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REVIEW ARTICLE

Neuronal-Astrocyte Metabolic Interactions: Understanding the Transition Into Abnormal Astrocytoma Metabolism

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Abstract

Brain function depends on complex metabolic interactions among only a few different cell types, with astrocytes providing critical support for neurons. Astrocyte functions include buffering the extracellular space, providing substrates to neurons, interchanging glutamate and glutamine for synaptic transmission with neurons, and facilitating access to blood vessels. Whereas neurons possess highly oxidative metabolism and easily succumb to ischemia, astrocytes rely more on glycolytic metabolism and hence are less susceptible to lack of oxygen. Astrocytoma cells seem to retain basic metabolic mechanisms of astrocytes; for example, they show a high glycolytic rate, lactate extrusion, ability to flourish under hypoxia, and opportunistic use of mechanisms to enhance cell division and maintain growth. Differences in metabolism between neurons and astrocytes may also extend to astrocytoma cells, providing therapeutic opportunities against astrocytomas, including sensitivity to acetate, a high rate of glycolysis and lactate extrusion, glutamate uptake transporters, differential sensitivities of monocarboxylate transporters, presence of glycogen, high interlinking with gap junctions, use of nicotinamide adenine dinucleotide phosphate for lipid synthesis, using different isoforms of synthetic enzymes (e.g. isocitrate dehydrogenase, pyruvate carboxylase, pyruvate kinase, lactate dehydrogenase), and different glucose uptake mechanisms. These unique metabolic susceptibilities may augment conventional therapeutic attacks based on cell division differences and surface receptors alone.

Key Words: Astrocyte, Glioma glycolysis, Hypoxia-inducible factor, Lactate, Metabolism, Mitochondria.

INTRODUCTION

In normal brain, astrocytes and neurons form a complex, symbiotic relationship for both maintenance of neuronal function as well as support of brain metabolism (1–5). Most aerobic metabolism within the CNS is performed within neurons owing to their high ATP requirements, particularly after neuronal activation, with astrocytes forming a smaller metabolic compartment in comparison. Astrocytes are important in buffering neurons from the bloodstream, forming part of the blood-brain barrier (BBB), and hence assisting in providing glucose to the extracellular space as the primary initial metabolite for the brain, in addition to storing glycogen for buffering hypoglycemia (6–10).

Astrocyte metabolism primarily functions using ATP derived from complete glycolysis, with lactate extrusion into the extracellular space as the end point (11–15). For example, extracellular glucose in the human brain varies between 0.5 and 1.5 mmol/L, whereas extracellular lactate levels approach at least approximately 2.5 mmol/L compared with ~0.75 mmol/L systemically (16, 17). This high extracellular brain lactate provides a key metabolite for neuronal aerobic metabolism, in addition to direct glucose utilization by neurons. Lactate levels are balanced between astrocyte extrusion and neuronal uptake and metabolism in a complex time course linked to neuronal activation (13, 18).

Astrocytes maintain homeostasis of the extracellular space and are able to sense high levels of neuronal metabolic demand through a variety of intercellular messenger molecules, including glutamate, K^+, nitric oxide (NO), hydrogen peroxide (H_2O_2), and ammonia, among others (Fig. 1) (1, 3, 9, 11). Mitochondrial function and the citric acid cycle (also known as the tricarboxylic acid [TCA] cycle) in astrocytes are very different from that of neurons, with astrocyte metabolism focusing on synthetic or cataplerotic pathways (i.e. intermediates are removed from the citric acid cycle), particularly for synthesis of glutamine for neuronal use (3, 9, 10, 19). A large aspect of astrocyte functioning is focused on glutamate uptake from spillover of glutamate into the extracellular space after neuronal synaptic activation. Rapid glutamate uptake via transporters into astrocytes functions to limit synaptic activity of glutamate, much like acetylcholinesterase, which limits acetylcholine function at neuromuscular junctions. To complete this cycle, astrocytes are critical to maintain glutamine extrusion for subsequent neuronal reformation of glutamate, and much of this glutamine is derived from the TCA cycle in astrocytes via α-ketoglutarate (α-KG) synthesis. Thus, astrocytes are primarily anaerobic in terms of ATP generation via anaerobic glycolysis and net lactate extrusion (although they can also generate ATP from the TCA cycle), whereas neurons primarily function with high levels of aerobic mitochondrial metabolism. This low sensitivity to hypoxia is evident by the well-known capability to culture astrocytes (or astrocytoma cells) for up to 24 hours from adult brain tissue (even cadaveric), whereas neurons survive less than 3 to 5 minutes in an anoxic environment (20).

