolysis and the Krebs cycle, and subsequent oxidative phosphorylation, will result in the production of at least 15 times more ATP. Furthermore, enhanced glycolysis imposes an immediate conversion of the glycolytic-derived pyruvate into lactate, which, despite being an energetic valuable molecule, is promptly discarded by the cell. Why would an active proliferating cell in need of a significant energy supply, waste such a metabolic asset and shift from mitochondrial respiration toward glycolysis? To understand this paradox, one must first consider the dynamic regulation of metabolism.

Metabolism results from the integration of cellular needs, with external cues (morphogens, substrates, oxygen, etc.). Cancer cells have become relatively independent from some of these external signals (such as growth factors) to maintain their (uncontrolled) growth. This integrative regulation makes metabolism pliable, with cells adopting distinct metabolic modes. If a cell is terminally differentiated, it will adopt a catabolic mode where its metabolism is adjusted for maximal energetic efficiency, and substrates are completely oxidized to feed the mitochondrial energetic machinery with sufficient reducing equivalents for ATP production in aerobicosis. This catabolic mode provides the cells with appropriate energetic charge to sustain their functions; this is particularly true for high energy-requiring phenotypes, such as cardiomyocytes and neurons.

On the other hand, if a cell is committed to rapid proliferation, ATP and NADH are not its only required commodities. Pathways such as glycolysis and the Krebs cycle do not operate as strict unidirectional metabolic avenues serving energetic purposes. Rather, they serve as metabolic hubs closely communicating with several other metabolic branches engaged in the production of distinct molecules some of which serve as molecular precursors for the synthesis of lipids, proteins, DNA and RNA (Feron, 2009; Vander Heiden et al., 2009). A proliferating cell will then adopt an anabolic state assuming a compromise between energy production and the synthesis of molecular precursors necessary for cell growth and subsequent division. Importantly, glycolysis is a much faster process to obtain ATP, and sufficient energy loads are generated if sufficient amounts of glucose are present, such as is the case of in vivo stem cell cultures (or for some stages of embryo development in vivo). In fact, an increase in ATP levels and a decrease in the AMP/ATP ratio would be detrimental for glycolytic fluxes, as it would lead to AMPK inactivation and cells would slow down the glycolytic process. Adding to this, ATP is also a known direct allosteric inhibitor of glycolytic enzymes so its levels should not rise uncontrollably, and must be balanced by ATP consumption. Moreover, a metabolic shift toward glycolysis has also been proposed as a mechanism to protect the cell from increased reactive oxygen species produced in mitochondria (Kondoh et al., 2005a, b).

**Metabolic pathways conferring anabolic advantages to the Warburg effect**

Glucose that is transported inside cells is rapidly activated in the cytosol by hexokinase-mediated phosphorylation, rendering the molecule usable by subsequent enzymes, and hampering its removal from the cell. The resulting glucose-6-phosphate can be isomerized to fructose-6-phosphate or serve also as a substrate for another catabolic process, the Pentose Phosphate Pathway (PPP; Fig. 3). This set of reactions branching from glycolysis yields the important production of ribose-5-phosphate and NADPH. While ribose-5-phosphate is necessary for the de novo synthesis of nucleotides, the NADPH electron donor is equally vital for the reductive synthesis of lipids or for the regeneration of reduced glutathione, a major cell antioxidant. The importance of the PPP for cell growth is well established, as seen for example in the fact that human
fibroblasts deficient for glucose-6-phosphate dehydrogenase (the first enzyme in the PPP and responsible for catalyzing one of the steps yielding NADPH) proliferate less and show evidence of early senescence when compared with control cells, a phenotype related to an increment in oxidative stress (Ho et al., 2000).

The hexosamine biosynthetic pathway is also necessary for cell growth and survival (Wellen et al., 2010). This pathway recruits fructose-6-phosphate from glycolysis and yields N-acetylglucosamine in a glutamine and acetil-CoA dependent fashion (Fig. 3). N-acetylglucosamine is the substrate for protein glycosylation and was shown to be necessary to...
maintain growth factor receptor expression, which in turn would enable cell growth and integration of glutamine and glucose metabolism.

