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GLUT-1 Overexpression in Neoplastic Cells

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Abstract: The aim of this literature review is to present the current state of knowledge on the basis of research on the structure, regulation, and influence of GLUT-1 protein overexpression in the diagnosis and therapy of cancer. For this purpose, the search engine for information on the presented issue was searched in the PubMed online databases. Based on the available data from 100 different publications, attempts were made to prove the influence of GLUT-1 overexpression on the appearance of various types of cancer. As a result of the analysis, it was found that the basic feature of neoplastic cells is the acceleration of glucose metabolism combined with the inhibition of the oxidative phosphorylation process. The increased rate of glycolysis compensates for the slight increase in the energy of anaerobic respiration, which allows cancer cells to continue their uncontrolled growth and proliferation processes. It is mediated by glucose transporters called GLUT-1 for increased cellular glucose uptake. Overexpression of GLUT-1 proteins, in particular those regulated by hypoxic states, has been described in many types of cancer. Numerous reports indicate a correlation between the level of GLUT-1 expression and the degree of malignancy of the tumor. Regulation of GLUT-1 levels is a major factor influencing glucose metabolism in cancer cells, making it a potential chemotherapy target.

Keywords: GLUT-1; Warburg effect; cancer metabolism.

1. Introduction

GLUT-1 belongs to the family of facilitating glucose transporters. It is involved in regulating tissue-specific glucose uptake and metabolism in the liver, skeletal muscle, and adipose tissue to ensure homeostatic control of blood glucose [1] and is also located in the mitochondria [2].

The GLUT-1 family (gene name SLC2A) includes helper transporters that move hexose molecules down the concentration gradient in a tissue and substrate-specific manner. There are 14 members in this group, numbered according to the order of discovery and divided into three subfamilies according to sequence similarity, characteristic elements, and functional features. GLUT-1 is an integral, hydrophobic membrane protein that consists of 492 amino acids with a molecular weight of 54 kDa. It helps transport glucose, galactose, mannose, glucosamine, and ascorbic acid. GLUT-1 consists of 12 hydrophobic transmembrane α-helices with N and C terminus on the cytoplasmic side and a glycosylated extracellular loop between 1 and 2 transmembrane helices. GLUT-1 is present in human tissues with the highest expression levels

in the plasma membranes of proliferating cells during early embryonic development. After birth, in the suckling phase, it is localized in high concentrations in the brain, skeletal muscle, and myocardium, and in the post-suckling phase and beyond into the adult stage, there is a decrease in GLUT-1 expression, except in the brain, and an increase in tissue-specific isoforms. In human erythrocytes, it accounts for about 5-10% of the total membrane protein. It is found widely in cells that make up various blood-tissue barriers. Retinal pigment epithelial cells, choroid, iris, squamous parasite, retinal cells, lens fiber cells, iris microcirculation endothelial cells, and outer segments of photoreceptor cells (to a lesser extent) of the blood-retinal barrier. GLUT-1 expression is regulated mainly by blood glucose concentration, cell signaling mechanisms, and hormones. In hypoglycemic states, GLUT-1 is upregulated in tissues such as the brain, which helps supply the main energy source [3, 4].

Reduced glucose transport activity causes inappropriate use of energy substrates and is associated with insulin resistance and type 2 diabetes. An important feature of cancer cells is increased glucose uptake. GLUT-1 is an important target in cancer treatment because cancer cells upregulate and promote basal glucose uptake in most cell types, thus ensuring the flow of sugar into the metabolic pathway. Deregulation of GLUT-1 is associated with many diseases, including cancer and metabolic diseases. Some natural products can be used as a source of glucose uptake inhibitors, and resveratrol is a naturally derived molecule with various properties. It can act as an antioxidant and anti-proliferative agent in cancer cells [5, 6]. Glucose, the main energy source for all cells, is transported into the cells by glucose transporters. These transporters are of two types, namely sodium-dependent. These transporters are present in a tissue-specific pattern and have substrate specificity. Among these transporters, GLUT-1 is ubiquitous in all body tissues and helps with basal glucose uptake. It performs many physiological functions in the body since embryo implantation and is also perceived to be associated with pathologies, including cancer [7].

Glucose is the main energy source for mammalian cells and produces ATP through glycolysis and oxidative phosphorylation. It is also a precursor of amino acids, nucleotides, and lipids. Glucose crossing the cell membrane into the cytoplasm is the rate-limiting step in glucose metabolism mediated by the family of glucose transporters [8].

