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The Warburg Effect Revisited—Lesson from the Sertoli Cell

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Abstract

Otto Warburg observed that cancerous cells prefer fermentative instead of oxidative metabolism of glucose, although the former is in theory less efficient. Since Warburg's pioneering works, special attention has been given to this difference in cell metabolism. The Warburg effect has been implicated in cell transformation, immortalization, and proliferation during tumorigenesis. Cancer cells display enhanced glycolytic activity, which is correlated with high proliferation, and thus, glycolysis appears to be an excellent candidate to target cancer cells. Nevertheless, little attention has been given to noncancerous cells that exhibit a "Warburg-like" metabolism with slight, but perhaps crucial, alterations that may provide new directions to develop new and effective anticancer therapies. Within the testis, the somatic Sertoli cell (SC) presents several common metabolic features analogous to cancer cells, and a clear "Warburg-like" metabolism. Nevertheless, SCs actively proliferate only during a specific time period, ceasing to divide in most species after puberty, when they become terminally differentiated. The special metabolic features of SC, as well as progression from the immature but proliferative state, to the mature nonproliferative state, where a high glycolytic activity is maintained, make these cells unique and a good model to discuss new perspectives on the Warburg effect. Herein we provide new insight on how the somatic SC may be a source of new and exciting information concerning the Warburg effect and cell proliferation.

Keywords

Warburg effect; Sertoli cell; glycolysis; lactate; testis; spermatogenesis

1. INTRODUCTION

Otto Warburg observed that glucose metabolism in cancer cells presents some specific characteristics very distinct from those of cells in normal tissues.[1, 2] Warburg reported that

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CONFLICTS OF INTEREST

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cancer cells, unlike most normal cells, convert glucose to lactate even in the presence of sufficient and physiological oxygen levels to support mitochondrial oxidative phosphorylation. That was intriguing since most cells, in the presence of oxygen, metabolize glucose to carbon dioxide through the Krebs cycle by oxidation of pyruvate derived from glycolysis. This reaction produces NADH that is used as fuel to maximize ATP production by mitochondrial oxidative phosphorylation, with minimal lactate production. Thus, there are considerable differences in the metabolic behavior of "Warburg" cells versus normal cells. Normal differentiated cells only produce high lactate levels under anaerobic conditions, while cancer cells produce high levels of lactate[3] regardless of oxygen availability. Thus, in contrast to normal differentiated cells, which primarily rely on mitochondrial oxidative phosphorylation to generate energy, cancer cells obtain their energy by aerobic glycolysis, a process known as "the Warburg effect". Warburg also postulated that glycolytic activity in cancer cells was similar to that observed in early embryonic cells, illustrating that cancer cells may present a primitive metabolic pattern.[1] Proliferation is undoubtedly related to the unique metabolic characteristics generally associated with cancer cells. Many unicellular organisms that present high proliferative activity use fermentation, the microbial equivalent of aerobic glycolysis, illustrating that aerobic glycolysis can produce sufficient energy to maintain cell proliferation.

A cell that undergoes proliferation must replicate all of its cellular content to produce two viable daughter cells. For that purpose, several factors and special conditions are needed. Among those, large amounts of ATP and energy, nucleotides, amino acids, and lipids are required for biomass replication. Within the testis, biomass replication is a crucial event, essential for the species maintenance and propagation. Thus, spermatogenesis, the process of sperm production and maturation, is under strict control. In that process, the somatic Sertoli cell (SC) is a key element since SCs create the blood testis barrier (BTB), and they provide nutritional and structural support for the developing germ cells. SCs also protect spermatogenic cells from the host immune response and block the entry of leukocytes into the seminiferous epithelium (for review[4]). Thus, these cells are responsible for the formation of an immune-privileged environment in the testis.[5, 6] To accomplish all these functions, the SC presents some distinctive characteristics not always explored by researchers.

One of the most important events during spermatogenesis is the metabolic cooperation between the SC and the developing germ cells. The somatic SC presents a high glycolytic flux to ensure the production of high lactate levels and factors required for the developing germ cells. Indeed, the SC metabolic behavior aligns with Otto Warburg observations in cancer cells. However, besides the Warburg-like metabolism, the SC presents a very important characteristic related to their maturation. It is dependent on the species, but SCs can only proliferate during a specific time period and in all species (including humans) they cease to divide at adulthood. Thus, SC is a somatic cell that, from a metabolic point of view, has Warburg-like metabolic behavior without the primary deleterious characteristic of Warburg effect: mitotic proliferation. Herein we propose to present an overview of that topic by exploring the Warburg effect and its significance to cellular homeodynamics with special emphasis to the testicular somatic SC and its unique metabolic and cell cycle characteristics.

2. THE WARBURG EFFECT POSTULATIONS

The first observations by Otto Warburg raised several intriguing questions. One of the most interesting findings was that proliferative cancer cells present a high glycolytic flux even in the presence of normal oxygen levels. Aerobic glycolysis is an inefficient way to generate ATP since conversion of a single glucose molecule to lactate generates only two ATPs, whereas mitochondrial oxidative phosphorylation generates up to 36 ATPs per molecule of glucose. The advantage of this metabolic process for high proliferative cells such as cancer cells is unclear. Recently, it was proposed that this occurs as an adaptation to intermittent tumor hypoxia.[7] However, cancer cells prefer glycolytic metabolism before being exposed to hypoxia.[8, 9] Tumor cells generate the majority of ATP from glucose whereas normal cells generate ATP through mitochondrial oxidative phosphorylation using several other substrates besides glucose, such as fatty acids and other metabolic intermediates.

Although cancer cells exhibit elevated glycolytic flux, it does not imply that cancer cells only use the glycolytic pathway for ATP generation. This led us to discuss the other interesting finding proposed by Otto Warburg: mitochondrial respiration inhibition. After the initial postulations that led to the well-known "Warburg effect," the author hypothesized that mitochondrial respiration and oxidative phosphorylation could be inhibited in cancer cells, which in turn would lead to glycolysis stimulation as a compensatory mechanism.[1] Mounting evidence has shown that aerobic glycolysis and suppression of mitochondrial metabolism occur in cancer cells, and metabolic reprogramming has been suggested as a hallmark for cancer.[7, 10] However, mitochondrial defects in cancer cells are not as abundant as it would be supported by this Warburg model and there are multiple signals that promote glycolysis, rather than mitochondrial inhibition.[11] Fructose 1,6-biphosphate, an important intermediate in glycolysis, is well known to induce a decrease in the activity of mitochondrial complexes III and IV.[12] It was also shown that a knockdown or disruption of lactate dehydrogenase A (LDHA) in cancer cells leads to stimulation of mitochondrial respiration and reduced cell proliferation under hypoxia, suppressing tumorigenicity.[13, 14] The inhibition of LDH mainly blocks the production of lactate from pyruvate, which can be transported into mitochondria for ATP production, stimulating the Krebs cycle machinery activity. Thus, these findings support the notion of mitochondrial respiration inhibition in cancer cells. However, it was demonstrated that some glioma, hepatoma, and breast cancer cell lines possess not only fully functional mitochondria, but also that the main ATP supply in these cells is obtained from mitochondrial oxidative phosphorylation.[15–17] Interestingly, some cancer cells can switch between glycolysis and oxidative metabolism depending not only on the presence or absence of glucose but also other environmental conditions.[18–20] Therefore, the metabolic switch from mitochondrial oxidative metabolism to glycolysis in most cancer cells may not be directly linked to inhibition processes, but rather be the result of interactions between a complex network of metabolic signals and pathways. While glycolysis stimulation and its relation with cancer cell proliferation is widely accepted, the decrease in mitochondrial oxidative phosphorylation remains a matter of debate. In the next subtopics we focus on the connection between glycolysis and cell proliferation. Within the testis, the somatic mature SC presents a Warburg-like effect without high proliferative

activity and therefore, can provide useful information to understand the crossroads between these two crucial events.

