THE DARK SIDE OF GLUCOSE TRANSPORTERS IN PROSTATE CANCER: ARE THEY A NEW FEATURE TO CHARACTERIZE CARCINOMAS?

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SHORT TITLE: THE ROLE OF GLUT TRANSPORTERS IN PROSTATE CANCER

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ABBREVIATIONS: 2DG: 2-deoxyglucose; AR: Androgen Receptor; AMPK: AMP-activated protein kinase; ATM: Ataxia telangiectasia mutated; CaMKKβ: Calcium/calmodulin-dependent protein kinase kinase beta; CRPC: Castration Resistant Prostate Cancer; DHT: Dihydrotestosterone; FDG: Fluro-D-glucose; G6PDH: Glucose-6-phosphate dehydrogenase; GLUT: Facilitative glucose transporter; GSTP1:Glutathione-S-transferase pi 1; HGPIN: High-grade prostatic intraepithelial prostate; HIF: Hypoxia-inducible factor; HK: Hexokinase; HMIT: H^+/myo-inositol transporter; IGF: Insulin-like growth factor; IGFR: Insulin-like Growth Factor Receptor; IR: Insulin Receptor; LKB1: Liver kinase B1; OXPHOS: Oxidative phosphorylation; PCa: Prostate Cancer; PET: Positron Emission Tomography; PFK: 6-phosphofructo-2-kinase; PFKFB2: 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2; PGC1α: Proliferator-activated receptor gamma coactivator 1-alpha; PPP: Pentose Phosphate Pathway; PSA: Prostate Specific Antigen; SGLT: Sodium/glucose transporters; SHBG: Sex-Hormone Binding Globuline; SLC: Solute Carrier; SRC2: Steroid Receptor Coactivator 2; T2DM; Type 2 Diabetes Mellitus; TCA: Tricarboxylic acid; TP53: Tumor Protein p53; TRAMP: Transgenic Adenocarcinoma of Mouse Prostate; TXNIP: Thioredoxin-interacting protein; ZIP1: Zinc-regulated transporter/iron-regulated transporter-like protein 1.

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ABSTRACT

One of the hallmarks of cancer cells is the increased ability to acquire nutrients, particularly glucose and glutamine. Proliferating cells need precursors for cell growth and NADPH reducing equivalents for survival. The principal responsible for glucose uptake is facilitative glucose transporters (GLUTs), which usually are overexpressed in cancer cells. Besides their role in glucose uptake, GLUT transporters are able to transport other compounds such as dehydroascorbic acid or uric acid. They play a major role in tumor progression and cellular processes such as regulated cell death. The prostate gland has the particular characteristic of being more glycolytic than other non-pathological tissues given an accumulation of citrate in the seminal fluid and the inhibition of m-aconitase that affects to Tricarboxylic Acid Cycle. In prostate cancer (PCa), androgens increase glucose uptake, upregulate GLUT transporters such as GLUT1 and GLUT3 and stimulate AMPK pathway, suggesting a possible connection between glycolytic and androgenic signaling. Interestingly, diabetes is not a risk factor of PCa, as it is in other cancers, while insulin stimulates progression and IGF1 pathway plays an important role in PCa progression. It was recently found that PCa cells overexpress GLUT4 and, more importantly, that it seems to be related to the castration-resistant phenotype, though little is known about its participation in tumor progression. This review will focus on the role of GLUT transporters along with PCa progression, and the involvement of GLUT4 on castration-resistant phenotype transition would be considered.

INTRODUCTION

In the 20's, Otto Warburg described a phenomenon in tumors that it was called the "Warburg effect". This discovery took place even before ATP was discovered or glycolysis was formulated[1]. It is based on the enhancement of lactate production because of the increment of glycolysis independently of oxygen concentration[2]. Although Warburg related this effect with defects in mitochondria of tumor cells, later studies showed that mitochondria were not altered in tumor tissues and the phenomenon was related to proliferation and growth[3].

The interest in cancer metabolism has increased during the last few years and not only for its self-importance but also for its connection with other signaling pathways[4]. Before being considered a hallmark of cancer in 2011 by Hanahan and Weinberg[5], Kroemer and Poyssegeur suggested that all hallmarks of cancer have somehow relation with metabolism[6]. Recently, six hallmarks of cancer metabolism have been proposed by Pavlova and Thompson in 2016[7]. Metabolic alterations of cancer cells include an increased ability to acquire nutrients, assigned preferred metabolic pathways and alteration of differentiation pathways.

The increase of glycolysis carries a higher glucose uptake by glucose transporters (GLUT)[8]. Therefore, GLUT levels are usually related to tumor progression. Although most cancers drive with the traditional Warburg effect, there are differences among them, including those concerning GLUT transporters. The prostate is metabolically unique since the differentiated tissue is glycolytic instead of oxidative. Prostate cancer (PCa) transformation involves a metabolic switch to oxidative phosphorylation (OXPHOS) then, later, in the advanced castration-resistant phenotype turns again to glycolytic[9,10].

PROSTATE CANCER: AN HORMONE-SENSITIVE CANCER WITHOUT A DIAGNOSTIC BIOMARKER

PCa is the most common malignancy among men and the second leading cause of cancer death[11]. In 2012, more than 1.1 million cases were recorded (data are taken from GLOBOCAN, the most recent statistics from WHO)[12]. This means an 8% of total new cancer cases and a 15% of all affecting in men. Its prevalence is higher in the west (about 68% of new cases) perhaps due to lifestyle and environmental factors. However, age and race are also well-recognized risk factors[13]. Despite high incidence, PCa is usually characterized by a slow growth and unpredictable outcome. PCa is a disease with a mixed origin, and the absence of a biomarker impedes to know how to anticipate the outcome of the disease.