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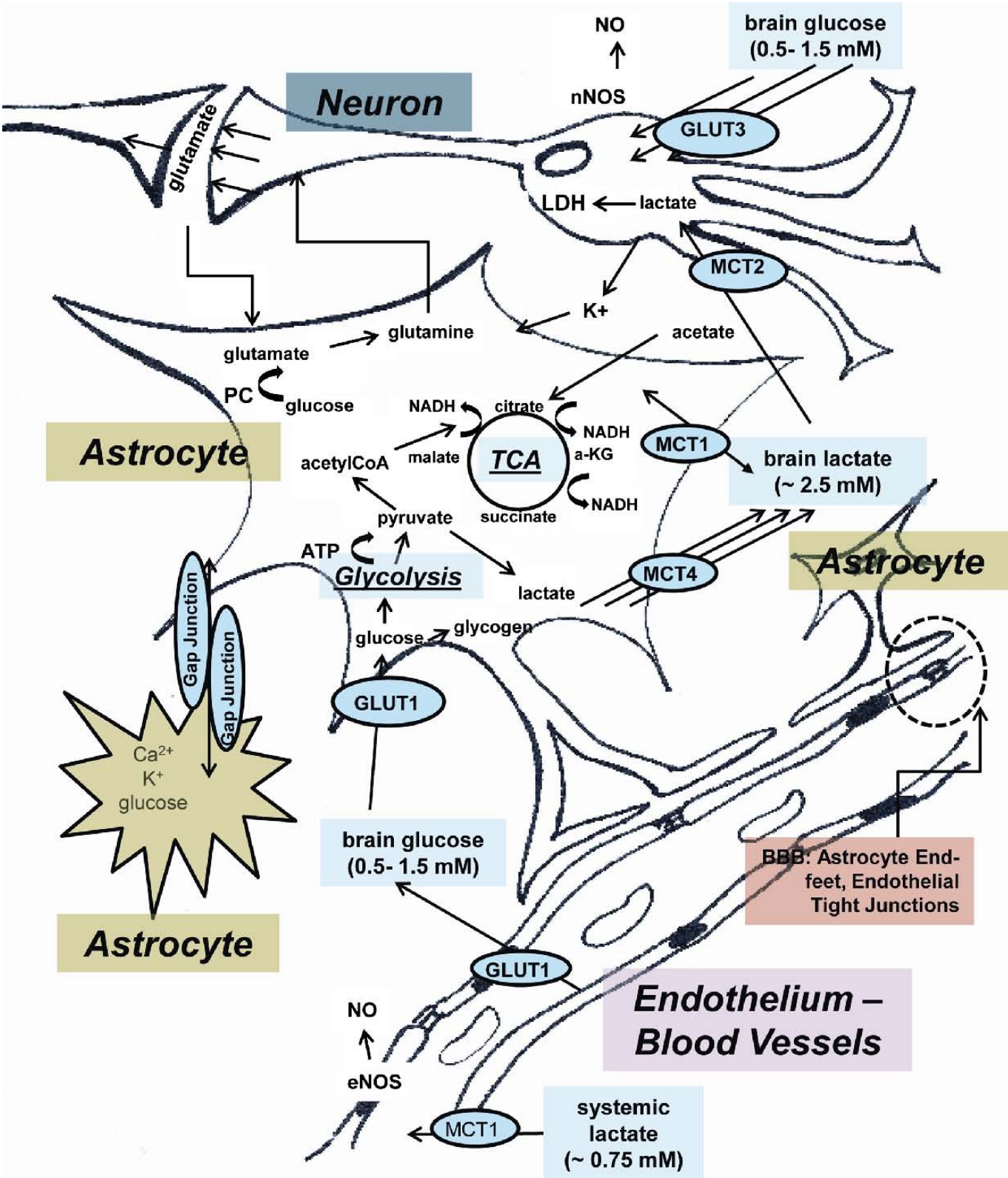
Astrocytoma cells maintain and extend these usual characteristics of normal astrocytes, with a high level of glycolytic functioning and lactate extrusion, and can flourish in a relatively anoxic environment (21). Astrocytoma cells likely share more characteristics with immature astrocytes than mature ones, but these basic metabolic functions remain common



to both. These characteristics follow the general trend noted for most cancer cells to develop a high rate of glycolysis and synthetic function to enhance cell replication, termed the *Warburg effect* (22–24). Astrocytomas are generally devoid of neurons, which is one reason why the extracellular lactate levels rise and extracellular acidity increases within the tumor beds. Astrocytoma cells are, however, clearly adept at survival as they infiltrate the neuropil of normal brain parenchyma. In addition, fast tumor growth leads to a hypoxic environment, which also favors tumor growth. Many of the invasive features of astrocytomas may depend on unique or distorted metabolism. Indeed, our laboratory has recently uncovered a novel genetic alteration that leads to the loss of the migration-related protein adherens junctional–associated protein, which directly interacts with enzymes in glycolytic pathways (unpublished data). The coupling of metabolic alterations and invasive properties is a growing field in astrocytoma studies.