Neoplastic cell energy and biosynthesis depend highly on glucose, which increases glucose transport and alters the main energy supply pathway from oxidative phosphorylation to glycolysis. These molecular and metabolic changes are also targets for cancer treatment. Small molecules inhibit basal glucose transport and cell proliferation and induce lung and breast cancer cell apoptosis without significantly affecting their normal cells [9].

Metabolic reprogramming promotes tumor growth and introduces a metabolic burden that can be used to treat cancer. Metabolic-targeted chemotherapy has been an effective treatment for cancer for decades, and the success of these therapies shows that there is a therapeutic window in combating malignant metabolism. Discoveries regarding the various metabolic relationships of tumors provide new strategies for treating altered metabolism, some of which are being evaluated in preclinical models or clinical trials. Metabolic changes occurring in neoplastic cells are of wide interest because of the many oncogenes and suppressor genes involved and because they reveal new therapeutic targets in inhibiting tumor growth [10, 11].

Several natural products can be used as sources of glucose uptake inhibitors, and resveratrol is a naturally sourced molecule with many properties that can be used as antioxidants and has anti-proliferative effects on cancer cells. Cancer cells show increased

absorption and consumption of sugar. Sugar transporters are not regulated in cancer cells, so they contain more sugar than normal cells [12, 13].

Neoplastic cells, unlike normal cells, are characterized by, among other things, independence from extracellular signals stimulating proliferation, unlimited replication potential, insensitivity to factors inhibiting proliferation and stimulating apoptosis, as well as the ability to angiogenesis, infiltrate surrounding tissues and create metastases [14]. Based on the literature data, there is a need to pay attention to other properties, such as genetic instability, avoiding immune responses, promoting inflammatory responses, and changes in cell metabolism [15]. Cancer cells are highly dependent on glucose for energy and biosynthesis and have been found to increase glucose transport and switch their main energy supply pathway from oxidative phosphorylation to glycolysis. These molecular and metabolic changes are also targets for cancer treatment [16]. The metabolic changes that occur during ontogenesis were described for the first time by the German scientist Otto Warburg [12, 13, 17]. He proved that in neoplastic cells, even with unlimited access to oxygen, glucose has increased uptake, and its changes lead to the production of lactic acid. This phenomenon is called oxygen glycolysis - the Warburg phenomenon. This researcher speculated that during carcinogenesis, mitochondria are damaged, and the oxidative phosphorylation that occurs in them is impaired [18].

The current literature data confirm that neoplastic cells have accelerated metabolism, high glucose demand, and increased glucose uptake [19]. Transport of glucose across the plasma membrane of mammalian cells is the first rate-limiting step in glucose metabolism and is mediated by the excitatory glucose transporter protein [20]. The available experimental data indicate that mitochondria work properly in most cancers, and the reasons for the different glucose metabolism should be sought elsewhere [21, 22]. Increased glucose transport in malignant cells is associated with increased and deregulated expression of a glucose transporter, characterized by overexpression of GLUT-1 and/or GLUT-3. By interacting with the GLUT-1 promoter enhancer, the oncogenic transformation of cultured mammalian cells leads to a rapid increase in glucose transport and GLUT-1 expression. In human studies, high GLUT-1 expression in tumors is associated with poor survival. Studies have shown that GLUT-1 or GLUT-3 expression cannot fully explain glucose transport in breast cancer, suggesting the involvement of other glucose transporters.

A new glucose transporter has recently been discovered in breast and prostate cancer. In human breast and prostate cancers and cell cultures, GLUT-1 is found inside and on the cell's surface. Therefore, the movement of GLUT-1 to the plasma membrane may contribute to glucose uptake. Several factors are related to regulating glucose transporters' expression in breast cancer. Hypoxia can increase GLUT-1 levels and glucose uptake. Estradiol and epidermal growth factor may play a role in the growth of breast cancer cells by increasing glucose consumption. Estradiol and epidermal growth factor also increase GLUT-1 protein levels in cultured breast cancer cells. Targeting GLUT-1 could provide new methods for the detection and treatment of breast and prostate cancer. Cancer cells show increased glucose uptake, a phenomenon used for prognostic and diagnostic imaging of various tumors using radiolabeled glucose analogs. However, we have not been able to channel glucose flow into treatment in a cancer-specific manner. Tumor-specific identification and targeting of GLUTs provide a promising method for blocking glucose-regulated metabolism and more complex signal transduction [23]. The glucose and glutamine metabolism in neoplastic cells are directly related [24, 25]. Most tumors and isolated tumor cell lines overexpress proteins from the

GLUT-1 family, presently found in the respective tissues of origin under non-neoplastic conditions [26].