Still, Prostate Specific Antigen (PSA) screening is the only predictive method employed in the clinic. However, its levels are dependent, among others, of obesity or age, being not always reliable[14]. A potential alternative is Prostate Cancer Antigen 3 (PCA3), overexpressed in primary PCa and metastases[15].

PCa is sensitive to hormones, mainly androgens. The active form of testosterone, dihydrotestosterone (DHT), mediates androgen receptor (AR) classical activation. Androgen responsive genes are involved in normal prostate architecture, homeostasis, and physiology but, in PCa, androgens promote proliferation and survival of cancer cells. Antihormonal therapy is, at first, a successful therapeutic approach[16]. However, PCa frequently becomes resistant to androgen deprivation, reaching a castration-resistant (CRPC) phenotype difficult to handle.

Contrary to other cancers, there is not a single pathway implicated in PCa progression nor a clear candidate as a biomarker. The homeobox gene *HOXB13* was

found as a predisposition gene[17]. Also, seventy-seven single nucleotide polymorphisms close to a noncoding region of the oncogene *c-MYC* and with the capacity to alter its expression, are also considered a prognosis marker[14]. In the 90% of PCa, Glutathione-S-transferase pi 1 gene (*GSTP1*) is hypermethylated, and it can be detected in urine[18].

More than half of the cases of PCa drive with androgen-driven ETS gene expression because of genomic rearrangements. However, ETS gene fusions need other events such as activation of PI3K/AKT pathway, which is mainly related to PTEN loss. Then, ETS-positive tumors are different from ETS-negative tumors, but *PTEN* and *TP53* mutation occurs in both types[14].

In CRPC, there are additional oncogenic pathways involved. The RAS/MAPK pathway and TGFβ3 are upregulated in patients with metastatic CRPC. In addition to AR signaling, the WNT/β-catenin and the Insulin-like growth factor (IGF) 1 pathways seem to play a major role in the most aggressive phenotype of CRPC[19].

Still, there is not a clear biomarker, as there is neither an effective treatment. Anti-hormonal therapies employed when cancer is still androgen-sensitive, and they go together with radiotherapy in localized or locally advanced carcinoma[16]. For CRPCs, docetaxel has been the preferred and the first-option chemotherapy during the last decade, but recent-discovered compounds have also been proposed as succesful[20].

Then, PCa is still a tumor without an effective prognostic and predictive biomarker and a curative treatment. However, targeting glucose metabolism has the potential to provide prognostic information and to treat PCa, since several glycolytic pathways are altered in the disease.

THE CURIOUS CASE OF GLYCOLYTIC METABOLISM IN PROSTATE

In differentiated cells, glucose in the presence of oxygen is predominantly employed to get their energetic requirements from OXPHOS in mitochondria. However, under hypoxia, lactic glycolysis is favored rendering only two ATP molecules from one molecule of glucose, instead of the 36 molecules obtained by OXPHOS[21]. In cancer cells, and in other proliferative cells, the rates of glycolysis and lactate production are enhanced. Even in the presence of oxygen, cancer cells select for glycolysis instead of OXPHOS to metabolize glucose in a process called "aerobic glycolysis"[3].

Aerobic glycolysis favors glycolytic pathways to produce the building blocks necessary to cell growth. Several oncogenic signaling pathways promote aerobic glycolysis and the increase of lactate secretion[22].

PCa, like other tumors, progresses with molecular alterations that cause an increase in glucose, glutamine and lipid metabolism. However, PCa is characterized by a particular metabolism of glucose that differs from the rest of carcinomas.

The prostatic fluid contains high levels of citrate because of the inhibition of m-aconitase, a tricarboxylic acid (TCA) cycle enzyme that converts citrate to isocitrate. This inhibition is driven by the overexpression of the zinc-regulated transporter/iron-regulated transporter-like protein 1 (ZIP1) in prostatic epithelial cells. Since the TCA cycle is somehow compromised in the prostate gland, glycolysis is favored (Fig 1). During tumor transformation, ZIP1 levels decrease, and OXPHOS is promoted[9]. However, this may only happen during the first steps of carcinogenesis. One study shows that the mitochondrial content does not change during carcinogenesis, but OXPHOS decreases with invasiveness[23].

Lactate production is usually associated with tumor progression in PCa. In addition to its catabolic products, pyruvate, and alanine, lactate measurement has been considered urine biomarker for non-invasive detection of PCa[24]. On the other hand, factors related to reverse Warburg effect were recently proposed as a marker to distinguish Gleason grades[25]. PCa cells employ interleukin-6 secretion to activate glycolysis in cancer associated fibroblasts, which, in turn, increase lactate secretion[26]. Lactate is consumed by OXPHOS-dependent PCa cells, having a role in redox homeostasis and angiogenesis[27].

Glucose metabolism has not been considered as important as glutamine or lipid metabolism in PCa progression, being the reason why it has been less studied. Several candidates have been proposed as potential metabolic targets. Multiple studies are underway employing inhibitors of lipogenesis, cholesterol metabolism, and glutamine metabolism[28]. Recently, the upregulation of the steroid receptor coactivator 2 (SRC2), which drives glutamine-dependent de novo lipogenesis, was proposed as an important co-regulator for PCa survival and metastases[29], and Sarcosine, an N-methyl derivative of glycine, is considered an important regulator of progression and metastases[30].