For the increased glycolytic flux to be maintained, cytosolic NAD$^+$ pools must be rapidly regenerated to serve as cofactors for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In cells reliant on mitochondrial OXPHOS, the NADH reducing equivalents are indirectly transferred to the mitochondria via the malate-aspartate shuttle or the glycerol 3-phosphate shuttle resulting in NAD$^+$ recycling. However, under hypoxic conditions or in aerobic glycolysis the bulk regeneration of NAD$^+$ occurs through the lactate dehydrogenase (LDH) catalyzed reaction converting pyruvate into lactate, which is ultimately excreted by the cell, a pattern identified in actively proliferating cells such as cancers and stem cells (Warburg, 1956; Thurlimann, et al. 2004; Pereira et al., 2011; Varum et al., 2011).

Some glycolytic intermediates may also serve as carbon sources for the synthesis of macromolecule precursors: pyruvate can be converted to alanine, phosphoenolpyruvate can generate tyrosine and phosphoglycerate dehydrogenase (PHGDH) can remove 3-phosphoglycerate from the glycolytic route redirecting it to serine synthesis, which in turn generate glycine and cysteine (Fig. 3). The conversion of serine to glycine leads to the donation of a carbon to the folate pool (tetrahydrofolate), which in turn will be involved in a variety of biosynthetic processes including the synthesis of purines, thymine and, for example, the regeneration of methionine (Berg et al., 2002; Vacanti and Metallo, 2013). Recent studies have shown that PHGDH is recurrently over-expressed in certain cancers and that the serine and glycine pathways are necessary for the proliferation of such cells. The shunting of 3-phosphoglycerate away from glycolysis would benefit cancer cell growth namely by limiting proliferation of such cells. The reductive reaction is an elegant adaptation of the cell to cancer cells in using this reductive pathway.

The mitochondrial Krebs cycle is an amphibolic hub to which major catabolism- and anabolism-related reactions converge (Fig. 3). Despite old assumptions probably rooted on Warburg's ideas that aerobic glycolysis was caused by irreversible damage to the mitochondria, we now know that this is not the case, as cancer cells may present an active Krebs cycle (DeBerardinis et al., 2007). Many of the metabolites involved in this cycle can serve as substrates for biosynthesis; for example α-ketoglutarate and oxaloacetate can be transaminated into the aminoacids glutamate and aspartate, respectively, which are precursors for other amino acids and nucleotides (e.g. glutamine, arginine proline and purine nucleotides in the case of glutamate and asparagine and pyrimidine nucleotides in the case of aspartate). Succinyl-CoA can be used in the synthesis of the porphyrin ring of the heme group. Conspicuously, citrate can be shunted to the cytosol where it can be converted back to acetyl-CoA by ATP-citrate-lyase (ACL) and be used for the necessary synthesis of lipids or to serve other purposes (discussed below). These reactions are critical for cell proliferation as the inhibition of ACL causes cell growth arrest in cancer cells in vitro and in vivo (Hatzivassiliou et al., 2005).

The voluminous cataplerotic reactions removing intermediates from the Krebs cycles in proliferating cells must be counterbalanced by anaplerosis, which replenishes those pools. Given that glucose is made of carbon, hydrogen and oxygen, other molecules must be incorporated to supply additional required elements, such as nitrogen, and glutamine is considered a major anaplerotic substrate. Studies in glioblastoma cells grown in aerobic conditions and presenting the Warburg effect revealed that glucose-derived carbon is the major source of the carbon skeleton to be incorporated in lipogenesis through citrate (DeBerardinis et al., 2007). Under those conditions glutamine, highly used via glutaminolysis, mostly served as an anaplerotic substrate, contributing significantly to the lactate and alanine pools (60% of all glutamine consumed) and less notably to lipogenesis. Glutaminolysis also yielded oxaloacetate to replenish intermediate pools and especially resulted in a robust NADPH production that was used to support biosynthesis (DeBerardinis et al., 2007). Glutamine has similarly been shown to be essential for mESC proliferation (Fernandes et al., 2010).