Cancer cells need continuous metabolic energy to continue their uncontrolled growth and proliferation. Accelerated glycolysis is one of the biochemical features of cancer cells. Recent work has shown that glucose transport and metabolism are essential for cancer cell survival after treatment, leading to poor prognosis. Glycolytic glucose degradation occurs before glucose transport across the cell membrane, a rate-limiting process mediated by a protein that promotes the glucose transporter. Tumors typically overexpress GLUT-1, especially the hypoxia-responsive proteins GLUT-1 and GLUT-3. Some studies have shown an association between GLUT-1 expression and the rate of proliferation, while other studies have shown that GLUT-1 expression may be of prognostic value. Hypoxic tumors are malignant with severe metastasis, high resistance to radiation and chemicals, and poor prognosis. The discovery of an oxygen-sensitive hypoxia-induced transcription factor enabled a new understanding of the molecular relationship between hypoxia and glucose metabolism disorders [27].

Increased glucose metabolism in neoplastic cells depends, inter alia, on increased expression of glucose-transporting proteins from the GLUT-1 family, enzymes such as phosphoglycommutase, on the degree of cell proliferation, their density and the presence of blood vessels in the tumor [28, 29]. So far, it has been observed that statin drugs can inhibit glucose uptake by neoplastic cells, including FDG uptake by neoplastic cells in PET testing [30].

1.1. GLUT transporters.

The number of known glucose transporters has increased significantly in the last 2 years. At least three to six Na + dependent glucose transporters (SGLT1-SGLT6; gene name SLC5A) have been identified. Likewise, thirteen members of the family of facilitating sugar transporters (GLUT1-GLUT12 and HMIT; gene name SLC2A) are currently recognized. These different transporters exhibit different substrate specificity, kinetic properties, and tissue expression profiles. The number of different gene products, together with the presence of several different transporters in some tissues and cells (e.g., GLUT1, GLUT4, GLUT5, GLUT8, GLUT12, and HMIT in white adipose tissue), indicates that glucose delivery to cells is a process of considerable complexity [31].

As previously mentioned, the GLUT-1 family of transporters consists of 14 proteins divided into three categories. Class I contains the most popular GLUT-1-GLUT-4 transporter. Class II includes the fructose transporter GLUT-5 as well as the GLUT-7, GLUT-9 and GLUT-11 transporters. The other transporters, GLUT6, GLUT8, GLUT-10, GLUT-12, and HMIT (HMIT transporters, H + coupled inositol), belong to class III. All GLUT transporters contain 12 hydrophobic α-helical transmembrane domains with large intracellular loops (between domains 6 and 7). These proteins' N- and C-terminal regions are on the cytoplasmic side. Class I and II GLUT proteins have an extracellular loop of N-glycosylation sites between transmembrane domains 1 and 2. N-glycosylation of GLUT-1 on Asn45 increases its stability and transport activity. Similar N-glycosylation sites for class III transporters exist between 9 and 10 transmembrane domains.

All GLUT proteins transport energy-independent hexoses of different kinetics and substrate affinity along a concentration gradient. The hypoxia-induced GLUT-1 and GLUT-3 transporters and the insulin-dependent GLUT-4 transporters show the highest affinity for

glucose. Different tissues of the body have different expression properties for the GLUT transporter. It helps transport glucose, galactose, mannose, glucosamine, and ascorbic acid [32-34].

The discovery of the important role of many signaling pathways related to glucose or glutamine metabolism in cancer progression started the search for new anti-cancer therapeutic strategies based on these phenomena [35, 36].

1.2. The role of GLUT-1.

GLUT-1 plays an important role in embryo implantation through increased expression in the endometrium and the basolateral surface of polarized trophectodermal cells and the internal mass of cells under the influence of estrogen and progesterone hormones. Maternal hyperglycemic diabetes is known to reduce the expression of GLUT-1, which activates Bax, leading to apoptosis and thereby causing embryo death. It plays an important role in the transport of glucose between the mother and the placenta and regulates the transport of glucose across the placenta to the fetus, increasing its expression in syncytiotrophoblastic cells and the surface of trophoblastic microvilli. This function of GLUT-1 is supported by the coordinated expression of GLUT-3 in the syncytiotrophoblastic layer. In adults, it is responsible for providing glucose for energy production in red blood cells and the brain. In muscles and adipose tissue, it supports glucose transport in the basal state. It also protects against insulin-resistant glucose uptake in skeletal muscle, to which oxidative stress contributes by regulating reactive oxygen species. On the mitochondrial membrane, its expression helps transport ascorbic acid to the mitochondria, which protects cells against oxidative damage [37].