However, glucose metabolism in PCa is different to other tumors given their close relation to AR signaling. Glycolysis differs between androgen-sensitive and insensitive cells, being tumors more aggressive more glucose-dependent[31,32]. Also, AR regulates several genes that are closely related to glucose consumption and biomass production[33]. Thus, an increased activity of several glycolytic enzymes by androgens has been found. Hexokinase-2 (HKII) phosphorylation is stimulated by androgens via protein kinase A signaling while 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 (PFKFB2) is stimulated by direct binding of AR to *PFKFB2* promoter. Activation of PFKFB2 causes a constitutive activation of 6-phosphofructo-2-kinase (PFK2), which is

involved in the second irreversible reaction of the glycolytic pathway[34]. Another isoform of PFK2, PFKFB4, was considered an important regulator for PCa survival[35]. HKII is also involved in the increase of glucose metabolism after androgen deprivation in PTEN/Tumor protein p53 (TP53) deficient PCa cells[36]. Thus, HK inhibitors such as 3-bromopyruvate or ionidamine are being tested in clinical trials[37]. Moreover, Pentose Phosphate Pathway (PPP) is promoted by an AR-mTOR mediated mechanism, maintaining Glucose-6-phosphate dehydrogenase (G6PDH) levels higher during PCa progression[38]. Overall, androgen signaling stimulates both glycolysis and anabolic metabolism

Androgens positively regulate glycolysis via Calcium/calmodulin-dependent protein kinase kinase beta (CamKKβ), which activates AMP-activated protein kinase (AMPK)[39]. AMPK is a metabolic regulator that promotes migration, cell growth and survival of PCa cells[40,41]. Because of AMPK activation, androgens activates peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), which is connected with mitochondrial biogenesis (Fig 2). Although PGC1α is overexpressed in patient samples, it was proposed as an antimetastatic factor[42]. Since PGC1α favors an oxidative metabolism, its loss could be related to the acquisition of a glycolytic and more aggressive phenotype[43].

Interestingly, high blood glucose drives with low levels of AR[44]. Thus, this might explain the surprising inverse relation between diabetes and PCa that will be discussed below.

ARE GLUT SUFFICIENT TO SUPPORT PROSTATE CANCER GROWTH AND SURVIVAL?

Since cancer cells show a high demand for nutrients for cell growth, the uptake has to be higher. In non-pathological tissues, cells become quiescent when the resources are scarce, but cancer cells lose this control, being always addicted to nutrients. Principal nutrients are glucose and glutamine[3].

There are two different types of transporters for glucose: Na^+/glucose transporters (SGLTs, *SLC5A*) and facilitative glucose transporters (GLUTs, *SCL2A*). Among the 12 members of SGLTs, only SGLT1 and SGLT2 are proposed as responsible for glucose uptake in some cancer cells[45].

GLUT transporters internalize glucose by a mechanism of facilitated diffusion. There are 14 members (GLUT1-12, GLUT14 and H^+/myo-inositol transporter –HMIT-). They transport other compounds in addition to glucose, and that circumstance establishes the differences between members. They also differ in their affinity for substrate and tissue location. They are divided into three classes: Class I includes GLUT1-4 and GLUT14, class II are GLUT5, GLUT7, GLUT9 and GLUT11 and class III consist of GLUT6, GLUT8, GLUT10, GLUT12, and HMIT. Class I is the best characterized, and its members share the same bacterial ancestor –XylE-[46]. Class II is able to transport fructose and, class III is structurally different. They share common elements as 12 transmembrane α-helixes while the cytoplasmic N-terminus and C-terminus are less conserved among members[47].

GLUT transporters respond to metabolic and hormonal regulation, and several transcription factors are able to increase glucose uptake by overexpressing or locating GLUTs on the cell membrane. Furthermore, under hypoxia or nutrient deprivation, tumor cells overexpress at least one of the isoforms, being predominantly GLUT1[48].

GLUT1 is overexpressed under growth factor withdrawal, which makes cancer cells more resistant to apoptosis[49].

GLUT transporters are regulated by glycosylation or phosphorylation. It was well studied that GLUT transporters are N-glycosylated, which is associated with its ability to increase glucose uptake[50,51]. Phosphorylation was recently described, opening a new paradigm of GLUT regulation. It was shown a phosphorylation site at serine 490 for ataxia telangiectasia mutated (ATM) that promotes surface GLUT1[52] and at serine 226 for protein kinase C which is related with GLUT1 deficiency syndrome[53].

The important role of GLUT transporters is also linked to the uptake of other compounds than glucose. The best well known is dehydroascorbic acid[54], the oxidized form of vitamin C, and recently our group opens the possibility that melatonin might also enter into the cell via GLUT transporters[55]. The affinity for the different substrates can be dependent on the interaction of other transmembrane proteins[56,57].

GLUT1 is usually associated with poor prognosis in tumors[58]. However, not always this transporter is found overexpressed in cancer, and other GLUTs are instead involved in increasing glucose uptake[8]. GLUT-dependent glucose uptake has an important role in diagnosis by positron emission tomography (PET) imaging. The uptake of 2-deoxy-2-[^{18}F]-fluoro-D-glucose (^{18}F-FDG) is employed in PET to follow glucose uptake. This compound is phosphorylated by hexokinase (^{18}F-FDG-6P), but it cannot continue the glycolytic pathway, being accumulated into the cytoplasm. This methodology is valid as a diagnostic tool particularly when cancer drives with the classical Warburg effect[59].