3. THE CENTRAL ROLE OF LACTATE IN THE TESTIS

The testis are tightly compartmentalized organs, with low and ununiformed oxygen distribution.[21] Thus, it is not surprising that, throughout the different developmental stages, germ cells use different metabolic substrates.[22] Lactate is the central energy metabolite and its importance to male germ-cell energy metabolism has been comprehensively discussed (for review[23]). Notably, several glycolytic enzymes have testis-specific isoforms that are predominantly expressed in spermatogenic cells,[24] evidencing the importance of these processes in testis. Mature germ cells express all the enzymes of the glycolytic pathway, but they are dependent on lactate supply by SCs. However, in testis, lactate is more than just a substrate and plays other key roles. It has been reported that intratesticular infusion of lactate into the adult cryptorchidic rat testis results in a clear improvement of spermatogenesis by suppressing the loss of spermatocytes and spermatids.[25] Also, lactate has been reported to stimulate RNA and protein synthesis in spermatids,[26] supporting the notion this metabolite can control spermatogenic cell development via a complex pathway. The pharmacological deprivation of lactate has also been shown to decrease the viability of male germ cells.[27] Moreover, under physiological conditions, more than 75% of spermatogenic cells undergo apoptosis[28] and surprisingly, lactate has been reported to exert an antiapoptotic effect on male human germ cells in a dose-dependent manner.[29]

The pivotal role of lactate on spermatogenesis is widely known and it has been proposed that targeting lactate oxidation and/or production in vivo in testicular cells, could be a potent and effective contraceptive agent.[23] It has been shown that sperm from males that have been immunized with a B-cell epitope of LDHC, a testis-specific LDH isoform, had a reduced binding capacity and thus, LDHC could be a potential candidate for the development of a vaccine for male contraception.[30] In summary, developing germ cells have several particular metabolic requirements. Among these high lactate concentrations are needed to sustain spermatogenesis and normal testicular functioning. Within the testis, the somatic SC is responsible for lactate production by maintaining a high glycolytic flux.

4. SERTOLI CELL AT A GLANCE

Testicular function is regulated by several factors. Most of them are directly dependent on SC. The SC has two main, very distinct, functions: formation of the seminiferous tubules and the nutritional and structural support of developing germ cells (Fig. 1). Moreover, they are responsible not only for water transport but also for the control of the seminiferous epithelium luminal fluid composition.[31–34] Fully differentiated SCs regulate the flow of nutrients, growth, and other factors to the developing germ cells. This is achieved through the BTB, also known as SC barrier.[35, 36] This barrier, which is established between adjacent SCs, physically divides the seminiferous epithelium into basal and apical compartments. This organization is crucial for spermatogenesis since spermatids maturation into mature spermatozoa (spermiogenesis) and release of mature spermatozoa (spermiation) occurs in the apical compartment[37] (Fig. 1). Moreover, BTB undergoes a well-coordinated

restructuring to facilitate the transit of primary preleptone spermatocytes while differentiating into leptotene and zygotene spermatocytes (for review[38, 39]). The germ cells form close associations with SCs and multiple germ cells are in contact with a single SC. These associations are morphologically unique and each represents a defined stage of the epithelial cycle of spermatogenesis (for review[40]).

Importantly, developing germ cells have a complete metabolic dependence on SC. The SC is responsible for lactate production that is then exported to the developing germ cells. During that process, the SC is targeted by external and internal factors such as hormones,[41–43] cytokines,[44] AMP-activated kinase (AMPK)[45] among others (for review[46, 47]). For years, the metabolic cooperation between testicular cells was disregarded, but recent advances have highlighted the relevance of these processes to male fertility. For instance, it has been proposed that the effects of several diseases known to impair the male reproductive function, such as diabetes mellitus, may be due to changes in the metabolic cooperation between SCs and developing germ cells (for review[37, 48, 49]). Although several biochemical steps are involved in lactate production by SC, glucose and its intermediates play a crucial role in this process. In fact, SCs are well known for maintaining a high glycolytic flux to support the nutritional needs of the developing germ cells. Glucose is taken up from the extracellular space via glucose transporters (particularly GLUT1 and GLUT3)[50, 51] and is then converted into lactate. In this process, the LDH isoenzyme system (that reversibly catalyzes the interconversion of pyruvate to lactate) is a key player, as is widely accepted for cells using aerobic glycolysis. Lactate is then transported across the plasma membrane by the action of monocarboxylate transporters (MCTs). It has long been shown that the SC has highly active glycolytic machinery even in the presence of oxygen. Therefore, the first postulation of the Warburg observations in cancer cells is effectively confirmed in SCs. However, contrary to what is reported to occur in cancer cells, this increase in glycolytic flux is not followed by SC proliferation. In fact, SC maturation is accompanied by several changes and interestingly one of the most important is the progress from a proliferative to a nonproliferative state. Moreover, the doubling time of SCs in vitro averages 3–4 days, depending on plated cell density,[52–54] whereas cancer cells generally duplicate in a more reduced period.[55–57] In addition to this difference in cell behavior, SC does not present metastatic characteristics as some types of cancer cells.

5. SERTOLI CELL PROLIFERATION AND MATURATION

Failure of SCs to normally proliferate during male development results in reduced production of spermatozoa in adulthood and usually the absence of germ cells reflects abnormalities in SCs, such as maturation failure. Moreover, several reproductive disorders, such as testicular germ-cell cancer, cryptorchidism, hypospadias, and low sperm counts, have a common origin in fetal life and are often attributed to abnormal SC function[58, 59] (for review[60]). To this contributes the fact that SCs are the first cells to differentiate in the fetal gonad.[60] During puberty and adulthood, SCs are responsible for physical and metabolic support of germ cells[47] and without a proper SC function, the development of germ cells into spermatozoa will not occur (for review[33, 46]). This confirms that SC is a major player not only in testis formation but also in spermatogenesis. These events are separated in time, fetal versus adulthood life, illustrating that important changes occur in SC to allow steady

progress from fetal to adult function. This process is usually known as maturation. During this process, the SC loses the ability to proliferate and the assembly of inter-SC tight junction takes place (for review[60]). The BTB, or SC barrier, is then formed.

Interestingly, only immature SCs proliferate and thus, the final number of SCs is expected to be determined before adulthood. There are species-related differences (for extensive review[60]), particularly concerning the time period in which SCs can proliferate, however in all species SCs cease to divide at adulthood. Fetal and neofetal periods, as well as peripubertal period, are crucial for SC proliferation.[61] Although SC numbers are generally stable, it is recognized that they can be augmented under certain conditions.

There are several factors that determine the ability of SCs to proliferate and thus their number in the testis. Some of these factors are hormonally controlled and depending on experimental or physiological conditions, SCs can behave differently. For instance, follicle-stimulating hormone (FSH) and thyroid hormones have very distinctive effects on SCs. FSH is reported to increase the proliferation rate (measured as time for duplication of cultured cells number) in SCs while thyroid hormones are reported to regulate SC maturation by altering the period in which their proliferation occurs.[54, 62–65] Additionally, nonhormonal factors are also crucial for SC ability to proliferate (for review[66]). The number of SCs determines the number of germ cells and hence daily sperm production.[67] In the 1980s, Johnson and colleagues[68] quantified the number of SCs in the testis of adult men and correlated it with daily sperm production. They concluded that there was a linear correlation between those factors and SC number, but SCs displayed considerable variations among men. Later, Jensen and colleagues reported a high variation in blood concentration of inhibin B among men.[69] Inhibin B is a specific product of SCs[70] that was reported to be altered depending on SC proliferative activity.[71] This later work provided important evidence that mature SCs may be proliferative.