Because many features of immature astrocytes are preserved and even amplified within astrocytomas, these metabolic differences may be exploited for astrocytoma treatments. Unique keys to astrocyte metabolism include, for example, acetate, which neurons selectively can neither uptake nor metabolize. Astrocytes also form syncytia to facilitate buffering of the extracellular space, rapidly passing Ca^{2+}, K^+, glucose, water, and other molecules from astrocyte to astrocyte via gap junctions for homeostatic purposes. This characteristic can be exaggerated within astrocytomas as well. Thus, the hypothesis of this review is that astrocytoma cells retain the basic metabolic characteristics of immature astrocytes, but many of these are distorted and exaggerated, with less metabolic control possible, but which may provide unique pathways of susceptibility for possible understanding of invasiveness and treatment. This review describes normal metabolic functioning within the neuronal-astrocyte-vascular metabolic unit, followed by a discussion of how these metabolic differences between neurons and astrocytes may expose susceptibilities and provide therapeutic strategies for astrocytomas.

NORMAL NEURONAL-ASTROCYTE-VASCULAR INTERACTIONS – METABOLIC UNIT

Normally, neurons, astrocytes, and blood vessels function in a complex network, and neurons cannot function without this metabolic linkage and astrocyte support (Fig. 1). However, astrocytes can be cultured in isolation from neurons (20), although in isolated tissue culture, they develop characteristics different from either astrocytes in vivo or in the presence of neurons.

Specific Neuronal Mechanisms

Although a low level of basal metabolism is important for cellular maintenance, neuronal activation leads to increased energy requirements (4). Neuronal metabolism consists of multiple compartments, including submembrane glycolysis linked to membrane ion pumping (i.e. Na/K ATPase, K-ATP channels, etc.), aerobic glycolysis for pyruvate generation for aerobic metabolism, and NADH/ATP generation via TCA cycle within mitochondria (1). In addition to glucose uptake (moderated by both endothelial cells and astrocytes), there is also considerable lactate uptake for aerobic metabolism, in a complex pattern (1, 13, 18). Neurons are highly dependent on aerobic metabolism and oxygen utilization, and they may succumb within a few minutes if deprived of substrate (glucose, lactate, oxygen), as in cerebral ischemia (25). As part of neuronal activation, there is spillover and release of byproducts that can then secondarily influence the surrounding cells and blood vessels, thereby affecting their metabolism. These mediators of astrocyte and vascular coupling include NO generation, K^+ release, glutamate release, H_2O_2, and lowering of extracellular lactate and oxygen, all contributing to astrocyte activation and a secondary (delayed) vascular response to enhance local blood flow and substrate delivery (3, 8–11, 26–28). This secondary vascular response, for example, also leads to enhanced oxygen, lactate, and glucose levels after neuronal activation, typically beyond what is needed for recovery (4, 13, 18). Neurons are adapted to this role, with lactate dehydrogenase (LDH) isoforms, for example, favoring lactate to pyruvate conversion (29), and monocarboxylate transport (MCT2) receptors on neurons augmented for pyruvate and lactate uptake at much higher affinity than those on astrocytes (MCT1 and MCT4) (5, 12, 30–32).

Astrocyte Mechanisms

Astrocytes form the boundary between the vascular network, substrate delivery, and neurons. Astrocytes connect blood vessels to the extracellular space and neurons: both astrocytes and blood vessels have similar glucose (Glut1) and monocarboxylate (MCT1) transporters, and astrocytes have end-feet on blood vessels that reinforce the blood brain barrier (i.e. beyond tight junctions between endothelial cells). Astrocytes are thought to be critically involved with glucose transport into the extracellular space, leading to a moderate level of glucose, partly due to limitations imposed by saturable transporters (i.e. 0.5–1.5 mmol/L) (9, 16, 17). Astrocytes also demonstrate an extensive network of gap junctions for extracellular homeostasis (i.e. Ca^{2+}, K^+, glutamate, and glucose buffering), which is enhanced in tissue culture (9, 33). Because astrocytes buffer the extracellular space, there are a variety of metabolic intermediates; once neurons are activated, these

FIGURE 1. Schematic of the important metabolic interactions between the cellular elements of the brain. A neuron is shown above and the primary roles of astrocytes are illustrated in terms of metabolic processing (center), as an interface to blood vessels and blood brain barrier (right), and as a syncytium with gap junctions interposed between individual astrocytes (left). Metabolites and their concentration in the extracellular space (glucose, brain lactate, and systemic lactate), and transporters (glucose Glut1 on endothelium and astrocytes, glucose Glut3 on neurons, monocarboxylate MCT1 on endothelium and astrocytes, MCT2 on neurons) are shown. α-KG indicates α-keto-glutarate; BBB, blood-brain barrier; eNOS/NO, endothelial nitric oxide synthetase and nitric oxide; LDH, lactate dehydrogenase; nNOS/NO, neuronal nitric oxide synthetase and nitric oxide; PC, pyruvate carboxylase; TCA, tricarboxylic acid cycle.

intermediates then signal to astrocytes and blood vessels to enhance the support for substrate repletion as well as continued activation without exhaustion. Candidates for metabolic signaling include K^+ (released after all action potentials), glutamate (uptake into astrocytes via glutamate transporters as well as direct receptor function on astrocyte glutamate receptors—i.e. metabotropic receptors), ammonia, lactate levels, oxygen levels, and diffusible factors (i.e. NO, H_2O_2, superoxide).