GLUT TRANSPORTERS AS CLINICAL TARGETS IN PROSTATE CANCER

In diagnosis, classic ^{18}F-FDG-PET has not been considered useful in primary PCa tumors, so the increased glycolysis and GLUT overexpression have not been recognized as relevant as in other tumors. However, now it is assumed that its utility is dependent on the stage of the disease. Also, the high activity by urine in the adjacent urine bladder overlaps the signal in prostate[60]. Thus, the assumption that ^{18}F-FDG-PET is not valid because PCa is not a glycolytic carcinoma should be discarded.

In PCa, it seems that exists a possible balance among GLUT transporters, being the majority-produced transporter dependent of the step of the disease (Table 1). GLUT1 is found overexpressed in PCa cells, being the highest levels found in androgen-independent cells[32]. Since GLUT1 is usually the transporter overexpressed in tumors, it has been the most studied in PCa. Although higher GLUT1 levels were also found in non-tumor tissues[61], it seems that GLUT1 is related to aggressiveness because it is usually overexpressed in poorly differentiated tumours[62]. Also, GLUT1 is connected with recurrence after radical prostatectomy[63]. This overexpression seems to be dependent of hypoxia more than androgenic regulation. Also, the stromal levels of GLUT1 have been employed to classified PCa by Gleason score and to indicate the presence of a tumor area undetected by biopsia[25]. Furthermore, GLUT1 is involved in the increase of glucose uptake by inflammatory cells in PCa[64] and it was recently described that GLUT1 overexpression in PCa might be mediated by the reduced levels of microRNA-132[65]. Regarding its intracellular localization, GLUT1 was also found in Golgi Apparatus where it could have a role in supplying glucose to the prostatic fluid[66].

On the other hand, GLUT3 is activated via caveolin-1 in AR-negative cells[67] and GLUT12, which is also considered an insulin-dependent transporter, was found in PCa but not in non-tumor samples[66], suggesting a similar role than other insulin dependent transporters as GLUT4 in tumorigenesis.

Fructose consumption in the modern diet is increasing, and it is considered a cancer risk factor. In tumor cells, fructose is differently metabolized than glucose, being mainly employed for nucleic acid synthesis[68]. However, in PCa, fructose was not considered as a risk of metastasis[69]. Fructose is secreted in seminal vesicle, so high concentration is found in the dorsal prostate and coagulating glands (rodent anatomy)[70]. GLUT5, the main fructose transporter in addition to GLUT2, is produced in the apical membrane of secretory cells in normal tissue and high-grade intraepithelial neoplasia (HGPIN). Furthermore, *SLC2A7*, *SLC2A9*, and *SCL2A11* mRNAs were found in PCa but only *SCL2A11* mRNA levels increased in PCa tissue respect benign prostate[61]. Altogether, fructose uptake might have a role in PCa progression, particularly at early stages.

In PCa cells, androgen stimulation increases both GLUT1 and GLUT3, increasing glucose uptake and the secretion of lactate[71]. However, these transporters, particularly GLUT1, are downregulated by DHT in non-tumor Sertoli cells[72]. Thus, hormonal regulation of GLUT1/3 seems to be tissue-dependent.

Interestingly, androgens and antiandrogens are able to interact with GLUT1 at the external opening since GLUT1 and the ligand-binding domain of AR share sequence homologies[73], establishing the idea that regulation is not only via signaling pathway. Other members such as the fructose transporter GLUT5 also seems to be under androgenic regulation. Using the antiandrogenic flutamide in *Scotophilus healthy*, GLUT5 production is reduced in testis[74].

As previously discussed, androgen signaling activates AMPK. As a metabolic regulator, AMPK regulates GLUT transporters[75]. Activated AMPK inhibits activation of thioredoxin-interacting protein (TXNIP), which binds to GLUT1 avoiding its

289 expression and translocation (Fig 2)[76]. Consequently, the insulin-independent GLUT1,
290 and consequently glucose uptake, is also hormonal regulated.

291 Besides AMPK, PI3K/AKT/mTOR pathway has a major role in glucose
292 metabolism through its activation by insulin and IGF1. AKT1 stimulates glycolysis by
293 an increase in both the expression and translocation of GLUT transporters in addition to
294 the phosphorylation of glycolytic enzymes such as hexokinase and
295 phosphofructokinase[8,77]. On the other hand, PTEN, the inhibitor of PI3K/AKT pathway,
296 is able to reduce SLC2A1 expression directly[78].

297 Hypoxia-inducible factors HIF1 and HIF2 are involved in the cellular response
298 to low oxygen concentration in PCa[79]. HIF1 promotes the transcription of the majority
299 of glycolytic enzymes and GLUT transporters, mainly GLUT1 and GLUT3[8].

300 *KRAS* is frequently mutated in PCa, and its rearrangements were mainly
301 involved in metastases[80,81]. Tumors with mutations in this gene drive with an increasing
302 rate of glycolysis and higher use of glycolytic products in other anabolic pathways[82].
303 Mutation of *KRAS* and *BRAF* are related with GLUT1 overexpression in cancer[83].

304 TP53, known as "genome guardian" has a dual role in glucose metabolism. On
305 the one hand, it activates HKII expression but, on the other hand, it inhibits glycolysis
306 by the overexpression of TP53-inducible glycolysis and apoptosis regulator TIGAR[84].
307 TP53 reduces *SLC2A1* and *SLC2A4* transcription[85], and it interacts with *SLC2A12*[86]. On
308 the other hand, the loss of TP53 upregulates GLUT3[87]. *P53* mutations are usually found
309 in PCa and as a consequence, a higher expression of GLUT transporters was
310 described[88].