2. Glucose Transporters as Therapeutic Targets in Cancer

2.1. GBM glioma.

Glioblastoma multiforme (GBM) is the most aggressive subtype of malignant glioma. The current standard of care involves a combination of cytoreduction by surgical resection followed by radiotherapy with concomitant and adjuvant chemotherapy (temozolomide). The role of bevacizumab in treatment is still under ongoing research and debate. Despite aggressive treatment, these tumors are undoubtedly fatal, especially in older people. Moreover, tumors present in the pineal gland are extremely rare, accounting for only 0.1-0.4% of all adult brain tumors, and this location complicates treatment. GBM is one of the most aggressive forms of brain tumor. According to the current standard of care, the prognosis for survival is 15 months, with a five-year survival rate below 3%.

A better understanding of the molecular mechanisms leading to cell growth and GBM survival could lead to developing new approaches to treat and combat disease. Basigin-2 protein induces the expression of matrix metalloproteinase enzymes, and its expression level is positively correlated. In 2011, Ramos-Vara *et al*. reported that a variant of Basigin splicing, called Basigin-3, may have an inhibitory function after binding to Basigin-2 in human hepatocellular carcinoma cells, as it reduces tumor growth, invasion, and expression of MMPs [38]. GBM is the most common malignant primary brain tumor. It contains a sub-population of undifferentiated stem cells with self-renewal and carcinogenic potential contributing to tumor initiation, invasion, recurrence, and therapeutic resistance [39]. Malignant gliomas are fatal neoplasms that display a cell hierarchy with cancer stem cells at the apex. Glioblastoma stem cells are not evenly distributed but located in specialized niches, suggesting that the tumor

microenvironment regulates the neoplastic stem cell phenotype. Indeed, recent studies show that hypoxia and its molecular responses regulate cancer stem cell maintenance. Acidic conditions, independent of limited oxygen supply, favor the expression of markers, self-renewal, and tumor growth. They exert a paracrine effect on tumor growth by developing angiogenic factors. Low pH conditions increase this expression associated with the induction of 2α hypoxia-inducible factor, a GSC-specific regulator. Induction of HIF2α and other GSC markers by acidic stress can be reversed by increasing pH in vitro, suggesting that increasing intra-tumor pH may be beneficial for targeting the GSC phenotype. All our results suggest that exposure to low pH promotes malignancy by inducing a phenotype of cancer stem cells and that culturing cancer cells at a lower pH, reflecting the endogenous conditions of the tumor, may better preserve cellular heterogeneity found in tumors [40]. High-grade gliomas constitute the vast majority of all gliomas, including glioblastoma multiforme. Despite enormous efforts in developing multimodal therapies, the overall prognosis remains poor; however, the survival time varies considerably between patients. The nature of diffuse penetration into the surrounding parenchyma of the brain creates a dilemma for neurosurgeons between extensive surgical resection to eliminate as many cancer cells as possible and adverse effects related to brain function. The heterogeneity of cytology and gene expression makes it difficult to coordinate an effective therapy that works for each patient [41].

Glioblastoma Multiforme is the most common and deadly type of brain cancer. To identify genetic alterations in GBM from experimental data, 20,661 protein-coding genes were sequenced, the presence of amplification and deletions was determined using high-density oligonucleotide arrays, and we performed gene expression analyses using next-generation sequencing technology in 22 human tumor samples. This comprehensive analysis led to the discovering of various genes not known to be altered in GBM. First of all, recurrent mutations in the active site of isocitrate dehydrogenase 1 were found in 12% of GBM patients. Mutations occurred in a high proportion of young patients and in most patients with secondary GBM and were associated with increased overall survival. These studies demonstrate the value of objective genomic analyses in characterizing human brain cancer and identifying a potentially useful genetic change for GBM classification and targeted therapy [42].