Furthermore, non-canonical reactions in the Krebs cycle have been observed as cancer mechanisms to support cell growth. The oxidation of isocitrate to α-ketoglutarate is catalyzed by isocitrate dehydrogenase (IDH) and requires the presence of NAD$. Three isoforms of IDH have been identified, and IDH3 is accepted to be responsible for the canonical conversion of isocitrate. The other two isoforms, existing in the mitochondrial matrix (IDH2) or in the cytosol (IDH1), are NADPH dependent and capable of facilitating the reductive reverse conversion of α-ketoglutarate to isocitrate (Mullen et al., 2012). Certain cancers have been shown to have a significant flux through the reductive pathway especially, when growing under hypoxia or when challenged with mitochondrial poisons (Wise et al., 2011; Metallo et al., 2012; Mullen et al., 2012). This adaptation does not merely reflect an isotopic exchange between metabolite pools but functions to supply the cell with sufficient carbon precursors to enable a robust lipogenesis. Aerobic glycolysis, hypoxia and the inhibition of the mitochondrial OXPHOS all result in a decrease in glucose-derived carbon entrance into the Krebs cycle with those molecules being mostly transformed into lactate. Glutamine is then exploited as the major de novo lipogenic precursor (Fig. 3), and the reductive reaction is an elegant adaptation of the cell to boost the rapid conversion of glutamine-derived carbons to citrate, supporting cell growth (Wise et al., 2011; Metallo et al., 2012; Mullen et al., 2012). However, more insights in the regulation of these reactions are needed and it would be interesting to understand if stem cells are akin to cancer cells in using this reductive pathway.

**Shared molecular basis of the Warburg effect**

Cancer cells and stem cells thus share regulatory pathways, some of which are influential for their identity and regulate their metabolic profiles. Although many molecular factors are conceivably implicated in the Warburg effect and its regulation, we will provide a brief overview on some of the most prominent ones.

**HIF**

One of the most uncontested and documented factors related to aerobic glycolysis is hypoxia inducible factor (HIF) (Semenza, 2010a, b; Barbosa et al., 2012). HIF transcription factors establish the primary instrument mediating cellular adaptation, and consequent survival in hypoxic conditions. The spectrum of activated responses is broad, including a pro-angiogenic action, the control of gene methylation and notably the active promotion of the metabolic shift from oxidative phosphorylation toward an oxygen independent glycolysis (Xia et al., 2009; Semenza, 2010a, b). This could render the cell metabolically independent from O2 and reduce the risk of cellular damage due to oxidative stress. HIF-mediated metabolic reprogramming occurs via the up-regulation of genes encoding critical enzymes for glucose uptake and the glycolytic pathway, as well as the repression of genes related to OXPHOS.
Particularly, HIF-1α was shown to directly activate the expression of PDK, which inhibits the pyruvate dehydrogenase complex. This diverts pyruvate away from the mitochondria, reducing mitochondrial O₂ consumption and enhancing lactate production (Kim et al., 2006; Pandreou et al., 2006). Biosynthetic pathways branching from glycolysis have been suggested to be elevated due to the HIF-1α-favored expression of a less active pyruvate kinase isoform (Vacanti and Metallo, 2013). HIF-1α also activates genes responsible for mitochondrial autophagy (Semenza, 2010a,b). HIF-1α and HIF-2α are broadly expressed in several cancers and are fundamental at distinct stages of tumorigenesis (Kondoh, 2008; Semenza, 2010a, b). Similarly, HIF participates in the maintenance of stem cell identity, namely by interacting with core pluripotency transcription factors (Covello et al., 2006; Hu et al., 2006; Forristal et al., 2010; Mohyeldin et al., 2010; Moreno-Manzano et al., 2010).

Importantly, stem cells and cancer stem cells reside in niches that will influence their physiology (Mohyeldin et al., 2010) and the Warburg effect has been justified as a cellular adaptation to hypoxia that is present in solid tumors and in some stem cell niches. But this notion is not coherent with the location of spermatogonia, or with fact that these cells maintain enhanced glycolysis under nearly 20% O₂, or with the generation of cancers appearing in well oxygenated environments such as in the case for leukemia and lung cancers (Vander Heiden et al., 2009). HIFs are now known to be controlled by multiple genetic factors, including oncogenes, tumor suppressing factors and signaling kinases (Kondoh, 2008). Most importantly, an increasing number of factors that stabilize HIF proteins under normoxia are being reported, some of which could serve as good tools for metabolic reprogramming control by stem cells and cancers, as we will discuss.