311 Since the inhibition of glycolysis by 2DG (2-deoxyglucose) causes an
312 activation of autophagy in patients with CRPC, its use in the clinic has to be considered

in combination with autophagy inhibitors[89,90]. Then, targeting glucose metabolism could be an option to treat PCa. However, consulting the current clinical trials in prostate (data are taken from www.clinicaltrials.gov), only 1% has a direct association with glucose metabolism, being one directly related with GLUT transporters (Fig 3).

Nevertheless, most studies are focused on inhibiting glucose uptake by blocking GLUT transporters. Currently, the recent crystallization of GLUT transporters, the better knowledge of the mechanism of inhibition and the development of GLUT-specific inhibitors open a new approach for the treatment of cancer that develops with increasing glucose uptake. Glucose deprivation kills cancer cells by different mechanisms[91]. The consequences of blocking glucose transporters, besides the downregulation of glycolysis, are the inhibition of cell growth, cell cycle arrest and FAS-induced cell death[92,93]. In fact, fasentin, a compound that inhibits GLUT1, increases apoptosis by sensitizing cells to FAS-ligand death receptor signaling in PCa cells[94].

Although there are not clinical trials focused on specific compounds that block GLUTs in PCa, some compounds that inhibit progression are well-known blockers of GLUT transport, particularly flavonoids. They play an important role in PCa prevention since their phytoestrogen activity and have a promising application as adjuvant treatment[95].

THE INSULIN-DEPENDENT GLUT4 TRANSPORTER IN PROSTATE CANCER: A LINK BETWEEN DIABETES AND PROSTATE CANCER?

Although the insulin-dependent glucose transporter GLUT4 has not been considered as important as GLUT1 in cancer, recent studies show the critical role that might play in several types of tumors. This transporter was described by our group in

PCa culture cells[96] for the first time. We found that phytoestrogens regulate GLUT1[96] in androgen sensitive LNCaP cells GLUT4 in androgen-insensitive PCa cells while showing a possible balance between both transporters dependent on the phenotype of the cells. We have found an increase production of GLUT4 by androgen-insensitive cells (unpublished data). In fact, GLUT4 has been already detected in the prostate of Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice[97]. However, in this model, the relevance of GLUT4 in tumor progression is under investigation.

It has been described that testosterone stimulates GLUT4-dependent glucose uptake in human skeletal muscle cells, cardiomyocytes, and 3T3-L1 adipocytes independently of AR signaling[98–101]. In adipocytes, it was confirmed that this regulation occurs through Liver kinase B1 (LKB1)/AMPK signaling. As previously described, AMPK phosphorylates the Rab-GTPase TBC1D1, which triggers GLUT4 externalization[102]. On the other hand, endometrial GLUT4 levels decrease after DHT treatment, and they are inversely related to AR expression in polycystic ovary syndrome[103].

Since GLUT4 is regulated by androgen-independent mechanisms in other tissues, its regulation in PCa might not be dependent on androgens or AMPK signaling. On the other hand, IGF1/insulin pathway regulates insulin-dependent transporter, which suggests its possible role in the regulation of GLUT4 in PCa. In this sense, it implies that this transporter could be more relevant in androgen-insensitive phenotype as our group previously suggested. In addition, GLUT4 might be an intermediate in the effect of insulin on PCa and might have some role in the inverse relationship between diabetes and PCa.

PCa risk is related to lifestyle being high-fat diet connected to its progression and aggressiveness. Hyperinsulinemia, which is usually associated with insulin resistance, is also related with a higher PCa risk[104–106]. Interestedly, insulin levels are higher in PCa patients[107], and insulin receptors (IR) have been found in PCa epithelial cells[108]. Moreover, epidemiological studies show a significantly decreased risk of PCa in long-standing type 2 diabetes (T2DM)[109]. However, it has also been reported that diabetic men have a more aggressive PCa but their PSA levels remain low, avoiding its early detection[110].

Circulating insulin and testosterone levels are correlated in male[111]. On the one hand, higher insulin levels decrease the production of sex-hormone binding globulin (SHBG), increasing free and biologically active testosterone[112,113]. Moreover, the sex hormone promotes insulin production in beta-cells by the extranuclear activity of AR[114] (Fig 4A). Since this positive feedback, insulin signaling would be related to androgen promotion of tumor growth. However, the IGF1 pathway is usually overexpressed in PCa and, this pathway promotes AR hormone-independent activation which supports a role of insulin-dependent glucose transporters, as GLUT4, in androgen-independent tumor growth (Fig 4B).

Under hyperinsulinemia, T2DM is usually treated using metformin, which activates AMPK in the liver[115]. Most of the current clinical trials connecting glucose metabolism and PCa are focusing on the treatment with metformin in androgen deprivation therapy. In several cancers, metformin itself is being considered a potential treatment. Since metformin inhibits the mitochondrial complex I, it was reported a metabolic switch towards glutamine metabolism in PCa[116]. However, it was shown that metformin induces apoptosis in the presence of other compounds such as the

antiglycolytic 2DG[117] or the anti-androgen bicalutamide[118], and it is particularly effective in CRPC[119]. Interestingly, metformin also inhibits androgen-induced IGF1 receptor (IGF1R) overexpression[120] so that GLUT4 transporter might be altered by this treatment.