It has been reported that mature rat SCs can incorporate ^{3}H-thymidine,[72] suggesting that SCs in rodents may be mitotically active but in a tightly regulated manner. Others have also shown that SCs may sustain, to some extent, their proliferative activity. When SCs were transplanted into SC impaired or defective testicles, these transplanted SCs were found to be proliferative, capable of populating the testis and even restoring spermatogenesis.[73, 74] In vitro studies showed that adult human SCs have proliferative potential[31] expressing some important factors and a number of markers of multipotent mesenchymal cells.[52] Recently, it has been proposed that the SC population is more dynamic than previously considered[75] and thus, manipulation of SC number can open new possibilities in the treatment of infertility. Hazra and colleagues used unbiased stereological techniques to enumerate total testicular cell numbers in mice and reported that a significant SC proliferation occurs during late postnatal-pubertal development[76] challenging the conventional postulation, based on DNA labeling of S-phase cells,[65, 77, 78] that rodent SCs cease to divide in midpostnatal life.[60] Thus, it is expected that as new improved techniques to assess cell number in vivo may evolve, new findings concerning SC number and proliferation during fetal, postnatal, pubertal, and adulthood may arise and contribute to new postulations.

It is widely accepted that in male rodents and humans, SCs begin to proliferate during fetal development and, in rat testis, they increase about 30-fold during the first 3 weeks after birth.[79, 80] Interestingly, SC proliferation rate decreases in rats and mice 5–15 days after birth.[77, 78, 81] Then, between the days 14–21, SCs undergo a differentiation process marked by the secretion of several proteins needed for germ cells and by BTB formation.[79] Although these time periods appear to be straightforward in mice and rats, the same is not the case in primates, including men, in which SC expansion is expected to occur in two periods. The first is very similar to rodents, however a second period is reported to occur during puberty when spermatogenesis is initiated[82, 83] (for review[79]). Thus, SC function switches to support germ-cell differentiation, meiosis, and spermatid transformation at a species-specific time. Overall, this switch from immature proliferative state to mature nonproliferative state is accompanied by several morphological and functional changes (for review[84]). Additionally, it is controlled by complex endocrine, paracrine, and autocrine factors (for review[75]). Intriguingly, the factor and mechanism that ultimately trigger SC exit from cell cycle to become nonproliferative, remains unknown and is a matter of debate. Finally, abnormal SC development or proliferation has been associated with disorders of testicular function and the presence of characteristics analogous to an undifferentiated state has been linked to testicular cancer and male infertility.[85–88] SC tumorigenesis only contributes to 4% of all testicular tumors,[89] which are relatively rare.[90] Thus, the mechanisms responsible for SC abnormal proliferation and tumorigenesis remain largely unknown. Nevertheless, recent studies have shown that they can be dependent on the proapoptotic CCDC6 protein loss[91] or might be related to the aging associated loss of SMAD4.[92] On the other hand, it was also recently reported that retinoblastoma protein is not associated with the development of SC tumorigenesis.[93] Since SC abnormal proliferation and maturation are associated with impaired spermatogenesis, male infertility, and testicular tumorigenesis, it is imperative to identify the mechanisms responsible for such processes.

6. LINK BETWEEN CELL METABOLISM AND PROLIFERATION: AN UNANSWERED QUESTION

It has been proposed that glycolysis may serve not just as a metabolic pathway to attain energy, but also as a crucial adaptation to facilitate the uptake and incorporation of nutrients into biomass needed to produce new cells.[94] This has been highlighted by reports showing that certain cancer-associated mutations are essential to acquire and metabolize nutrients rather than ATP production through glycolysis.[94] Nutrient availability also influences acetylation status of metabolic enzymes, modulating the enzymatic activity.[95] Moreover, uncontrolled proliferation is associated with stimulation of growth factors and cancer cells acquire genetic mutations that functionally alter the receptor-mediated signaling pathways. These alterations may be responsible for an increase in nutrient uptake and activation of metabolism, promoting cell survival and growth.[96, 97] Growth factor signaling regulates the activity of glycolytic enzymes that have a critical role in shifting glucose metabolites to pentose phosphate pathway (PPP) as well as to nucleotide and biosynthesis pathways.

The controlling step of metabolic pathways is substrate supply. Cells in culture usually depend on glucose and glutamine as carbon, nitrogen, free energy, and reducing equivalents

precursors that are necessary to support cell growth and division[94] (Fig. 2). Most of the carbon for fatty acid synthesis is derived from glucose, thus a large part of the glucose taken up by cells is required and shifted to macromolecular precursors such as acetyl-CoA for fatty acids synthesis. In this process, glucose is converted to acetyl-CoA in the mitochondrial matrix. Glucose metabolism derived precursors such as acetate, a well-known crossroad metabolite, can be converted into acetyl-CoA in the cytosol and in mitochondria by acetyl-CoA synthase,[98] and are pivotal for the synthesis of fatty acids and cholesterol. Noteworthy, active growth and acetyl-CoA levels are highly correlated, and it has been recently reported that acetyl-CoA induces cell growth and proliferation.[99] This observation provides evidence that acetyl-CoA can be used as a metabolic control point to the cell cycle in proliferating and nonproliferating cells.[100]

The importance of acetyl-coA as a metabolic crossroad between glycolysis and lipogenesis in proliferating cells has been a matter of debate,[101] since cancer cells do not acquire fatty acids from dietary sources. In addition, fatty acid synthase has been proposed as a mechanism by which cancer cells can restore the redox imbalance promoted by increased glycolytic flux.[102] Interestingly, it has been recently reported that in vitro cultured mature SCs secrete high amounts of acetate in a mechanism that is under strict hormonal control.[103] It was proposed that acetate is important to maintain the high rate of lipids synthesis that occurs during germ-cell development. The SC is responsible for synthesis and export of several factors, including metabolic intermediates that support spermatogenesis. Thus, SC production of acetate that is then exported to the extracellular space, where it can be utilized for de novo lipids synthesis, is a possible candidate as a checkpoint of spermatogenesis. Nevertheless, the exact role for acetate in spermatogenesis remains to be identified.

Glucose metabolism and cellular growth control are processes linked to phosphoinositide 3-kinase (PI3K) signaling pathway. This pathway is essential for the conversion of amino acids into proteins (via mTOR signaling) and also for glucose uptake and metabolism (through AKT signaling) by regulating transporter expression and phosphofructokinase (PFK) activity.[97] This pathway, extensively reviewed by Zoncu and colleagues,[104] has been suggested as the crossroad between high glycolytic rates and cell proliferation. Additionally, mTOR inhibition shifts glucose metabolism toward mitochondrial respiration,[105] illustrating that mTOR is a key player in these processes. The mTOR signaling pathway includes association of mTOR with Raptor (regulatory-associated protein of mTOR) and other subunits to create a multiprotein complex called mTOR complex 1 (mTORC1).[106] Another crucial signaling molecule in the mTORC1 pathway is the ribosomal protein S6 kinase (rpS6). Recently, it was reported that rpS6 regulates BTB restructuring during specific stages of spermatogenesis.[107] The authors provided clear evidence that rpS6 regulates BTB dynamics through changes in the recruitment of proteins at the SC–SC interface. mTOR can also be associated with Rictor (rapamycinin-sensitive companion of mTOR) and other partners forming the mTORC2.[108] It has also been reported that mTORC2 is a key regulator of BTB.[109] Thus, it is evident that SC, the key component of BTB, is under tight control from the mTOR pathway. Although the involvement of mTOR in the metabolic control of SC has not yet been elucidated, it likely provides key information that connects cell metabolism and the mechanism(s) responsible for proliferation control in SC.