Astrocytes can also store reserve energy as glycogen, whereas neurons cannot (3, 6, 7). Glycogen has been implicated indirectly in neuron function, for example, buffering low glucose conditions (6). Note that glycogen reserves can be built up with extra substrate, such as glucose, pyruvate, or lactate, which can facilitate intrinsic glucose incorporation into glycogen rather than being consumed as an energy substrate (7). Glycogen release in the brain may be directly coupled primarily to low levels of extracellular glucose rather than hormonal factors. Glycogen is subsequently released either as glucose into the extracellular space or as lactate, after being routed through glycolysis.

Astrocytes are also specialized for glutamate uptake to buffer the high rate of neuronal release and limit postsynaptic neuronal activation because glutamate is highly toxic in the extracellular space (3, 10, 19). Inasmuch as neurons rapidly require glutamine in return for subsequent vesicular synthesis of glutamate, the bulk of the astrocytic TCA cycle is devoted to synthesis of α-KG, which is needed for glutamine synthesis rather than for energy production (3). A secondary result is also ammonia recycling between neurons and astrocytes. In addition to glutamine synthesis, astrocytes also have several key enzymes that differ from those in neurons, with respect to lipid synthesis, pyruvate carboxylation (to generate glutamate indirectly from glucose), nicotinamide adenine dinucleotide phosphate (NADPH) utilization, and LDH. For example, neurons can use ketone bodies during both early development and later in life, whereas astrocytes are more directly dependent on glucose (34).

Most ATP within astrocytes is for cell pumping requirements to maintain ionic exchange, likely arising from glycolysis, with rapid extrusion of lactate via MCT4 (12) and replenishment of the extracellular space (13, 18). There is clearly some energy derived from the TCA cycle because astrocytes are exquisitely sensitive to fluoroacetate, a highly toxic astrocyte poison that is converted only within astrocytes to fluorocitrate within mitochondria. However, many filopodia of astrocytes are too thin to allow for mitochondria, thereby creating spatial subdivisions that likely have different metabolic needs (2, 3).

Vascular coupling between neurons, astrocytes, and blood vessels is primarily due to diffusible factors, particularly NO, but which also hinges on a number of other factors, such as extracellular lactate (28). Other diffusible factors include reactive oxygen species (ROS), typically byproducts of neuronal metabolism such as superoxide, which is rapidly converted into H_2O_2 by superoxide dismutase. Oxygen itself is also a diffusible signal into blood vessels that regulates function and metabolism (26, 35). A secondary consequence of these ROS is that a control system is needed to handle the byproducts, which may also be damaging to cells. This antioxidant system includes glutathione, which requires NADPH from $NADP^+$-linked isocitrate dehydrogenase for functioning, as well as superoxide dismutase and catalase.

Astrocytes in Isolation

The local metabolic unit functions in a cooperative fashion, with neurons requiring considerable energy substrates (glucose, lactate) and glutamate synthesis from substrates (glutamine) derived from astrocytes and blood vessels; these, in turn release, a number of factors that must be buffered and can function as signals, including glutamate, K^+, NO, superoxide, and H_2O_2 (Fig. 1). In the absence of neurons (e.g. in isolated tissue culture), astrocytes develop even more robust syncytia with enhanced gap junctions compared with those in vivo. Extruded lactate can eventually be retransported back into astrocytes if the level rises sufficiently to match the low-affinity MCT1 and MCT4 receptors present on astrocytes, particularly higher than 5 to 8 mmol/L (30–32). Because there is less glutamate uptake, much less glutamate-glutamine recycling occurs and internally produced α-KG can be used directly for synthetic cell functions. Thus, astrocytes function differently in isolation because much of the extracellular buffering function is not needed.

Vascular and Extracellular Space Mechanisms

Because astrocytes can span between small blood vessels, they can facilitate substrate delivery to neurons through transporters into extracellular space, gap junctions, and likely glycogen reserves (9). Blood vessel diameter can change rapidly in response to both diffusible and extracellular factors (thereby modulating substrate delivery to local metabolic units), primarily through feedback from neurons and astrocytes (28). The BBB effectively divides the brain extracellular space from the systemic circulation, except for transported factors, particularly glucose, but transport can be saturated. For example, it has been shown in adult humans that systemic lactate is nearly completely isolated from brain extracellular lactate (16, 17) because of the limited MCTs present in adults. In infants, in contrast, there is considerable interchange because of a high level of MCT1 to allow ketone bodies to be taken up into the CNS. Monocarboxylate transports may also be upregulated in adults when they are on a ketogenic diet (34).

Astrocyte Summary

Astrocytes normally function as part of a metabolic unit in which their function is critical to support and enhance neuronal substrate delivery, assist in communication to blood vessels, and buffer byproducts resulting from neuronal activation (3). By their very nature, astrocytes are much less reliant on oxidative metabolism. Instead, they primarily function via complete glycolysis with lactate extrusion. The cataplerotic TCA cycle is key to glutamate-glutamine synthesis and recycling. There are a number of metabolic differences between neurons and astrocytes that, if fully present in astrocytoma cells, may offer key therapeutic opportunities.