Most of the current research on human brain tumors focuses on the analysis of the molecular and cellular mass of the tumor. However, there is overwhelming evidence in some malignancies that a tumor clone is heterogeneous for proliferation and differentiation. In human leukemia, the neoplastic clone is organized into a hierarchy derived from rare leukemic stem cells, which have high proliferative and self-renewal potential and are responsible for maintaining the neoplastic clone. The enhanced self-renewal capacity of brain tumor stem cells is highest in the most aggressive clinical specimens of medulloblastoma compared to low-grade gliomas. BTSC identification provides a powerful tool for studying the neoplastic process in the central nervous system and developing therapies [32, 33]. Cancer cells self-renew under clonal conditions and differentiate into neuron-like, glial, and abnormal cells with mixed phenotypes [43].

Oral squamous cell carcinoma can grow rapidly and without restriction. Therefore, hypoxic tissue areas are common in these malignant tumors and contribute to cancer progression, treatment resistance, and poor outcomes. The expression of the HIF and GLUT proteins in TSCC appears to be associated with the severity of the disease and the presence of metastases. Additional studies are needed to evaluate these proteins' diagnostic and prognostic

uses. Some glucose transporters are overexpressed in aggressive, rapidly growing tumors [44,45].

Treatment for laryngeal cancer includes radiation therapy, surgery, chemotherapy, or a combination thereof. Functional treatment of laryngeal cancer is a major challenge due to its resistance to chemotherapy and radiotherapy and the tendency to local recurrences. Finding ways to inhibit cancer energy supply is an increasingly attractive proposition. GLUT-1 is the major glucose transporter in solid tumors and has been the subject of cancer research. Increased expression of SLC2A1 in head and neck cancers correlates with lymph node metastasis, poor survival, and clinical grade, and suppression of SLC2A1 expression by antisense oligodeoxynucleotides was found to reduce glucose uptake and inhibit Hep2 cell proliferation.

Table 1. GLUT-1 overexpression in neoplastic cells.

Lp.	Type of cancer	Reference number
1.	GBM glioblastomas	[38-43]
2.	A brain tumor	[32, 33, 43]
3.	Oral squamous cell carcinoma	[44,45]
4.	Laryngeal cancer	[43]

2.2. GLUT-1 inhibitors.

Cancer cells rapidly increase their energy requirements, increasing the level of the human glucose transporter. This upregulation suggests that GLUT-1 is the target of therapeutic inhibitors to fight many types of cancer. Inhibitors, the inward open structures of WT-hGLUT-1 crystallized with three different inhibitors: cytochalasin B, a nine-membered bicyclic ring fused to a 14-membered macrocycle, hGLUT, and two previously undescribed Phe-amide inhibitors. Despite the very different chemical backbones, all three compounds bind in the central cavity in an open state to the inside of hGLUT-1, and all binding sites coincide with the glucose binding site. The inhibitory activity of compounds was determined for members of the hGLUT family, hGLUT1-4, using cellular assays and compared with homology models for these hGLUT members. This comparison revealed a likely basis for the observed differences in inhibition among family members. We indicate regions of the hGLUT proteins that can be targeted for isoform selectivity and show that the same regions are used for inhibitors with very different structural backbones. The structures of inhibitors with the hGLUT1 complex provide important insight into the design of more selective inhibitors, particularly hGLUT and especially hGLUT1 [46, 47].

Resveratrol (3,5,4′-trihydroxystilbene or RSV) is a natural polyphenol product that has attracted much attention mainly for its anti-cancer, anti-inflammatory, and cardioprotective properties. RSV is structurally similar to tyrosine kinases, known inhibitors of GLUT-1. To investigate the relationship between the RSV and this transporter. Using kinetic assays, we observed for the first time that RSV inhibited glucose uptake in human U-937 and HL-60 leukemia cell lines by direct interaction with the inner surface of GLUT-1 in a non-competitive mode.

Regarding RSV and glucose uptake, studies in various human ovarian cancer cells showed that treatment with RSV was able to inhibit glucose uptake, lactate production, Akt and mTOR signaling, and cell viability depending on the dose and time used. There are many studies on RSV and glucose uptake in neoplastic cells and pathological conditions such as insulin resistance. Most experiments were performed in vitro and in vivo using labeled glucose analogs. In cancer cells, resveratrol inhibits glucose uptake, promoting an anti-cancer effect. Still, in pathological conditions such as insulin resistance or diabetes mellitus, resveratrol