7. ROLE OF ALTERNATIVE FUELS FOR PROLIFERATIVE AND NONPROLIFERATIVE LACTATE-PRODUCING CELLS

ATP production and growth can be diminished when glucose is scarce. In cancer cells, there is a continual glucose and nutrient supply from circulating blood. Nevertheless, there are alternative fuels, rather than glucose, that can support lactate production. In several conditions, cancer cells and SCs use amino acids and glycogen to maintain ATP production. Additionally, the role of pathways that replenish the Krebs cycle intermediates, known as anapleurotic pathways, cannot be disregarded, as their contribution for cellular metabolism can be vital. Among the several metabolites these pathways can supply, glycolysis-derived pyruvate and glutaminolysis-derived α-ketoglutarate are of extreme importance to provide anapleurotic substrates for specific Krebs cycle reactions. This is even more important in in vitro studies where culture conditions and media concentration can exert a stoichiometric pressure in cells to work toward some pathways over others.

One of the most important substrates for cancer cells and testicular SCs is glutamine (Fig. 2). The oxidation of glutamine and leucine has been reported to yield much of the required energy by SCs,[110] although the importance of this substrate is far from being disclosed. In cancer cells, the glutaminolysis is not only well reported, but has a known relation with proliferation and the specific metabolic characteristics of these cells. For instance, it has been reported that cultured glioblastoma cells convert 90% of glucose and 60% of glutamine into lactate and alanine.[111] The high glutamine consumption is also an interesting feature of cancer cell energy metabolism[112] and glutaminolysis has been reported to be significantly increased in cancer cells.[113, 114] This can be due to several reasons. Glutamine is known to be involved in anabolic pathways and can also be degraded in the Krebs cycle to generate ATP[115, 116] (Fig. 2). Glutaminolysis includes a step in which glutamine is converted to glutamate and chronic hypoxia results in elevated glutamate and reduced glutamine.[117] Glutamate can be converted to α-ketoglutarate and enter the Krebs cycle supplying crucial intermediates to fatty acids and amino acid synthesis (Fig. 3). Glutamate can also be directly converted to glutathione, which is essential for redox state homeostasis.

In SCs, glutamine inhibits the incorporation of alanine into proteins,[118] which may be crucial since alanine can be converted to pyruvate that is also a substrate used by SCs to maintain lactate production. It has been reported that SCs can metabolize not only glutamine but also several other amino acids such as alanine, leucine, and valine that significantly alter SC metabolic behavior[118] (Fig. 3). Remarkably, in SCs, glycine is converted to serine and incorporated in phospholipids,[118] a peculiar process since these cells, when matured, do not proliferate. The role of lipids and amino acids in SC proliferation deserves much attention in future studies since SC mitotic activity is dependent on maturation state and thus, proliferative capacity. For instance, in vitro cultured immature SCs present higher rates of oleate oxidation to CO_2[119] illustrating that progression of immature to adult mature state is accompanied by slight metabolic alterations that allow these cells to produce lactate at high rates without proliferation.

The role of glycogen in the secluded environment of proliferative and nonproliferative lactate-producing cells has been somewhat disregarded by investigators. However, it has

long been reported that cancer cells present high glycogen content[120] and reduced glycogen synthase kinase-3 (GSK3) activity.[121] GSK3 is responsible for glycogen synthase inactivation and thus reduces glycogen synthesis. Moreover, glycogen phosphorylase is overexpressed under hypoxic conditions,[122] evidencing an important role for glycogen in cancer cell metabolism, especially under conditions of insufficient vascularization.[123] It has also been proposed that glycogen may have a key role in SCs. The presence of glycogen and glycogen phosphorylase activity has long been reported in SCs.[124, 125] Although the relevance of glycogen in cancer cells and SCs remains unclear, it is expected that glycogen can be used to maintain the high glycolytic flux in conditions of glucose deprivation (Fig. 3).

The alternative fuels, rather than glucose, have been less studied in proliferative and nonproliferative lactate-producing cells. Nevertheless, their role is expected to be crucial not only for lactate production but also for cell cycle arrest/stimulation.

8. GLYCOLYTIC FLUX CONTROL IN SERTOLI AND CANCER CELLS

Cancer cells and SCs prefer aerobic glycolysis over mitochondrial phosphorylation even though aerobic glycolysis is an inefficient way to produce ATP. Nevertheless, the lower ATP yield of glycolysis comparative to mitochondrial oxidative phosphorylation is partially compensated by increased rates of glycolytic flux. This flux can be controlled by several factors. Glucose transport is known to be a rate-controlling step of glycolysis.[126] The high-affinity glucose transporters, GLUT1 and GLUT3, are overexpressed in several cancer cells[127] and their inhibition impairs tumor cells growth.[128] In SCs, it has also been reported that modulation of GLUT1 and GLUT3 expression may have a key role in the hormonal regulation of spermatogenesis,[129] particularly concerning insulin action.[41, 103] The high glycolytic fluxes that these cells exhibit are intimately related with the activity of the enzymes participating in the glycolytic pathway.[130, 131] In fact, the glycolytic flux rate is controlled by different mechanisms such as enzyme content and activity, substrate availability, allosteric effectors, and modification in regulatory enzymes.[132]

Several authors reported that maintenance of a high rate of aerobic glycolysis is mainly achieved through upregulation of enzymes and transporters associated with glucose uptake and catabolism such as GLUT1, pyruvate kinase (PK), and LDH.[94, 133–136] Additionally, metabolic shift in cancer cells is often associated with altered expression in genes and proteins that regulate glycolysis and oxidative phosphorylation. These alterations may be due to several factors including nuclear factor kappa light chain enhancer of activated B cells (NF-kB) and hypoxia inducible factor (HIF)-1, as well as p53.[137] For instance, it has been reported that HIF-1 can regulate the expression of several glycolysis-related enzymes and transporters including GLUT1, GLUT3, hexokinase (HK), LDH, and MCT4.[138, 139] Cells using aerobic glycolysis exhibit high ratios of ATP/ADP and NADH/NAD^+ and if these ratios are disturbed, cell growth can be impaired and apoptosis stimulated.[140, 141] In normal proliferating cells, when ATP levels drop due to diminished glucose metabolism, cells can undergo cell cycle arrest and catabolic reactions occur.[142, 143] For instance, changes in ATP/ADP levels lead to activation of p53 through AMPK pathway.[144] The cellular activation of p53 can inhibit the Warburg effect and promote oxidative phosphorylation. The loss and/or mutation of p53 results in decreased oxygen consumption and stimulation of

glycolysis, the main metabolic characteristic of tumor cells.[145] Moreover, p53 downregulates glucose transporters, particularly GLUT1, GLUT3, and GLUT4.[144, 146, 147] The mitochondrial DNA copy number and mitochondrial mass are also maintained by p53.[148, 149] In SCs, the role of p53 remains largely unknown but it is well known that sex hormones modulate lactate production by these cells and also downregulate p53 expression.[43, 150] Therefore, it is possible that p53 can also be a regulator of glycolytic flux in SCs.