CONCLUDING REMARKS

After almost 100 years, the metabolic switch to aerobic glycolysis is still under study in oncology. This change is accepted in most of the cancers, but PCa has been considered particular from the metabolic point of view because the role of glucose metabolism in the first steps of progression was less important. Now, several studies are showing that glucose metabolism plays an important role also in prostate carcinogenesis but in a different way than in the rest of cancers. Androgens, despite increasing glycolysis, are involved in anabolism promoting PPP and mitochondrial biogenesis. Thus, androgen dependent tumors are oxidative at first, shifting to glycolysis only in the latest stages of the disease. Then, contrary to other tumors, glucose transporters, and in particular GLUT1, are only overexpressed in the most aggressive tumors that usually drive with hypoxia and with a higher glycolytic activity (Fig 5). Since insulin signaling is related to PCa progression, it seems possible that insulin-dependent glucose transporters might play a relevant role in the progression of the disease. A role of insulin in PCa cancer is suggested by clinical observation since diabetes is inversely related to PCa and some clinical trials are ongoing using the antidiabetic metformin. This could be a good reason to look into the possibility of insulin-dependent glucose transporters act as principal players in those stages of the disease that do not depend on androgens. Glucose transporters have not been considered relevant in prostate cancer progression because glucose metabolism is different in prostate gland than in other differentiate tissues. However, the importance of these transporters has been recently

409 considered since the relevance of increasing nutrients uptake including glucose is
410 clearly demonstrated in prostate cancer. From now on, the employment of components
411 of glucose metabolism, including metabolites, enzymes or transporters to characterize
412 carcinomas will be an area of interest that should be exploited and carefully considered.

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Table 1: Expression of GLUT transporters in non-malignant and tumor prostate

Glucose transporter	Expression in prostate	Location	References
GLUT1	Non-malignant prostate/Aggressive tumors	Plasma membrane, cytoplasm, Golgi system. Secretory and luminal epithelial cells/Basal cells	61,62,63,66
GLUT3	CRPC		7
GLUT4	PCa	Plasma membrane, cytoplasm	97
GLUT5	Non-malignant prostate/ Overexpression in HGPIN	Plasma membrane (apical zone of epithelial cells)	61
GLUT7	Non-malignant prostate/ Overexpression in PCa (mRNA levels)		61
GLUT9	Non-malignant prostate/PCa without overexpression (mRNA levels)		61
GLUT11	Non-malignant prostate/PCa with overexpression (mRNA levels)		61
GLUT12	PCa	Plasma membrane, cytoplasm	66

FIGURE LEGENDS

Figure 1: Zn accumulation in the mitochondria of non-malignant prostatic epithelial cells. High Zn uptake in prostatic epithelial cells is due to the overexpression of ZIP1. Zinc is accumulated in mitochondria where it inhibits aconitase, TCA-cycle enzyme that converts citrate to isocitrate. Citrate excess is secreted into the prostatic fluid.

Figure 2: Androgen regulation of AMPK in prostate cancer cells. Androgen stimulation activates CAMKKβ, which phosphorylates AMPK. AMPK is responsible for promoting glucose uptake via GLUT1, glycolysis, and mitochondrial biogenesis in PCa cells.

Figure 3: Clinical trials related to glucose metabolism in prostate cancer. (A) Number of clinical trials focused on metabolism and glucose metabolism in cancer and, particularly, in prostate cancer. (B) Number of current clinical trials (recruiting and active) focused on metabolism and glucose metabolism in cancer and, particularly, in prostate cancer.

Figure 4: Possible molecular pathways regulated by insulin in prostate cancer. (A) Positive feedback regulation between insulin and testosterone. Since insulin reduces SHBG synthesis in the liver, circulating active testosterone levels increase and stimulates insulin release by beta-cells in pancreas via extranuclear AR activity (B) Insulin stimulates testosterone production, which may activate AR signaling, and activates PI3K/AKT pathway by itself or through IGF pathway. This last activation might lead GLUT4 regulation in PCa cells. SHBG = Sex-hormone binding globulin. Test =Testosterone.

Figure 5: Tumor progression in prostate and GLUT expression along it. The non-pathological prostate is characterized by high glycolytic activity and GLUT1 expression because of impairment in TCA cycle. In the first stages of tumorigenesis, OXPHOS is favored, and circulating insulin levels increase in comparison with the non-tumorogenic prostate. This may be connected with the expression of the insulin-dependent transporter GLUT4. In poorly differentiated PCa tumors, glycolysis and GLUT1 overexpression are again promoted, being correlated with low oxygen levels.