increases glucose uptake and insulin sensitivity, promoting antidiabetic effects. Other studies in neural cells have shown that RSV inhibits glucose uptake, promoting neuronal glucose regulation and insulin sensitivity. Among other mechanisms of action, RSV also attacks a large number of intracellular molecules involved in the control of the cell cycle and the induction of apoptosis. RSV is an attractive candidate for cancer treatment due to its unique ability to influence the mTOR / AMPK pathway at various levels. RSV has a strong short-term effect on metabolism by inhibiting mTOR and S6 ribosomal protein kinase and activating AMPK. The type of cell death observed in RSV-treated cancer cells has been described as apoptosis or autophagy. Van Ginkel *et al*. concluded that elevated RSV levels lead to tumor regression and extensive tumor cell death. The basic mechanism involves the direct activation of internal and external apoptotic pathways. Thus, in normal adipocytes, it has been observed that RSV induces apoptosis at concentrations above 20 μM, while in insulin-resistant adipocytes, RSV stimulates glucose transport via SIRT1-AMPK-Akt. These results suggest that RSV may behave differently depending on the dose used and the cell type and metabolic state. Recently, Dai *et al*. RSV has been shown to inhibit the growth and proliferation of MGC-803 gastric cancer cells in a dose- and time-dependent manner by reducing the expression of genes related to the Wnt pathway, such as β-catenin, c-myc, and cyclin D1. In turn, Kleszcz *et al*. No inhibition of c-myc gene expression by resveratrol was observed in FaDu throat cancer cells, but the doses used were significantly lower.

With regard to autophagy, many studies show that RSV induces autophagy and cell death in cancer cells when they are depleted of nutrients and that RSV can act by eliciting a hunger-like signaling response. Indeed, activation of the JNK pathway by RSV leads to the induction of genes involved in both the early and late stages of autophagy in CML cells. RSV may also affect the mitochondrial membrane potential, the respiratory chain, and the synthesis of ATP [22]. Glucose transport inhibitors mimic glucose deprivation and work by inhibiting primary glucose transport. These inhibitors can complement and replace traditional glucose deprivation, which cannot be used in animals, as new tools to study the effects of glucose transport and metabolism on cancer and normal cells [48, 49].

Low molecular weight GLUT-1 inhibitors have been described in the literature, including resveratrol [50], naringenin [51], phloretin [42], WZB117 [53], salicyl ketoximes [54], thiazolidinedione [55], STF-31 [56] pyrazolopyrimidine [57], and phenylalanine amides [58]. The principle of synthetic chemical lethality was used to demonstrate the sensitivity of VHL-deficient kidney cancer cells to the inhibition of glucose uptake by STF-31. WZB117 was able to inhibit glucose uptake by A549 cancer cells and their proliferation in a dose-dependent manner. All these results emphasize the potential of GLUT-1 inhibition in cancer treatment [59,60].

The experimental studies conducted so far show that RSV induces apotheosis in ovarian cancer cells by impaired glucose uptake, a process involving the transport of GLUT-1 in the plasma membrane regulated by Akt [61]. Glucose transport inhibitors mimic glucose deprivation and act by inhibiting primary glucose transport. These inhibitors can complement and replace traditional glucose deprivation, which cannot be used in animals, as new tools to study the effects of glucose transport and metabolism on neoplastic and normal cells [62].

Another very important inhibitor is BAY-876. It is a potent and selective GLUT1 inhibitor. BAY-876 showed good in vitro metabolic stability and high oral bioavailability in vivo. GLUT-1 overexpression has been reported in many types of human cancers, including

cancers of the brain, breast, colon, kidney, lung, ovary, and prostate. It has been correlated with advanced stages of cancer and poor clinical outcomes [63].

It has been known for 80 years that the growth of cancer cells in the energy process is supported by increased glucose metabolism. This phenomenon suggests the need for a corresponding increased glucose uptake across the plasma membrane by enhancing glucose transport proteins, SGLT proteins, and GLUT proteins. The results of many studies have shown that the expression of glucose transporters, especially GLUT-1, is increased in various malignancies. GLUT-1 overexpression has been found to be associated with tumor progression. GLUT-1 overexpression has been found to be associated with poor overall survival in various malignancies [64-66].