One of the main Warburg postulations was related to mitochondrial malfunction and oxidative phosphorylation depletion. These processes are related to changes in NADH/NADPH ratio and thus, intracellular redox balance disturbance. It has been reported that respiratory chain components, as well as Krebs cycle enzymes, are sensitive to reactive oxygen species (ROS) levels.[151, 152] Interestingly, it has been suggested that proliferating cells using aerobic glycolysis may have protective mechanisms against oxidative stress.[153] This protection is due to NADPH/$NADP^+$ balance that is produced through PPP, which in turn is derived from the glycolytic pathway. Thus, increased glycolysis is responsible for PPP activation and increased levels of an important indirect antioxidative by-product: NADPH. This has been widely suggested in proliferating cells since they are known to possess increased glucose-6-phosphate dehydrogenase activity,[154] which is the rate-limiting step in PPP. In the 1980s, Grootegoed and colleagues[110] reported that SCs incubated in the absence of hormones did not present a PPP operating at its maximum rate. However it was reported that hormonal signals may accelerate glucose metabolism via PPP in these cells and it is well known that within the testis, SCs are the main hormonal targets. Therefore, PPP is expected to be very active in SC in vivo. In germ cells, PPP is reported to be essential for maintaining biosynthesis of nucleotides, production of NADPH, and ribose-5-phosphate that are necessary for RNA.[22]

Interestingly, cancer cells exhibit a high basal level of oxidative stress. This has been associated with activation of oncogenes, loss of tumor suppressors, and also to the microenvironment that a tumor creates.[155] A novel mechanism by which ROS impairs β-cell function in type 2 diabetic individuals has been proposed.[156, 157] High glucose levels activate Hif1α, thus a switch to lactate production and impaired glucose oxidation and insulin secretion are observed. This increase in lactate production, despite regular oxygen availability, is very similar to the Warburg effect described in cancer cells,[158] evidencing that ROS may also be important in the glycolytic flux control of both proliferative and nonproliferative cells known as lactate producers.

The use of glycolytic inhibitors may provide crucial information about glycolytic flux control in these cells. 2-Deoxyglucose is well known to inhibit glucose metabolism since it is phosphorylated inside cells by HK, generating 2-deoxyglucose-phosphate that accumulates and inhibits HK. Its action, at millimolar concentration range, is known to cause ATP depletion and cell death in a hypoxic environment.[159–161] 3-Bromopyruvate (3-BrPA) is a halogenated pyruvate glycolytic inhibitor that has a strong inhibition effect on HK, affecting mitochondrial respiration and leading to ATP depletion that leads to cancer cell death.[162, 163] Lonidamine is also known to inhibit aerobic glycolysis by inhibiting HK[164] and depleting ATP in a dose-dependent way.[165] Glufosfamine is a conjugate of

glucose and ifosfamide that once inside the cells releases the toxic ifosfamide resulting in cell death.[166, 167] It has also been reported that catechin (−)-epigallocatechin 3-gallate (EGCG), present in significant amounts in natural products such as tea, can target glutamine metabolism by inhibiting glutamate dehydrogenase under low glucose conditions.[168]

A thorough understanding of glycolytic flux control in cancer cells and SCs is not only important to understanding the progression of cancer and spermatogenesis, but also provide useful information concerning cell cycle regulation.

9. CANCER AND SERTOLI CELLS METABOLIC BEHAVIOR UNDER PATHOLOGICAL CONDITIONS ASSOCIATED WITH CARBOHYDRATE AVAILABILITY

One of the most important functions of SC is to ensure glucose transport and metabolism to produce lactate for the developing germ cells.[23] A deregulation of this metabolic behavior compromises the energy supply to germ cells and consequently affects male fertility. Several metabolic diseases are considered public health threats. In common, most of them present in their etiology insulin resistance and/or insulin absence, as well as inability to efficiently respond to insulin stimulation. Under these circumstances, SC metabolism undergoes important alterations (for reviews[37, 48]). The SC has specific insulin receptors (IRs),[169] and insulin is reported to increase the rate of lactate production in these cells.[170] SCs cultured in insulin-deprived conditions exhibit a modulation in glucose transporters (GLUT1 and GLUT3) to maintain lactate production.[41] Additionally, the first hours of insulin deprivation completely suppress acetate production.[103] The acetate produced by SCs is expected to play a crucial role in the maintenance of high rate of lipid metabolism by germ cells. Therefore, although SCs cultured in insulin-deprived conditions maintain lactate production through modulation of metabolism-associated enzymes and transporters, germ-cell development may still be compromised. These in vitro studies show that insulin may have a more significant role on spermatogenesis of individuals diagnosed with metabolic diseases than previously thought, although these findings require further investigation. There are some in vivo studies also showing evidence that carbohydrate availability is crucial for testicular metabolic functioning and fluids homeostasis. Indeed, we have recently reported that high-energy diets induce a prediabetic state altering testicular glycolytic metabolic profile promoting significant alterations not only in sperm parameters,[171] but also in bicarbonate transport mechanisms in the testis and the epididymis.[172]

Cancer cells are also known to possess an increased IR content, and under hyperinsulinemia conditions (such as those in diabetic individuals), certain tumors have increased activation of IR signaling pathways.[173, 174] It has been reported that insulin sensitizer, metformin, may protect against cancer development since patients with type 2 diabetes taking metformin present a lower risk of developing cancer compared to those not taking metformin.[175] Several authors have discussed the complex relation between metabolic diseases and cancer (for extensive review[176–178]). The use of metformin to control insulin levels avoiding cancer progression has also been discussed (for review[179]), but the local mechanisms responsible for cell arrest or progression in these conditions remain largely unknown. Moreover, it was

recently reported that metformin can modulate SC metabolism and redox state[180] improving the metabolic environment for spermatogenesis. The regulatory role of insulin and carbohydrate metabolism in mature and immature SC of individuals with a metabolic disease may open new insights on the possible mechanisms by which SC controls its proliferative activity under these unconventional circumstances of glucose metabolism and insulin signaling.

10. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

A connection between cancer cell metabolism and cell structure was previously hypothesized by Warburg[1] but, decades later, the complex interlink between metabolism and proliferation remains a hot topic for research. Increased glycolytic flux is undoubtedly a major characteristic of cancer cells, but whether this metabolic shift is a symptom or cause is under debate. However, the Warburg effect is a key alteration in cancer cell energy metabolism, which is associated with malignant transformation. In this review, we discussed the connection between the metabolic characteristics of high proliferative cancer cells and the nonproliferative testicular SC (summarized in Table I). Several issues should be taken into consideration and carefully evaluated. It is clear that metabolic adaptation in a malignant tumor goes far beyond the Warburg effect discussed herein. Although lipid metabolism was not extensively discussed in this review, in the last decade it has been recognized as an important issue in cancer metabolism (for review[155, 181]). Indeed, fatty acids can be used as energy fuel in growth and have a crucial role in the survival of cancer cells, particularly through the control of NADPH levels.[182] Interestingly, SCs can also fulfill their energetic needs by oxidative substrates[118, 183] and it has been reported that only a small fraction of pyruvate produced from glucose enters the Krebs cycle.[184] Instead pyruvate is converted to lactate, which is then exported to germ cells to serve as the energy source.[185, 186] Additionally, it has been reported that phagocytized spermatogenic cells by SCs are used as energy source mostly through β-oxidation, rather than glycolysis.[28] Thus, the role of β-oxidation in both cells types should be carefully evaluated and these studies will certainly reveal important aspects beyond the Warburg effect. Importantly, the role of dedifferentiation of both, cancer cells and SCs, is often overlooked and the mechanisms responsible for those processes may highlight key features of the metabolism of these cells. The acidification of extracellular microenvironment is also an issue that deserves attention in future studies. In cancer cells, it has been proposed that acidification may be responsible for the selection of malignant cells[7] and in SCs, the molecular mechanisms by which the tubular fluid is formed are still scarcely investigated even though the SC is the secretory component of the seminiferous epithelium. Finally, caution must be taken when assuming that all cancer cells display “the same” Warburg effect, since each particular cancer cell has specific needs and thus, some assumptions may be a matter of controversy concerning each type of cancer. When discussing the Warburg effect in the testis, one should take into account that the immature SC is morphologically and biochemically distinct from the fully mature SC.