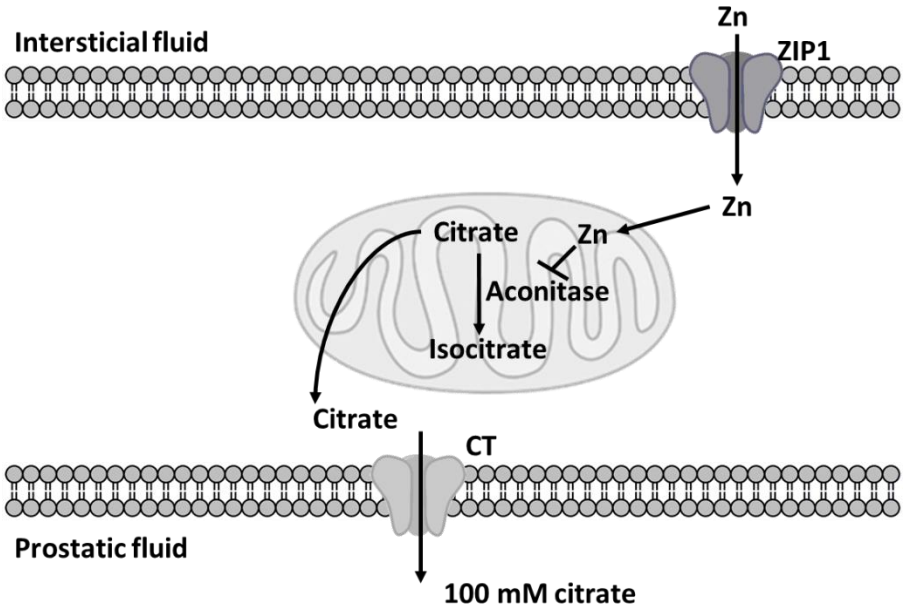
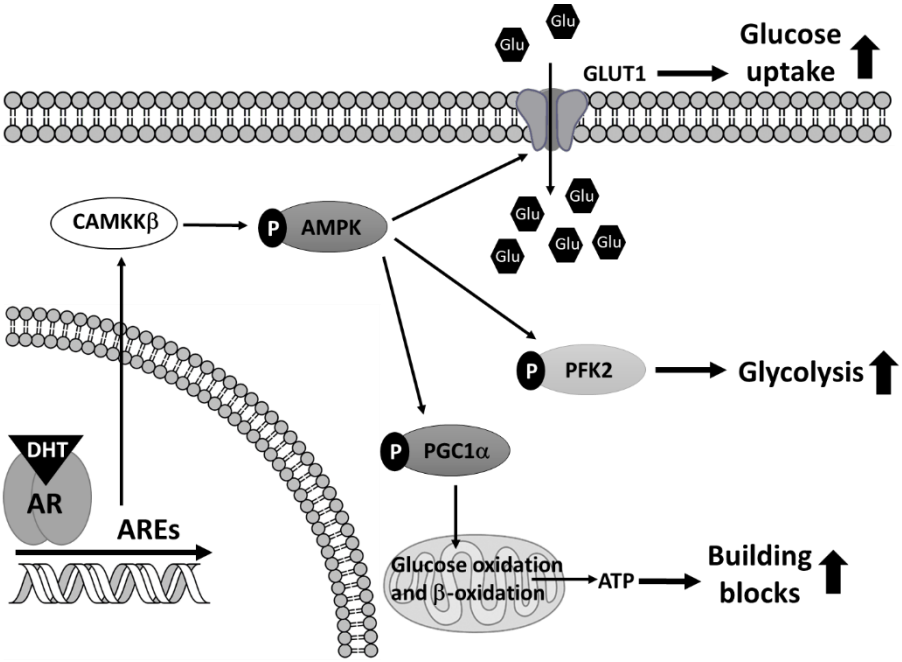
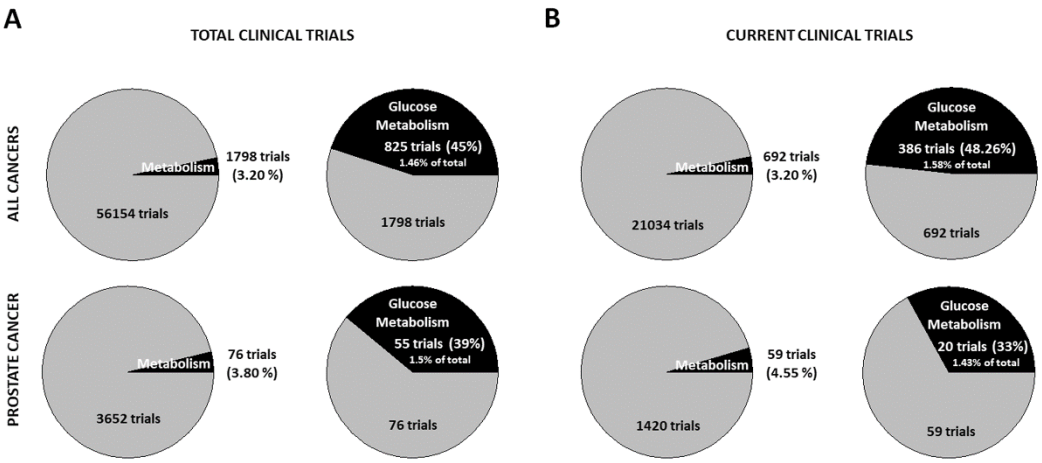