c-myc

The transcription factor c-myc belongs to the Myc family of proto-oncogenes, and more than one thousand of its target genes have been identified, accounting for 15% of all genes in the genome (Laurenti et al., 2009). c-myc regulates and integrates cell proliferation, cell cycle control and metabolism and its altered expression is implicated in a myriad of cancers. This transcription factor has regulatory functions in stem cells and is necessary for the self-renewal of mESC (Cartwright et al., 2005), establishing an axis with other factors to regulate genes related to pluripotency (Yeo and Ng, 2013). As discussed earlier, c-myc was also one of the four factors originally described to induce nuclear reprogramming during the derivation of iPSC (Takahashi and Yamanaka, 2006). This factor is known to induce the expression of enzymes involved in glycolysis, biosynthetic pathways as the PPP, and amino acid metabolism. Interestingly, c-myc also up-regulates mitochondrial biogenesis while decreasing O₂ consumption in proliferating cells, probably to promote Krebs cycle anabolic-oriented functions (Dang, 2010). Moreover, this protein favors the glutamine oxidative pathways, once again most likely to serve anabolic purposes, endorsing the use of glutamine as a carbon and nitrogen source (Dang, 2010).

UCP2

Recently, the idea that increased glycolysis results not from a permanent and irreversible damage to mitochondria but instead from the inability of this organelle to use the mitochondrial proton gradient to produce ATP, gained credence with studies demonstrating the action of the uncoupling protein 2 (UCP2) in both cancer and stem cells (Samudio et al., 2008, 2009; Zhang et al., 2011). UCP2 is an inner mitochondrial protein and serves as a shunt dissipating the mitochondrial proton gradient, hence uncoupling energy production from O₂ consumption. UCP2 has been shown to decrease glucose-derived pyruvate oxidation in the mitochondria, while promoting the use of other substrates in the Krebs cycle, such as glutamine (Samudio et al., 2009). UCP2 must also be repressed during stem cell differentiation, to allow metabolic maturation (Zhang et al., 2011).

When metabolism takes over

Mounting evidence substantiates that metabolism is not a mere foot soldier subordinated to the signaling pathways and transcriptional dynamics regulating stem cell/cancer physiology. The fact that some metabolites and cofactors themselves have regulatory roles, either at the metabolic level or by interfering in signaling and transcriptional processes, has strengthened this notion. Therefore, it is conceivable that metabolism can take advantage of these mechanisms to control the cell state, particularly by influencing the molecular decision-making processes such as those involved in tumorigenesis or in pluripotency versus differentiation.

Much of the control exerted in HIF signaling is related to the stability of the HIF-1α and HIF-2α subunits. In order to perform their function, HIF-1α monomers heterodimerize with the HIF-1β subunit. Alpha and beta subunits are constitutively expressed, but in normoxic conditions the degradation of the alpha subunit occurs due to the hydroxylation of proline residues carried out by prolyl hydroxylases (PHD) (Semenza, 2010a,b). PHD activity requires O₂ and α-ketoglutarate as substrates and non-heme iron as a cofactor (regenerated by ascorbate); hence hypoxia inactivates PHD and leads to the stabilization of HIF enabling its transcriptional regulatory function (Guzy and Schumacker, 2006). Normoxic stabilization of HIF can occur downstream of the activity of growth factors (Lu et al., 2002; Gatenby and Gillies, 2007) and nitric oxide (Semenza, 2010a,b). It is then equally reasonable to say that events disrupting PHD function through changes in α-ketoglutarate levels and binding would render HIF signaling active, even in normoxic conditions. This is what occurs for example with the metabolites succinate and fumarate, which inhibit PHD activity by competing with α-ketoglutarate for the catalytic center of the enzyme, as seen in some cancers (Semenza, 2010a, b). Interestingly, pyruvate and lactate are similarly capable of stabilizing HIF-1α in normoxia, establishing a feed-forward loop between HIF signaling and the glycolytic products that might be important in the malignant progression of cancer or for the metabolic status of stem cells (Lu et al., 2002; McFate et al., 2008).

On the other hand, most function-altering protein post-translational modifications (PTM), such as phosphorylation, glycosylation, prenylation, methylation and acetylation, make use of metabolic substrates, serving as an ingenious way for the cell to sense metabolic fluctuations and allow the integration of metabolic fluxes with cellular programs (Metallo and Vander Heiden, 2010). Importantly epigenetic modifications change global gene expression and modulate transcriptional networks involved for instance in pluripotency, differentiation and tumorigenesis. Furthermore, metabolism can control chromatin organization influencing the cellular transcriptional landscape.