NEURONAL-ASTROCYTE-VASCULAR METABOLIC UNIT VULNERABILITIES

A number of metabolic avenues that take advantage of the unique astrocyte (and astrocytoma) susceptibilities might

be useful for treatment of astrocytomas of various stages have now either been described or could be envisioned (Fig. 2). Recent reviews have summarized some of these approaches (36, 37). A recent discovery of isocitrate dehydrogenase (IDH) as being involved in earlier low-grade astrocytomas has also enhanced the possibility of the utility of metabolic approaches (38–41). There are a number of possibilities both for specific treatments and particularly to enhance the sensitivity of astrocytomas to more nonspecific treatments such as radiation therapy (42). These will be briefly summarized.

Acetate

Acetate is uniquely transported into astrocytes and is then converted to citrate within the TCA cycle. The astrocyte poison fluoroacetate maintains the acetate selectivity and provides a mechanism for directly separating astrocytic from neuronal metabolism on a short-term basis (3, 10). However, another acetate analog, dichloroacetate, has been suggested in early clinical trials as possibly following this route of selective toxicity for treatment of glioblastomas, through unique acetate susceptibility (43).

Glucose Uptake

Glucose uptake into astrocytes is normally via GLUT1 transporter, but neurons typically show GLUT3 transporters; GLUT3 has a higher affinity for glucose than GLUT1 (9, 33, 44). There are now several examples in which astrocytomas have switched to GLUT3 to accommodate a higher affinity for available glucose (44–47). However, because GLUT3 transporters are still critical for glucose uptake in neurons, an antagonist to GLUT3 may be useful, primarily in the vicinity of the tumor, to block local glucose uptake rather than on the entire brain; this would avoid neuronal toxicity from lack of glucose uptake.

Monocarboxylate Transporters

Neurons use MCT2, which has a much higher affinity for pyruvate (by a factor of >30) and lactate (by a factor of ~10), whereas astrocytes use MCT1, which has a low affinity for lactate; MCT4 appears to be involved in lactate extrusion from astrocytes (Fig. 1) (12, 18, 30–32). This low affinity for lactate uptake into astrocytes leads to extrusion and rarely any uptake at the normal extracellular level of ~2.5 mmol/L. However, if lactate levels rise to ~5 to 8 mmol/L, this reverses the concentration gradient, to foster lactate uptake by MCT1, thereby providing entry into glial cells rather than net extrusion. The normally low level of MCT1 present on the BBB in adults is different than that in young infants, in which high levels of MCT1 are present for uptake of ketone bodies during suckling (instead of glucose). Because astrocytomas may not be able to metabolize ketone bodies (i.e. β-hydroxybutyrate and acetoacetate) whereas neurons can, a ketogenic diet may provide a treatment effect by reducing available glucose levels, possibly starving astrocytoma cells of critical substrate (34, 46). A ketogenic (high-fat, low-carbohydrate) diet leads to hepatic production of ketone bodies and low systemic glucose, and the brain adjusts by upregulating the MCT1 transporters on the BBB (similar to infancy). In addition, as lactate levels build up within astrocytomas toward 5 to 8 mmol/L owing to the lack of uptake by neurons, the reversed uptake into astrocytoma cells may lead to alterations in intrinsic LDH within mitochondria, for rapid generation of pyruvate (48).

Glutamate Uptake and Transporters

Astrocytes have highly selective glutamate uptake for elimination of extracellular glutamate after synaptic release and then recycling of glutamine back into neurons (3, 19). This selectivity leads to different uses of TCA cycle for synthesis of α-KG and subsequently glutamine. Inasmuch as there is minimal glutamate recycling within astrocytomas owing to the paucity of neurons, these cells may use glutamate as an alternative energy source to glucose, using alternative pathways in different ways (49) possibly because of IDH1 mutations. Alternatively, glutamine could be metabolized directly for energy in addition to protein synthesis (46, 50). Specific antagonists to block such enzymes may prevent glutamate/glutamine from being scavenged for metabolic substrate in astrocytomas.