3. Immunohistochemical Method

Immunohistochemical techniques detect antigens in tissue sections using immunological and chemical reactions. The technique is very sensitive and specific, making it possible to detect many different antigens in many animal species [67]. Immunohistochemical techniques detect antigens in tissue sections using immunological and chemical reactions [68-70]. Immunohistochemistry is an important tool often used to diagnose several diseases in the pathology laboratory. The binding of formalin influences the quality and sensitivity of immunohistochemical staining, resulting in a variable loss of antigenicity known as the masking effect. While the sensitivity of immunohistochemistry is excellent for some antigens, other antigens, such as COX-1 and COX-2, are difficult to identify, especially in formalin-fixed paraffin sections. Antigen capture is an epitope re-exposure technique that detects masked antigens using standard immunohistochemical procedures. One common method involves partial enzymatic pre-digestion with trypsin or pepsin. In contrast, other non-enzymatic heat-mediated antigen recovery procedures or methods include pressure cookers, hot plates, or irradiation of tissue sections with microwave (MW) in water or various antigens. -recovering solutions. In this chapter, we will describe a technique that provides a more robust, much simpler approach to demonstrating the expression of cyclooxygenase-1 and cyclooxygenase-2 in frozen, vibratomic, or paraffinic sections and/or cells in culture.) Hypoxia-induced factor -1alpha (HIF-1alpha)) is a transcription factor that activates many genes, including vascular endothelial growth factor (VEGF) and glucose transporter-1 (GLUT-1) in response to hypoxia and promotes neoangiogenesis. HIF-1alpha VEGF and GLUT-1 expression can be analyzed by immunohistochemistry, and microvessel density is determined by immunostaining. HIF-1alpha was increasingly expressed from early to advanced stages of endometriotic adenocarcinoma in parallel with activation of its downstream genes, such as GLUT-1 and VEGF, and increased angiogenesis [68,69].

In previous studies, paraffin sections were immunologically stained with anti-GLUT-1 or GLUT-4 antibodies using the avidin-biotin-peroxidase complex method [70]. It was previously shown that GLUT-1 expression of the glucose transporter could be detected by immunostaining in tissue sections from anaplastic cancer [71].

Protein overexpression has been observed in many types of cancer, especially in the hypoxia-regulated GLUT-1 and GLUT-3 proteins. Since these transporters have a high affinity for glucose and can transport it efficiently, they are a key factor limiting glucose metabolism in cancer cells. We then reviewed the latest technological advances in resveratrol and other natural products, such as GLUT-1 inhibitors, focusing on targeted therapies for different types of cancer. Targeting GLUT-1 activity is a promising drug development strategy for treating

tumor growth. It is known that tumor cells have accelerated metabolism, high glucose demand, and increased glucose uptake. Glucose transport across the plasma membrane of mammalian cells is the first rate-limiting step in glucose metabolism mediated by the glucose transporter (GLUT, which stimulates the protein glucose transporter). Increased glucose transport in tumor cells is associated with increased and deregulated glucose transporter expression, characterized by overexpression of GLUT-1 and/or GLUT-3. In human studies, high GLUT-1 expression in tumors is associated with poor survival. Studies have shown that GLUT-1 or GLUT-3 expression cannot fully explain glucose transport in breast cancer, suggesting the involvement of other glucose transporters. A new glucose transporter, GLUT-1, has recently been discovered in breast and prostate cancer. In human breast and prostate cancers and cell cultures, GLUT-1 is found inside and on the cell's surface. Therefore, the movement of GLUT-1 to the plasma membrane may contribute to glucose uptake. Several factors are associated with the regulation of the expression of glucose transporters in breast cancer. Hypoxia can increase GLUT-1 levels and glucose uptake. Estradiol and epidermal growth factor may play a role in the growth of breast cancer cells by increasing glucose utilization. Estradiol and epidermal growth factor also increase GLUT-1 protein levels in cultured breast cancer cells [72-74].

4. Regulation of GLUT-1 Transporter Expression

Expression of the GLUT-1 transporter is mainly regulated by cell signaling mechanisms, blood glucose, and hormones. During hypoglycemia, GLUT-1 levels increase in tissues, including the brain, which helps deliver the main energy source. High GLUT-1 expression may be an independent prognostic marker for predicting shorter survival in various types of cancer [75, 76]. Increased MT expression has been reported to be associated with poor prognosis in various cancers [77]. The activity of enzymes such as lactate dehydrogenase (LDH) is perhaps the most common clinical enzyme used in cancer patients for prognostic purposes—enzymes or in combination with tumor markers or other factors [78]. GLUT-1 shows altered expression, e.g., in colorectal cancer (CRC) [79].