Epigenetics is based in specific acetylation and methylation of histones and DNA. Histone acetylation normally associated with gene expression is mediated by histone acetyltransferases (HAT), which require cytosolic acetyl-CoA as a substrate and are therefore dependent on its availability.
The cytosolic acetyl-CoA pool can be fed by the mitochondrial citrate through a mitochondrial shunt and subsequent ACL conversion of this cytosolic citrate to acetyl-CoA. Acetyl carnitine, another mitochondrial-derived molecule, has also been shown to serve as an additional substrate for nuclear acetylation of histones (Madiraju et al., 2009). Opposing the action of HATs are histone deacetylases (HDAC), comprised of several protein families one of which includes the NAD$^+$-dependent sirtuins, enzymes that respond to the intracellular redox state (Hitchler and Domann, 2009). Histone and DNA methylation are also intrinsically linked with metabolism. Firstly the action of histone and DNA methylases depends upon the availability of S-adenosylmethionine (SAM) that serves as a methyl group donor, and is regenerated through the methionine cycle that communicates with the folate cycle (involved in the synthesis of glycine from serine, as previously noted) and with the reaction regenerating glutathione (Hitchler and Domann, 2007). Secondly, some of the histone demethylases (such as the JmjC superfamily) and enzymes contributing to DNA demethylation (such as TET1) are equally reliant on α-ketoglutarate, oxygen and ascorbate akin to what was discussed for PHD (Metallo and Vander Heiden, 2010; Hitchler and Domann, 2012).

These are just some examples of converging points between metabolism and other cellular programs within the intricate regulatory network supporting the execution of cellular processes such as growth, self-renewal and differentiation.

**Metabolic adaptations in reproductive cancers**
The metabolic alterations previously discussed for cancer cells in general have also been reported as a recurrent aspect in reproductive system cancers, including breast, ovarian, cervical/uterine, vaginal/vulval, testicular and prostate cancers. Following the 2012 data from the European Cancer Observatory (see eaco.iarc.fr), breast and prostate cancers are the most prevalent forms of reproductive cancers in women and men, respectively, and consequently correspond to the reproductive cancer types causing higher mortality (Ferlay et al., 2013). In this section, we will focus on the characterization of the metabolic traits of these two types of cancers, as well as on those of gonadal cancers.

**Breast cancer**
The occurrence of aerobic glycolysis in breast cancer cells can be demonstrated by the importance of glucose metabolism, notably in terms of increased glucose uptake and modulation of the expression of specific glucose transporters (Brown and Wahl, 1993; Younes et al., 1995). In these studies, Glut1 (glucose transporter 1) expression was found to be particularly up-regulated in breast cancer cells, presenting both higher proliferation and histological scores (Younes et al., 1995). Furthermore the dependence of breast cancer cells on glucose was demonstrated by the apoptosis observed in cells grown under glucose deprivation (Lee et al., 1997) or submitted to 2-deoxy-α-glucose (2DG) treatment (Aft et al., 2002).

LDH expression and activity has been identified as another instrumental regulatory node in breast cancer metabolism. As previously noted, LDH is the enzyme responsible for the interconversion between pyruvate and lactate (Draoui and Feron, 2011) and five different isoenzymes can be assembled including LDH-A which is notoriously up-regulated in tumors presenting elevated activity (Balinsky et al., 1983; Koukourakis et al., 2003; Draoui and Feron, 2011). A reduction in LDH-A expression and activity has been shown to compromise cell proliferation, particularly under hypoxic conditions, and increase mitochondrial oxygen consumption. Furthermore when transplanted into female mice, the same LDH-A targeted cells were observed to generate considerably smaller tumors, causing less mortality. Interestingly, the authors suggest that the mutual regulation of LDH and mitochondrial activity occurs at the level of metabolite availability (Fandin et al., 2006).