Lactate Dehydrogenase

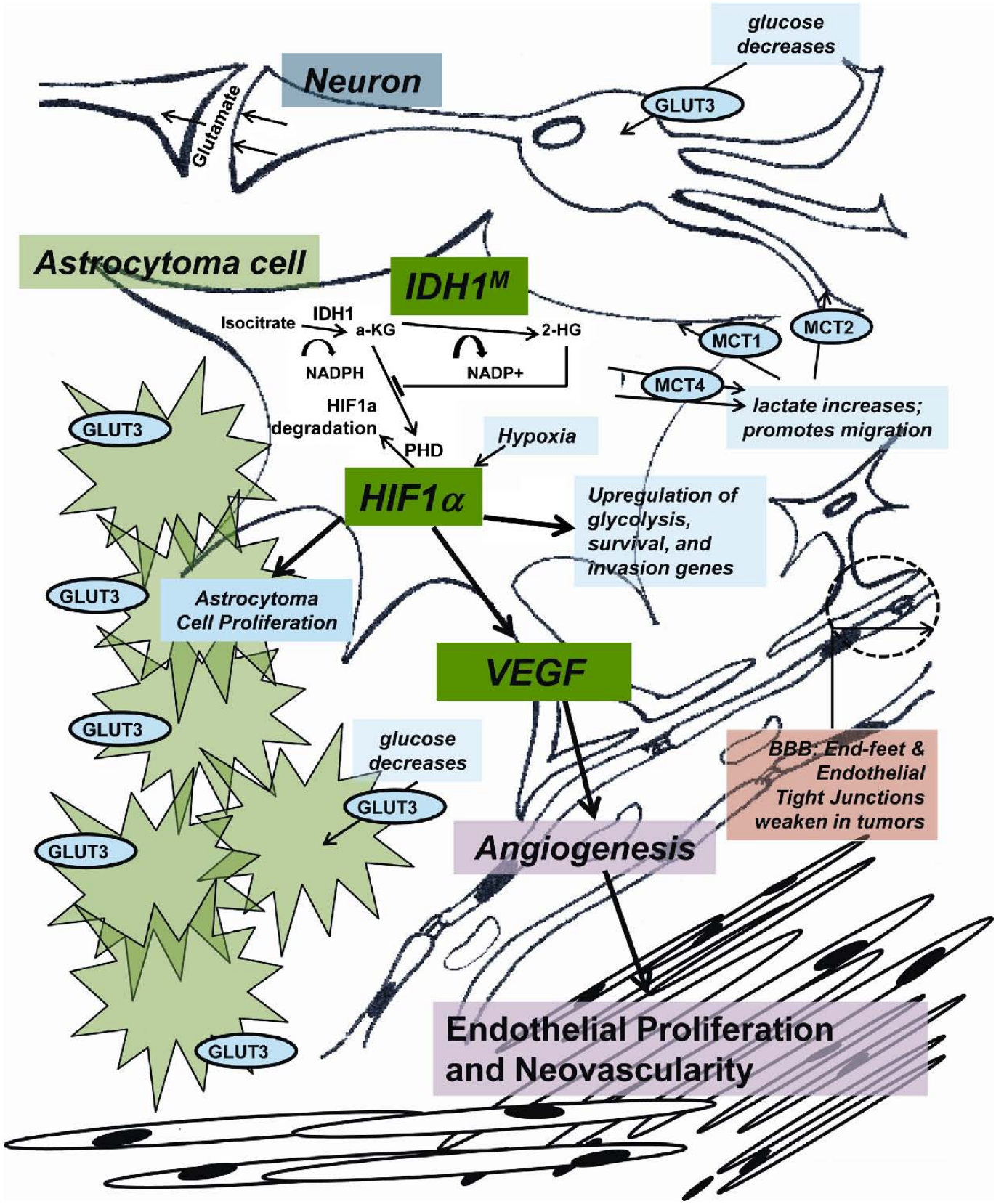
The usual cytoplasmic isoforms of LDH differ in function: neuronal LDH is biased toward reverse lactate to pyruvate conversion, whereas astrocytes primarily function with forward LDH of pyruvate to lactate conversion (29). If lactate builds up extracellularly and is reverse transported back into astrocytes, other LDH isoforms such as mitochondrial LDH may function to use lactate (48, 51). Targets such as LDH itself may serve to limit glycolysis (52). High extracellular lactate levels may also contribute to an acidic microenvironment within astrocytomas, as well as promote cellular migration through transforming growth factor–mediated regulation of matrix metalloproteinase-2 (53). High lactate levels have been noted in both MRI spectroscopy studies of astrocytomas as well as direct extracellular dialysis studies of these tumors (54–56). Such high lactate levels are clearly predicted by the highly active glycolysis scheme predicted in the *Warburg effect* (22). Thus, another metabolic target may be the astrocytic version of LDH to block pyruvate conversion into lactate.

Glycogen

Astrocytes exclusively demonstrate mechanisms for internal glycogen storage, on which a number of physiological processes may depend (6, 7). For example, glycogenolysis antagonists severely affect the brain's response to low glucose. Glycogen synthase may also be a target for astrocytoma cells by inhibiting gluconeogenesis (57). Antagonists for both glycogenolysis and glycogen storage are readily available but not yet tried to partially block astrocytoma cells.

Gap Junctions

Astrocytes form a syncytium where small molecules (e.g. glucose, K^+, and glutamate) can be diffused between multiple cells, moving metabolites away from a focal area to other uninvolved areas and possibly to and from blood vessels for disposition. These gap junctions may be important both to dissipate toxins (i.e. excess K^+) and to enhance glucose and



lactate delivery to neurons (9, 33). Gap junctions have several specific antagonists and may be very important in the disconnection of astrocytoma cells to prevent diffusion of substrate between tumor cells (33).