5. Discussion

Cancer cells change metabolism to promote growth, survival, proliferation, and long-term maintenance. A common feature of this metabolic conversion is increased glucose uptake and fermentation of glucose to lactic acid. This phenomenon is even observed in functioning mitochondria and is collectively referred to as the "Warburg Effect". The Warburg effect has been documented in the literature for over 90 years, and extensive research has been conducted over the past 10 years in which thousands of articles describe its causes or functions. While this is of great interest, the function of the Warburg effect is still unclear [80-83].

6. Conclusions

GLUT-1 is one of the major isoforms in the family of glucose transport proteins that facilitates the import of glucose into human cells to fuel anaerobic metabolism. Glucose transporters are important in biology because they are gates to one of the most important molecules in life, namely glucose. Drugs directed against glucose transporters are also potential antitumor agents [84,85].

HIF-1alpha, VEGF, and GLUT-1 expression can be analyzed by immunohistochemistry, and microvessel density is determined by immunostaining [31].

Glycolytic degradation of glucose occurs before the transport of glucose across the cell membrane, a rate-limiting process mediated by a glucose transporter-promoting protein, which belongs to the family of glucose transporter-promoting proteins. Tumors typically overexpress GLUT-1, especially the hypoxia-responsive proteins GLUT-1 and GLUT-3. There are also studies that have found an association between GLUT-1 expression and the rate of proliferation, while other studies have shown that expression may have a prognostic value. Hypoxic tumors are malignant with severe metastases, high resistance to radiation and chemicals, and poor prognosis [59].

The hypoxic-dependent GLUT-1 and GLUT-3 transporters transport both glucose and dehydroascorbic acid, thus playing an important role in regulating the functional status of the cell. As overexpression of both proteins is observed in many types of cancer, in the future, a more detailed understanding of the structure and regulation of GLUT transporters may affect the development of new methods of cancer diagnostics and therapy [86].

The GLUT-1 protein represents the family of proteins responsible for transporting glucose across cell membranes, an important substrate in cell metabolism. Under physiological conditions, this protein is present only in erythrocytes, cerebral vascular endothelial cells, and muscles [87]. The expression of this protein correlates with chronic tissue hypoxia and limited access to glucose; therefore, its presence is often found in neoplasms [88, 89]. For uncontrolled growth, cancer cells need a constant energy source, which is ensured by an accelerated process of glycolysis (anaerobic respiration). Anaerobic glycolysis is the process of metabolizing glucose into pyruvate and then into lactic acid. This process provides much less energy (two ATP molecules) than aerobic glucose combustion (36 ATP molecules). In many cancers, a high level of this transporter correlates with a shorter metastasis-free time and worse overall survival [90-94].

Malignant cells show increased glycolytic metabolism and, in many cases, increased glucose transporter gene expression. The expression of the glucose transporter GLUT-1 increases in colorectal cancer, and the degree of expression may be of prognostic importance [95]. Identifying and targeting tumor-specific GLUT-1 offers a promising approach to block glucose-regulated metabolism and more complex signaling [96]. A better understanding of the mechanistic links between cellular metabolism and growth control could lead to better human cancer treatments [97]. Expression of both isoforms of glucose transporter may contribute to the maintenance of human brain tumors, and the expression of the GLUT3 isoform may be closely related to malignant lesions of astrocytomas, especially with abnormal neovascularization accompanying gliomas [98].

Glucose metabolic activity closely mirrors the response to gefitinib treatment. FDG-PET may be a valuable clinical predictor early in treatment for therapeutic responses to EGFR kinase inhibitors [99], and thus IF-1alpha induces GLUT-1 expression [100].

Glucose transporters exhibit tissue-specific distribution and selective expression in various types of cancer. GLUT-1 is expressed at high levels in most cancers, and GLUT-3 is found mainly in the brain, supporting the development of GLUT-1-selective compounds. Thus, GLUT-3 is also overexpressed in many additional cancer types (beyond neural glioblastoma), such as breast and endometrial cancers, head and neck cancers, colon cancer, pancreatic cancer, non-small cell lung cancer, and thyroid cancer [101].

An important feature of cancer cells is the increase in glucose uptake. Thus, GLUT1 is an important target in cancer treatment because cancer cells upregulate GLUT1. This membrane protein facilitates basal glucose uptake in most cell types to ensure the flow of sugar

into metabolic pathways. Improper GLUT1 transport is associated with many disorders, including cancer and metabolic diseases [102]. A marker of hypoxia and a metabolic indicator that was used to examine the metabolic activity of cells is GLUT-1 [103.

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Conflicts of Interest

The authors declare no conflict of interest.

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