A recent study attempting to compare the metabolic profile of breast tumors and normal tissue by means of gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFM), presented a comprehensive metabolic map depicting changes in metabolites from control and affected samples at the level of distinct metabolic pathways (Budczies et al., 2012). This profiling procedure was capable of identifying many metabolites present at different concentrations in each condition, affecting many of the pathways previously identified as being altered in cancers. Notably they identified a robust regulation of glutaminolysis and a simultaneous decrease in the levels of all free fatty acids assessed, in the breast cancers. Although the latter result might seem difficult to integrate with the characteristic up-regulation of de novo fatty acid synthesis occurring in proliferative cells as mentioned earlier and particularly in breast cancer cells (Kuhaja et al., 1994), the authors suggest that the free fatty acids produced are immediately directed to the synthesis of membrane phospholipids. Additionally, the Kennedy pathway (responsible for de novo synthesis of the glycerophospholipids, phosphatidylcholine and phosphatidylethanolamine) has also been found to be up-regulated in breast cancer (Budczies et al., 2012) and differences in the lipid profiles of tumor and normal tissues have been observed (Hilvo et al., 2011). Furthermore loss of function analyses of the genes coding for the enzymatic machinery supporting these differences, have led to the identification of enzymes shown to be fundamental for breast cancer viability (Hilvo et al., 2011).

As mentioned earlier, citrate has a central role in lipid synthesis by providing the substrate for acetyl-CoA production. The non-canonical IDH reductive reaction directly converting α-ketoglutarate to isocitrate has also been identified in breast cancer cells (Metallo et al., 2012), demonstrating the metabolic adaptation of using glutamine and favoring rapid lipid synthesis in this cancer type.

An inevitable issue when discussing breast cancer is the estrogen receptor (ER) status. ER expression is limited to 6–10% of normal breast epithelial cells, but this percentage is greatly increased in about 60% of primary breast cancer cells. Of these ER-positive tumors, one third are responsive to anti-estrogen treatments, at least in an initial treatment phase (Hanstein et al., 2004). Besides engaging a large number of downstream transcriptional circuitries, estrogen stimulation in breast cancer cells is reported to induce a large array of metabolic alterations that contribute to the metabolic cancer phenotype and allow for their increased proliferation. Estradiol, the most active form of estrogen, enhances glucose and glutamine consumption with a simultaneously elevates lactate production (Forbes et al., 2006; Davison and Schafer, 2010). Additionally estradiol increases PPP fluxes by regulating the activity of glucose-6-phosphate dehydrogenase, once again favoring the biosynthetic pathways necessary for proliferation (Thomas et al., 1990; Forbes et al., 2006).

**Prostate cancer**
Prostate cancer is an obvious example of the involvement of metabolism during carcinogenesis and pathology progression. Surprisingly, the
metabolic remodeling occurring in prostate cancer is quite unique and does not follow the pattern noted above. The prostate gland is anatomically organized in functionally distinct regions, with 70% of the total tissue corresponding to the peripheral zone that exerts the gross of the organ glandular function. Accordingly the peripheral zone is also the most prevalent prostatic region affected by malignancy (Costello and Franklin, 2006). Normal human prostate tissue is committed to the production of the prostatic fluid which presents exceptionally high levels of citrate ranging from 40 to 150 mM (Humphrey and Mann, 1948; Kano and Sas, 1975; Costello and Franklin, 2000).

Compelling evidence for an altered metabolism during prostate carcinogenesis arises from the fact that the extensive accumulation of citrate observed in normal prostate peripheral zone is not maintained in cancerous areas. Indeed, normal peripheral zone tissue contains around 12 000–14 000 nmol/g of citrate contrasting with 250–450 and 200–2000 nmol/g present in other normal tissues and prostate cancer samples, respectively (Costello and Franklin, 2006). Furthermore, as in other cancers, choline metabolism is altered in prostate cancer, leading to a rise in the levels of choline-containing compounds in tumoral tissue, which may serve as essential membrane building blocks during cell proliferation (Bertilsson et al., 2012). The combined evaluations of the oscillations occurring in such metabolites during prostate cancer development have been useful as the molecular basis for the non-invasive detection of prostate cancer by magnetic resonance spectroscopic imaging (Kurhanewicz et al., 2002).

The significance and the molecular mechanism underlying citrate modulation in prostate cancer has been the subject of investigation. Peripheral zone cells have adopted unique metabolic conformations allowing them to function as citrate-producing cells such as a truncated oxidative pathway and its use as an end-product directed for secretion in normal peripheral zone cells have adapted unique metabolic conformations allowing them to function as citrate-producing cells such as a truncated oxidative pathway and its use as an end-product directed for secretion in normal prostate cells express high levels of RREB-1 even in early stage malignancy (Zou et al., 2013). Furthermore activation of sarco- |

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ETS gene fusion regulation which are known mediators of prostate cancer progression (Sreekumar et al., 2009).