Pyruvate Carboxylase and Alternative Metabolic Substrates

This enzyme is mostly situated within astrocytes and allows indirect conversion of glucose to glutamate for use in both the TCA cycle, but also particularly for synthesis of glutamate for eventual transition to neurons (3). The selective presence of this enzyme within astrocytes facilitates a much greater flexibility for glucose utilization than is present within neurons. Other enzymes that have been targeted as abnormal in astrocytoma cells include pyruvate kinase (the last step to pyruvate generation in glycolysis), which shows alternative splicing in astrocytomas (58) and other enzymes in glucose (21, 51, 59) and pentose cycle metabolism unique to astrocytomas (60). Inasmuch as other substrates may wane because of decreased substrate supply, even purines and pyrimidines may serve in metabolism for ATP generation in astrocytomas (61). These various enzymes may be antagonized with specific drugs, which are not likely to affect neuron functioning.

Ischemia

Neurons have high levels of oxidative metabolism and are very susceptible to hypoxia and ischemia: they can only tolerate lack of substrate for 3 to 5 minutes before cell death occurs, particularly if there is a metabolic demand superimposed (27). However, on exposure to a sublethal hypoxic or ischemic episode, neurons and astrocytes can develop resistance through activation of hypoxia-inducible factor (HIF1α), which enhances glycolysis, leads to increased blood vessel formation (via vascular endothelial growth factor [VEGF] release), and leads to the second ischemic episode being better tolerated (35, 62). However, astrocytes can survive in low oxygen conditions and can adapt to low glucose conditions by increasing affinity for glucose and a number of adaptive schemes, including managing metabolism with alternative substrates. Astrocytomas (and cancer cells in general) tolerate hypoxia very well, likely through HIF1α induction, which stimulates a wide range of events, including a higher efficiency of glucose uptake and glycolysis and enhanced VEGF for induction of angiogenesis. Although HIF1α is normally induced in neurons and astrocytes (63), it is currently actively targeted for its role in astrocytomas through a variety of inhibitors (45, 64–66). Selectivity for astrocytomas may arise through induction of enhanced oxygenation in the normal brain so as not to cause adverse effects throughout the brain.

Isocitrate Dehydrogenases

Some isoforms of these enzymes (IDH 1-3) catalyze the oxidative decarboxylation of isocitrate to α-KG in the TCA cycle (67). Within mitochondria, the relevant IDH isoforms use NAD^+ as a cofactor, which is converted into NADH and can then be oxidized in the electron transport chain to maintain ATP levels (18, 25, 27). IDH1 localizes to the cytoplasm, whereas IDH2 and IDH3 are found in mitochondria. IDH1 and 2 share 70% sequence homology. Cellular glucose sensing and TCA cycle function are clearly regulated by these enzymes. Pyruvate enters the TCA cycle and is converted to isocitrate, which exits the mitochondria via a citrate/isocitrate carrier. IDH1 and 2 reversibly convert isocitrate to α-KG and generate NADPH (Fig. 2). These products promote insulin secretion systemically but likely regulate glucose metabolism via other mechanisms in astrocytes. Conversion of α-KG also affects astrocyte glutamate and glutamine metabolism. The NADPH produced by these enzymes is an important reducing agent, essential for the reduction of glutathione for antioxidative protective mechanisms. IDH1 is more important for α-KG formation and seems to be more localized to astrocytes than neurons (68). IDH1 or 2 deficiencies lead to increased lipid peroxidation, oxidative DNA damage, peroxide generation, and decreased cell survival (69). Mutations in IDH1, particularly at arginine codon 132, are found in 50% to 94% of World Health Organization grade 2 and 3 astrocytomas and secondary glioblastomas that arise from lower-grade astrocytomas (67). IDH1 mutations are thought to occur before *TP53* mutations, one of the more common genetic alterations in these tumors, which have led to the theory that IDH1 mutations arise in the transition from a normal astrocyte to a tumor.

The functional consequences of IDH1 mutations may create vulnerabilities that can be exploited for astrocytoma therapies. Loss of normal enzymatic activity is clearly established via dominant inhibition of any wild-type enzymes during dimerization. More interesting is the possibility of a neomorphic or abnormal gain of function effect. Unbiased metabolite profiling has revealed that expression of IDH1 arginine132 mutations leads to production of 2-hydroxyglutarate (2-HG), a potential oncometabolite (49). One potential effect of 2-HG is the regulation of HIF1α that promotes tumorigenesis (Fig. 2). HIF1α is hydroxylated by HIF prolyl hydroxylase (PHD), which targets HIF1α for ubiquitylation and proteosome degradation. PHD requires oxygen and α-KG. By converting α-KG to 2-HG, mutant IDH1 lowers the availability of α-KG, which then decreases PHD activity (along with a hypoxic environment) and leads to HIF1α enhancement and/or stabilization. HIF1α functions as a transcription factor that will activate transcription of genes that promote metabolism, invasion,