In the last decade, there has been a growing interest in metabolomics. It is obvious that metabolomics has potential to reveal novel pathways and disclose the metabolic network that establishes the connection between cell proliferation and metabolic needs. Therefore, a growing number of studies are expected in metabolomics of both cancer cells and testicular

cells in the coming decade. This will significantly improve understanding of these cells and reveal possible candidates and/or pathways for future intervention. The increased glycolysis exhibited by cancer cells and SCs has been the starting point for the design of anticancer therapeutic strategies/new anticancer agents and male contraceptive design, respectively. However, the metabolic plasticity in these cells must be taken into consideration to develop metabolism-based therapeutics for cancer and infertility or to develop an effective male contraceptive.

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Biographies

Pedro F. Oliveira was conferred a Biochemistry degree in 1996 at the Biomedical Institute Abel Salazar, University of Oporto (ICBAS-UP). In 2004, he was conferred a PhD degree in Biomedical Sciences. In 2005, he began to work on a new line of research focused on Reproductive Biology, particularly in Sertoli cells physiology, at the Laboratory of Gamete Physiology and Ionic Transport, University of Porto. In 2009, he was hired as Assistant Researcher at the University of Beira Interior (CICS-UBI). Since then, Pedro F. Oliveira has published more than 50 publications, mostly as senior investigator, in leading peer-review journals in the areas of Biology of Reproduction, Urology, Andrology, and Biochemistry.

Several postdoctoral researchers and postgraduated students were awarded with fellowships and have been developing their research projects under his supervision. He is Principal Investigator and team member of several ongoing funded projects. He is also ad-hoc reviewer in several top journals and serves as editorial board member of some journals. His work has been focused on the metabolic cooperation among testicular cells and the hormonal regulation of those processes. His research team was the first to describe that human Sertoli cells exhibit an elevated production of acetate, which is highly susceptible to hormonal modulation, particularly by insulin. His team was also the first to report several alterations associated with prediabetes in testis and Sertoli cells, such as in PGC1-α/SIRT3 axis and mitochondrial bioenergetics. His laboratory is actively studying Sertoli cell metabolism in pathological conditions and how it is related with male subfertility/infertility.

Ana D. Martins obtained her degree in Biomedical Sciences at the University of Beira Interior, Portugal, in 2011. In the 2012, she completed her Master's degree in Biomedical Sciences and was integrated in the Endocrinology and Reproduction research area at Health Sciences Research Center, University of Beira Interior (CICS-UBI), Portugal. In 2012, she was hired as laboratory technician to support the project: "Can Hormonal (De)regulation of Ion Transporters in Human Sertoli Cells be Responsible for Infertility?" at Health Sciences Research Center, University of Beira Interior (CICS-UBI), Portugal, under the supervision of Prof. Pedro F. Oliveira. Currently she is a PhD student in Biomedical Sciences at the Abel Salazar Biomedical Sciences Institute at the University of Porto (ICBAS-UP), Portugal, under the supervision of Dr. Marco G. Alves and Professor Mário Sousa. She has ten publications in the last year (2013) in leading peer-review journals. Her publications are focused on Sertoli cell metabolism, with particular emphasis on the modulation of these cell's metabolic behavior under pathological conditions. Her major interests of research are focused on reproductive biology and testicular metabolism.

Ana C.Moreira obtained her PhD in Biosciences, Toxicology branch at the University of Coimbra in 2013. Her work was essentially focused on the effects of phytoestrogens in mitochondria to study the role of phytoestrogens as an alternative to hormone replacement therapy during menopause. In the last years she has collaborated with Drs. Bjoern Bauer and Anika Hartz from the University of Minnesota in USA to study the role of phytoestrogens in glucose metabolism and transport in the blood–brain barrier. Currently, she is a postdoc at Unit for Multidisciplinary Investigation in Biomedicine (UMIB), University of Porto, under the scientific supervision of Dr. Pedro F. Oliveira and Professor Mário Sousa. Her research interests are focused on hormonal control of Sertoli cell metabolism, particularly the role of ghrelin–leptin axis in nutrient membrane transport through Sertoli cell/blood–testis barrier and its importance in male (in)fertility. Ana C. Moreira is author or co-author of several papers in peer-reviewed journals, including Food and Chemical Toxicology, Mitochondrion, and Clinical Science.

C. Yan Cheng, PhD, is the Head of the Mary M. Wohlford Laboratory for Contraceptive Research at the Population Council's Center for Biomedical Research in New York. His lab uses techniques of protein biochemistry to study the role of Sertoli cell proteins on testicular function. His research interest in recent years has focused on the biology of blood–testis

barrier (BTB) and cell adhesion in the testis, and the development of male contraceptives mostly notably adjudin that exert their effects on cell adhesion in the testis. Adjudin is currently being actively investigated to serve as a male contraceptive, and intensive development is underway. His laboratory has recently identified a local regulatory axis known as the apical ES-BTB-basement membrane that coordinates and regulates cellular events across the seminiferous epithelium during the epithelial cycle of spermatogenesis. More important, a biologically active fragment released from the apical ES during its degeneration via the cleavage of laminin chains by metalloproteinase 2 at spermiation is shown to be a potent peptide to reversibly disrupt BTB function, causing reversible disruption of spermatogenesis, illustrating its potential as a contraceptive peptide. His other line of research focuses on the impact of environmental toxicants on male reproductive health, in particular the mechanism(s) by which toxicants undermine the BTB function, and how this impedes testicular function, causing subfertility and/or infertility in men.

Marco G. Alves obtained his degree in Biology at the University of Aveiro, Portugal, in 2005. In the same year he was hired as laboratory technician and joined the “Metabolic profiling and Toxicology group” and the “Intermediary metabolism group” at the Center for Neuroscience and Cell Biology, University of Coimbra (CNC-UC), Portugal. In 2006, he was awarded a PhD fellowship under the supervision of Prof. Rui A. Carvalho. In 2011, Marco G. Alves obtained his PhD degree in Biochemistry, Bioenergetics branch, at the University of Coimbra, Portugal. Later that year he was awarded a postdoc fellowship and moved to the Health Sciences Research Center, University of Beira Interior (CICS-UBI), Portugal to join the Endocrinology and Reproduction research area. In the last years he has been awarded with several fellowships and prizes and was/is a team member of several ended and ongoing funded projects. He is also ad-hoc reviewer in several top journals and serves as editorial board member of some journals. His main lines of research are focused on reproductive biology, diabetes, metabolism, metabolic modulation, and cellular metabolic profiles. He has more than 40 publications in the last years (2009–2013) in leading peer-review journals including some invited reviews. These publications are mostly focused on Sertoli cell metabolism and modulation of these cells metabolic behavior under pathological conditions.