Ovarian and testicular cancers
Similar variations of aspects discussed for breast and prostate cancer have also recently been described in other types of cancers in the reproductive system. For example, ovarian cancer cells have been found to be sensitive to glycolysis inhibitors such as 2DG (Hernlund et al., 2009; Sullivan et al., 2014), as well as to HIF inhibitors (Zhang et al., 2013). Additionally, the glycolysis enzyme, GADPH, has been suggested as a marker to indicate early disease progression in advanced serous ovarian cancer (Hjerpe et al., 2012), while SIRT1 might have a similar role in ovarian epithelial tumors (Jang et al., 2009). Interestingly, pyruvate uptake, oxygen consumption and mitochondrial activity seem to be increased as ovarian cancer cells detach and become more invasive (Caneba et al., 2012).

In terms of the male gonad, there are fewer studies, and although pluripotency markers have been described in male germ cell tumors (Ulbricht, 2005; Gillis et al., 2011; Chieffi et al., 2012), there is much less information available. Testicular germ cell tumors (TGCT) represent about 96% of all testicular cancers in men of ages ranging from 20 to 39 years old. These tumors may arise from a common precursor carcinoma in situ (CIS) state which presents a different differentiation potential (Loojenga et al., 2011; Bustamante-Marín et al., 2013). The possibility that the CIS that will develop into a TGCT derives from a spermatogonial stem cell or another less differentiated progenitor is still under debate. However, its reasonable to assume that the failure to repress pluripotency and cell cycle arrest opens a critical window of opportunity for this transformation to happen especially if we also consider that the metabolic phenotype of these cells and their localization within the testis could be beneficial for faster proliferation (Heaney et al., 2012; Silvan et al., 2013). Interestingly TGCT comprises three main different types of tumors. Type I germ cells tumors are teratomas and yolk sac tumors present in newborns and infants that represent two stages of development (PGC and gonocytes); these tumors are positive for Oct3/4. Type II TGCT comprises non-malignant tumors (seminomatous tumors) and malignant ones also known as germ cell cancers (GCC) (nonseminomatous tumors). They can be positive for Oct3/4, Sox2 or even Sox17. Type III germ cell tumors (spermatocytic seminomas, SC) have a different origin given that they arise from spermatogonia or spermatocytes (Eini et al., 2013). It is important to note that LDH has been suggested as another marker for seminomas, both in terms of metastasis and relapse (Powles et al., 2013; Ruf et al., 2013).

Possible metabolic strategies in cancer treatment
The recognition that cancer cells rely upon unique metabolic adaptations is now widely acknowledged and seen as a valuable therapeutic target in the attempt to control tumorigenesis. Consequently, intensive research has been carried in the pursuit of an effective strategy to target those metabolic reactions. We will try to summarize and clarify the efforts and breakthroughs in this area. One of the most extensively studied glucose metabolism-targeted pharmacological agent used in cancer diagnosis and treatment is the glucose analog, 2-DG. As noted previously, this compound competitively inhibits glucose transport and as it is converted by hexokinase into phosphorylated 2-DG, which is not metabolized any further to a significant extent, resulting in a decreased glycolytic output (Dwarakanath et al., 2009). The higher glucose use in tumor cells is needed, for example, for repairing radiotherapy damage. Hence it was predicted that glycolysis inhibitors, namely 2-DG, would increase radiotherapy efficiency (Jain et al., 1973). Subsequently there was a burst of experimental work using 2-DG as an anti-tumoral agent. In vitro experimental studies have shown that 2-DG inhibits cancer cell growth in a great number of cancers such as lung cancer (Fan et al., 2013), glioblastoma multiform (Pistoliato et al., 2010) or pancreatic cancer (Cheng et al., 2013). But most importantly, many clinical trials combining 2-DG with radiotherapy have been performed not only due to the anti-tumoral properties of this substance (Mohanti et al., 1996), but also because, by administrating it with the radioactive isotope fluorine-18, it functions as a radiotracer for the diagnosis, surveillance and metastasis detection of several cancers, such as cerebral glioma (Mohanti et al., 1996), gastric cancer (Ma et al., 2013), colorectal cancer (Chiewwit et al., 2013) and both prostate (Stein et al., 2010; Jadvar et al., 2013) and ovarian cancers (Manegold-Brauer et al., 2013). However, its clinical success has been limited due to a lack of efficacy given that in the presence of 2-DG cells increase autophagy and there have been reports of toxicity to the brain and heart, since such tissues will also uptake the compound (Stein et al., 2010; Schulze and Harris, 2012) (Stein et al., 2010; Schulze and Harris, 2012).