Figure 1

Figure 2





847 **Figure 3**

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849 **Figura 4**

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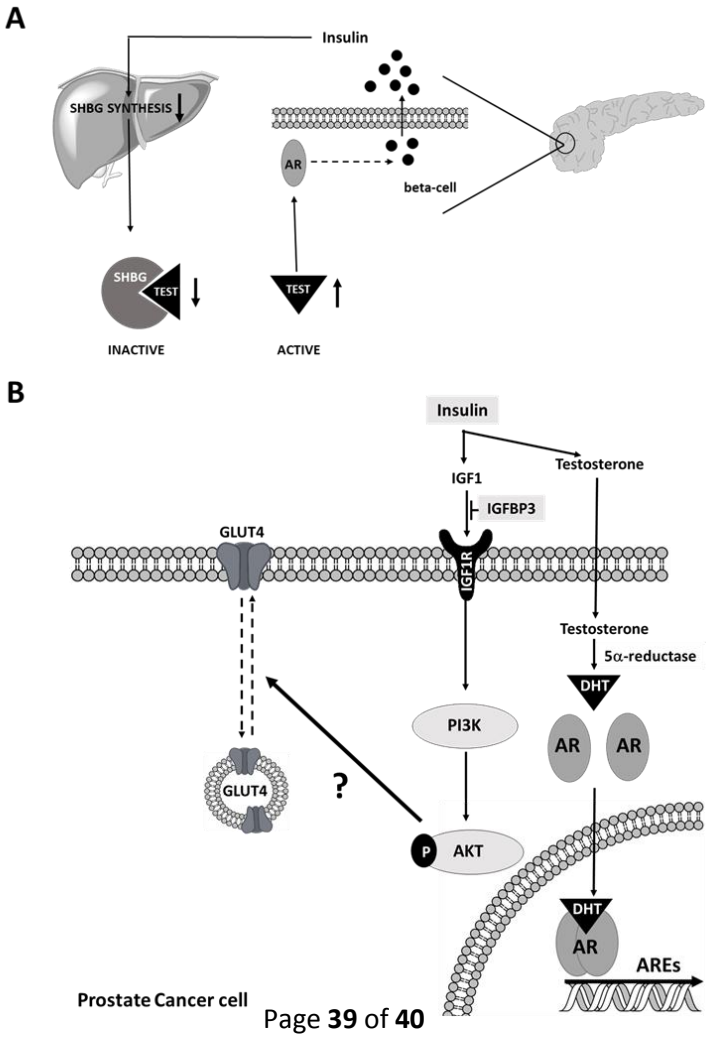
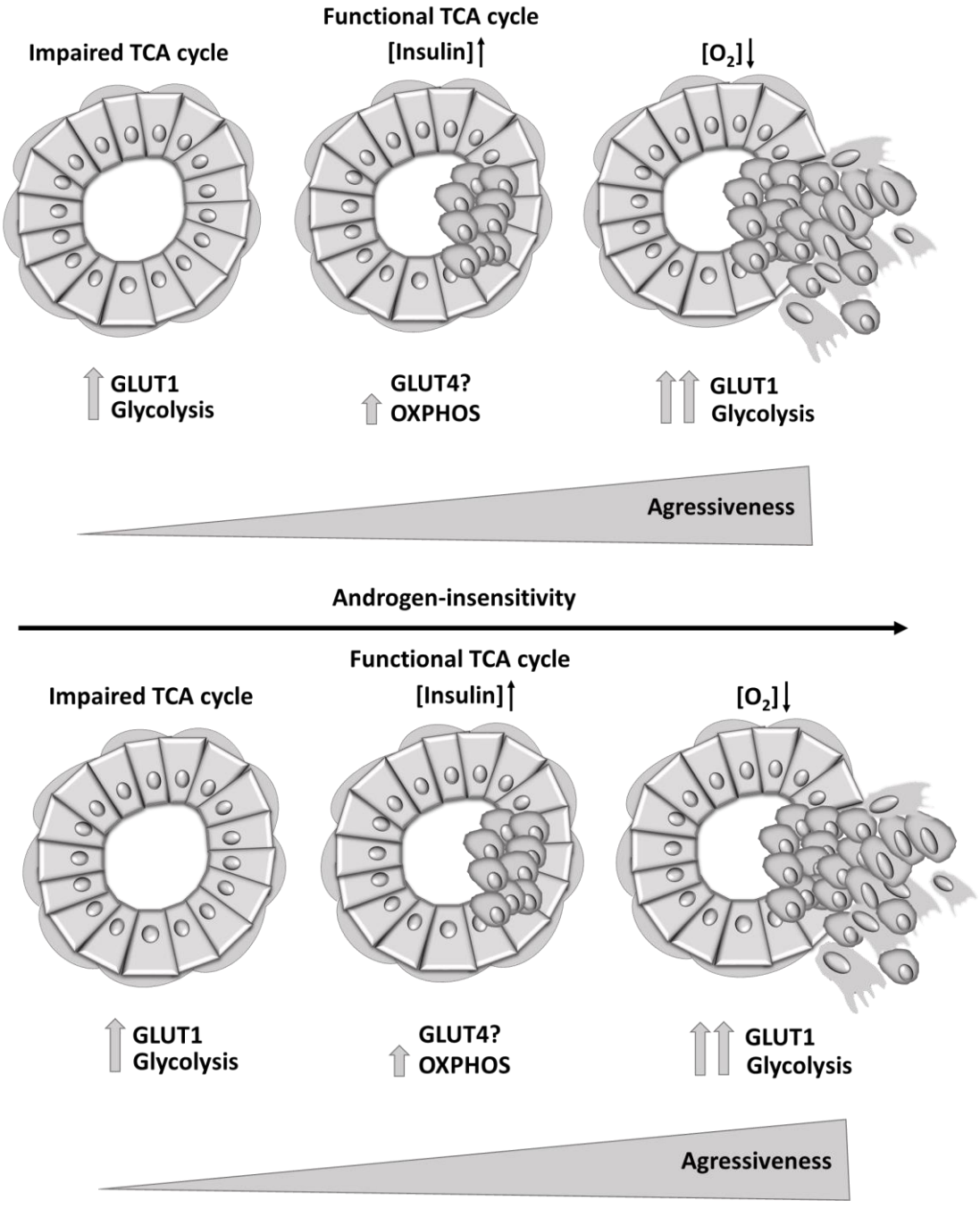


Figura 5


Impaired TCA cycle
Functional TCA cycle
[Insulin]↑
[O2]↓
GLUT1
Glycolysis
GLUT4?
OXPHOS
GLUT1
Glycolysis
Agressiveness
Androgen-insensitivity
Impaired TCA cycle
Functional TCA cycle
[Insulin]↑
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