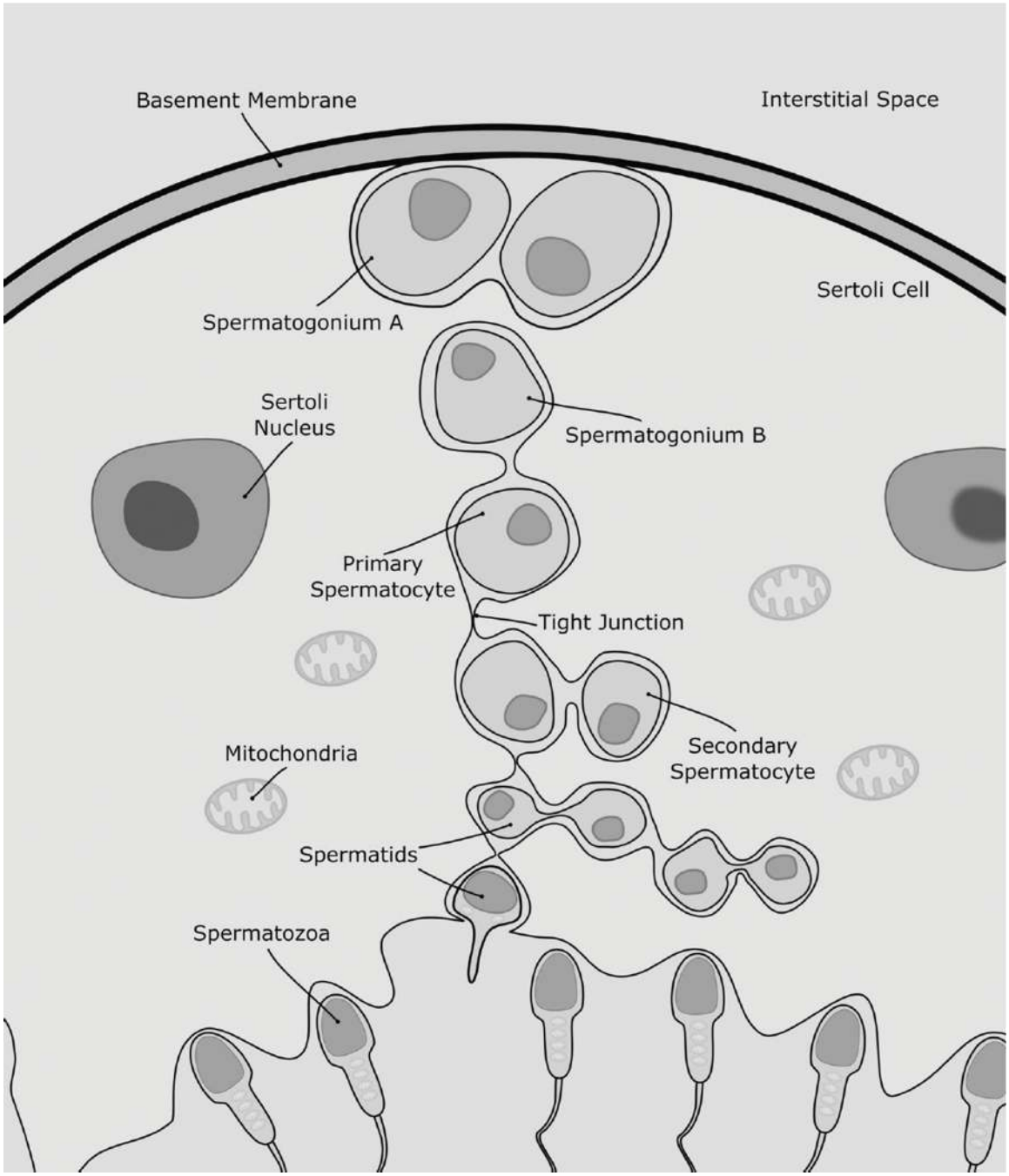

Figure 1.

Schematic representation of the seminiferous tubules. The Sertoli cell (SC) barrier physically divides the seminiferous epithelium into basal and apical (adluminal) compartment. Tight junctions are established between adjacent SCs forming the Sertoli/blood–testis barrier. The germ cells form intimate associations with SCs and a single SC is in contact with multiple germ cells. Fully differentiated SCs ensure physical and nutritional support for germ cells. The SCs control the entry of substances to the intratubular fluid and are responsible for the secretion of several essential factors for germ-cell development. SC is

responsible for the microenvironment where germ cells develop into fully mature spermatozoa.

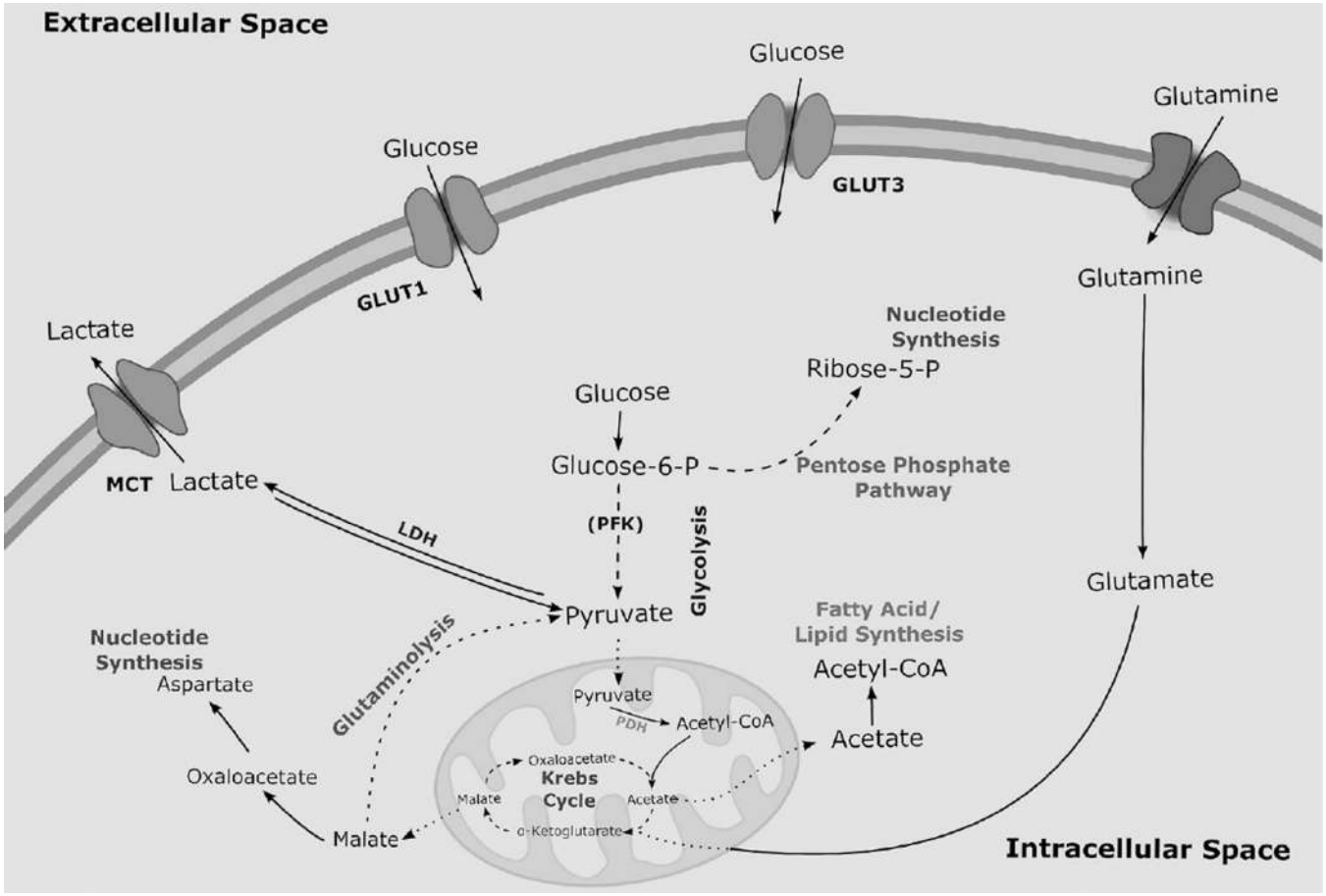

Figure 2.