Another example of a chemical compound acting on cancer metabolism is 3-BromoPyruvate (3-BrP). 3-BrP is a synthetic alquilant agent analog of pyruvic acid (Apfel et al., 1984). Although 3-BrP is a non-specific compound, most publications report that its targets are mainly: hexokinase II (HKII) (Ko et al., 2001; Chen et al., 2009), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Ganapathy-Kanniappan et al., 2009) and pyruvate dehydrogenase (PDH) (Maldonado et al., 1972). With such metabolic targets, 3-BrP is also regarded as a potential cancer therapeutic agent. Although there are no clinical trials described for this compound, several reports have shown its promising effects on different cancer types such as colon carcinoma cell lines (Irlund et al., 2008), glioma cells (El Sayed et al., 2012), leukemia and lymphoma cells (Chen et al., 2009), rat mammary tumors (Buijs et al., 2009) and many others (Pedersen, 2012).

Dichloroacetate (DCA) is also a promising compound. DCA is a pyruvate analog that inhibits pyruvate dehydrogenase kinase (PDK). When PDK is inhibited, pyruvate is preferably diverted to the mitochondrial Krebs cycle (Bonnet et al., 2007; Michelakis et al., 2008; Mathupala et al., 2009). Some studies have already been conducted and have confirmed that DCA may be a potential anti-cancer drug for non-small-cell lung cancer, glioblastoma cell lines (Bonnet et al., 2007), prostate cancer cells (Cao et al., 2008), neuroblastoma cancer cells (Vella et al., 2012) and breast cancer cells (Bonnet et al., 2007; Sun et al., 2010) due to its anti-proliferative and/or pro-apoptotic potential. Although DCA is not suitable to be patented, one phase II clinical trial has already been conducted and its published results seem promising (Michelakis et al., 2010); other clinical trials are ongoing (see ‘ClinicalTrials.gov’). Studies using RNA interference screening tools have been used to identify metabolic weaknesses in cancer cells, such as PHGDH. This enzyme is involved in the serine biosynthesis pathway, essential for the in vivo growth of breast cancer cells (Possennato et al., 2011) and is thus a possible therapeutic target (Locasale et al., 2011; Ye et al., 2012). Another possible target could be an isoform of
phosphofructokinase 2, called PFKFB4, which is important for the survival of prostate cancer cells (Ros et al., 2012).

Basically, a cancer cell has the ability to induce compensatory biosynthetic pathways in order to generate the limiting metabolites that the cell needs to divide. Another aspect of this plasticity relies on the exchange of metabolites between cancer cells and the surrounding tissues. All these characteristics bring an additional challenge to identification of the metabolic targets that are key players in supporting the survival of cancer cells.

Conclusions and Future Directions

Although many issues regarding signaling networks and the possible crosstalk between the mitochondria and the nucleus have yet to be deciphered, the reviewed studies suggest that there are uncanny similarities between PSCs and cancer cells at the metabolic level. Furthermore, metabolism is clearly more complex than originally thought, and, rather than just a bystander responding to genetic programs, it may also play a direct role in the cell status. Targeting metabolic pathways may therefore be a promising strategy for both the control of cancer cell proliferation and the regulation of stem cell physiology, in terms of manipulating stem cells toward relevant phenotypes that may be important for tissue engineering, and changing cancer cells to become less tumorigenic.

Clearly what is required is a better understanding of the metabolic pathways involved, and how they may inform the design and testing of more specific compounds and/or identify molecular targets whose up- or down-regulation may result in more controlled and predictable effects.

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Authors’ roles

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Conflict of interest

None declared.

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Metabolism in gametogenesis and cancer