Summary of the most important metabolic mechanisms proposed to occur in both cancer and Sertoli cells (SCs). The high-affinity glucose transporters (GLUT1 and GLUT3) are present in the plasma membrane and are responsible for adaptive mechanisms under unfavorable conditions of glucose/insulin deregulation. In normal conditions these cells metabolize glucose into pyruvate in a multistep reaction where phosphofructokinase (PFK) is known to play a key role. Pyruvate is converted into lactate, by lactate dehydrogenase (LDH), that is then exported through the action of specific monocarboxylate transporters (MCTs). The Krebs cycle is known to be repressed in these cells but, under specific circumstances, several intermediates are known to be essential to maintain lactate production and/or the proliferative/nonproliferative activity of the cells. Glutaminolysis is represented, as it is a well-known source of pyruvate and is associated with tumorigenesis of cancer cells, as well as SC normal functioning. The action of pyruvate dehydrogenase (PDH) in acetyl-CoA formation is also highlighted since fatty acids and lipid synthesis occur at high rates in SC and cancer cells. The pentose phosphate pathway is also represented since it is stimulated by high glycolytic fluxes and both SCs and cancer cells present a high capacity for biosynthesis of nucleotides.

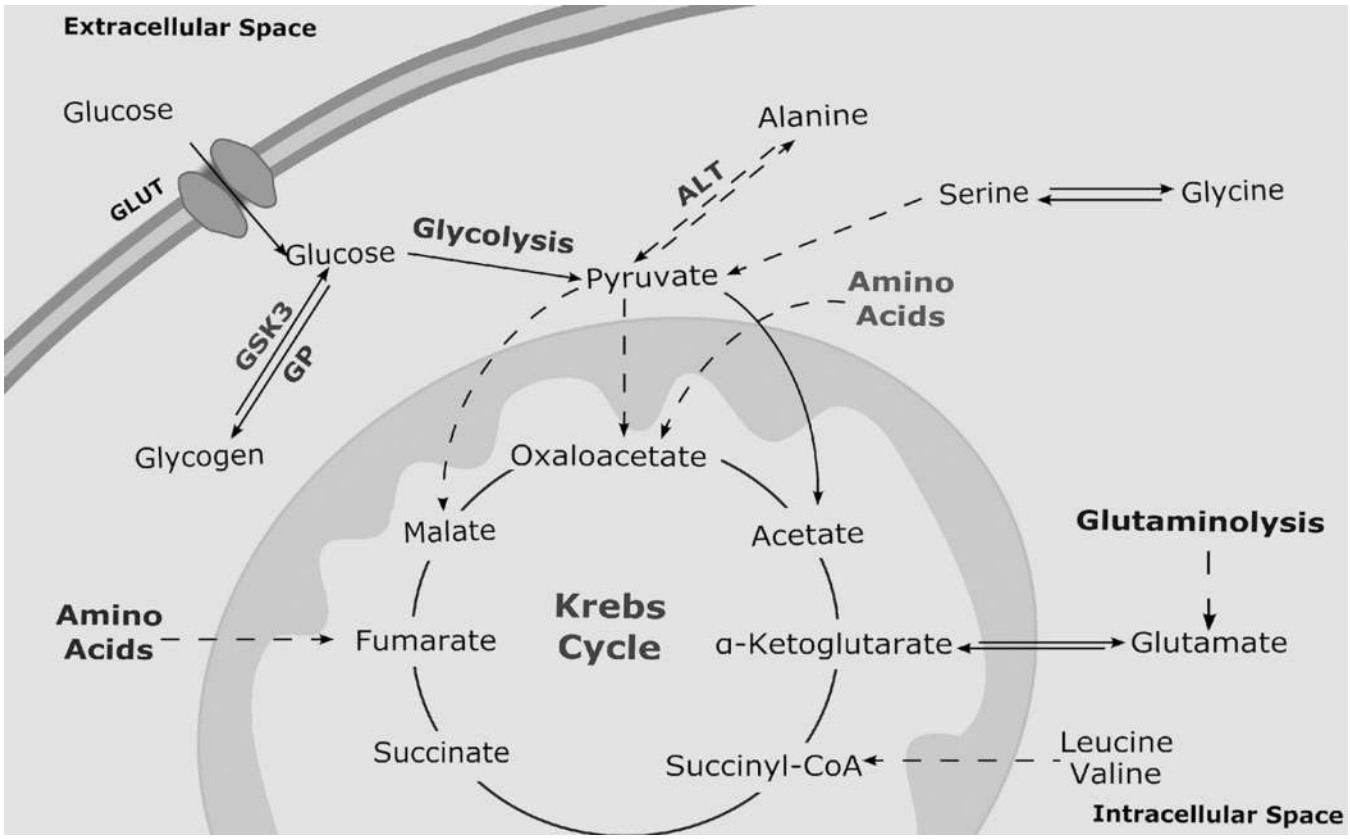

Figure 3.

Summary of alternative fuels for Sertoli and cancer cells. In several conditions, these cells can use alternative fuels, rather than glucose, to maintain energy and biomass production. Glycogen metabolism is mainly controlled by glycogen synthase kinase-3 (GSK3) and glycogen phosphorylase (GP) activities, and may be essential to overall metabolism of these cells. Glycogenolysis- and glycolysis-derived pyruvate can enter the cycle at multiple points such as malate, oxaloacetate or acetate, it thus plays a crucial role. On the other hand, glycine and serine can also stimulate pyruvate production. Interestingly, the pathways that replenish the Krebs cycle intermediates, also known as anapleurotic pathways, are somewhat ignored in metabolic studies with these cells. Nevertheless, under certain conditions, several Krebs cycle intermediates are also used by these cells. For instance, glutaminolysis-derived α-ketoglutarate and succinyl-coA derived from leucine and valine are of extreme importance. Moreover, several amino acids can replenish the Krebs cycle at fumarate and oxaloacetate. Thus, the role of alternative substrates, rather than glucose, should deserve special attention since it may unveil important resemblances and differences between proliferating and nonproliferating lactate-producing cells.

Table I

Sertoli cell as a Tool to investigate cancer cell metabolism and physiology

Physiological characteristics	Sertoli cell	Cancer cell	References
Proliferation rates (in vivo)	↓	↑	55, 60, 65, 77, 79, 94
Cell duplication time (in vitro)	↑	↑	15, 52, 53, 55
Motility behavior	↓	↑	15, 52, 53, 55
Glycolytic flux	↑	↑	7, 13, 27, 46, 47, 101, 126
ATP/ADP ratio	↑	↑	140, 141
NADH/NAD^+ ratio	↑	↑	42, 43, 140, 141
p53 glycolytic flux regulation	+	+	43, 140, 145, 150

↑,high; ↓, low; +, feature characteristic.