FIGURE 2. Schematic of the subversion of normal cellular metabolic interactions for the maintenance and growth of astrocytoma cells. Rapid astrocytoma tumor growth and division lead to hypoxia and depletion of extracellular glucose, causing a switch to the more sensitive Glut3 glucose transporter, increases in extracellular lactate, promotion of migration, upregulation of HIF1α to enhance glycolysis, upregulation of VEGF to enhance angiogenesis, and blood-brain barrier breakdown. Many ordinary characteristic astrocyte enzymatic and cellular processes are enhanced with this transformation into a tumor phenotype, yet still may provide susceptible points of metabolic treatments. 2-HG indicates 2-hydroxyglutarate; α-KG, α-keto-glutarate; BBB, blood-brain barrier; HIF1α, hypoxia-inducing factor; IDH, isocitrate dehydrogenase (IDH1M is a mutated version); PHD, HIF prolyl hydroxylase; VEGF, vascular endothelial growth factor.

angiogenesis, and ultimately more survival in astrocytoma cells. In addition, IDH1 mutations could lead to a selective advantage for tumor cells through glucose sensing. Less IDH enzyme activity leads to lower cytosolic NADPH levels, which would signal a low nutrient status in glucose sensing pathways. Tumor cells would compensate by increasing nutrient consumption or perhaps by blocking differentiation. Lastly, IDH1 mutations would increase mutagenesis that favors tumorigenesis. Unchecked oxidative stress would lead to mutations from ROS exposed to the genome.

Astrocyte Cell of Origin

Clearly, astrocytes have the capability to divide, but the actual origin of these cells has remained unclear. When neurospheres are isolated from subventricular zones (SVZ) and plated on substrate without additional growth factors, their natural fate is to become astrocytes (70). Brain-derived neurotrophic factor and other growth factors were required to induce neuronal differentiation (70). Neurosphere formation requires 2 key elements: 1) removal from the in vivo environment, which presumably exerts too much contact inhibition of growth and proliferation and oxygen into a flask with no substrate (i.e. uncoated plastic), and 2) addition of growth factors, particularly epidermal growth factor (EGF) and/or fibroblast growth factor. Even many years later, the natural fate of these cells in vivo remains to be fully delineated. It has recently been noted that astrocytoma neurospheres can also be cultured from samples of tumors, but they have more extensive chromosome derangements and other astrocytoma abnormalities (71). The ability to culture astrocytoma neurospheres from tumors suggests that the primary differentiation of astrocytes from the SVZ via asymmetric division has itself become deranged (72). Because these SVZ cells are presumably lifelong, there may be a consideration that, as the organism ages, there may be subsequent chromosomal abnormalities within a subpopulation of the SVZ cells themselves. A recent study of aged neural progenitor cells (which can also form neurospheres) suggests that aged cells are more likely than fresh cells to develop into astrocytomas, particularly after 12 to 18 months (73). This finding may highlight the common experience that, as individuals age, they are much more susceptible to developing malignant astrocytomas, particularly glioblastomas (37). Intrinsic SVZ cells also demonstrate sensitivity to EGF, which they require for growth. In many instances, EGF receptors are either overexpressed or mutated into constitutively active receptors in astrocytomas, demonstrating a link to inherent stem cells (36). There have been a large number of clinical trials of astrocytoma treatments focusing on EGF receptors (36, 37). These observations support the idea that similarities may exist between astrocytoma cell and normal immature astrocyte metabolism, because they may have common cells of origin.

Other Approaches

Other metabolic approaches include susceptibility to ROS and redox systems for cell maintenance such as thioredoxin (74), manipulation of the mitochondrial membrane potential via a selective action on astrocytoma cells to alter the metabolism (75), and possible intervention to the action of diffusible signals such as NO, which may be abnormal in astrocytomas (76). There is some concern that CD133, which is generally considered to be a marker of stem cells in neurospheres (along with nestin), may have a component that is related primarily to metabolic stress (77). CD133 clearly is not an ideal target for astrocytoma stem cells because CD133-negative cells that can give rise to astrocytomas have been discovered. In addition, neurons may favor switching of glycolysis into metabolic pathways such as pentose phosphate, which leads to enhanced glutathione and protection from ROS, rather than use glucose metabolism for energy (78). Alternative energy substrates to glucose and the induction of low glucose conditions, such as during a ketogenic diet, may thus considerably enhance toxicity to astrocytoma cells while minimally affecting neurons if alternative substrates for oxidative metabolism can be supplied rather than glucose.

Astrocytoma Summary

There are clearly many mechanisms associated with astrocytoma formation, including several different chromosome abnormalities, mutations in metabolic enzymes (38), increasing rates of mitosis, angiogenesis, and alterations of growth factor receptors, which all have treatments directed specifically toward these mechanisms. However, further treatments might include delineation of abnormal enzymes or those in which mutant enzymes may alter function, therapy directed specifically at astrocytoma stem cells, other aspects of metabolism pertinent to astrocytes (and particularly astrocytomas), and the local astrocytoma cell environment (e.g. the susceptibility to invade surrounding tissue). Astrocytoma cells do seem to behave like native immature astrocytes in many respects, demonstrating primarily glycolytic and synthetic metabolic pathways, lactate efflux, enhanced glucose uptake, low oxygen conditions, HIF1α upregulation, and likely high gap junction presence. These features are also in the absence of neurons because neurons disappear rapidly within the confines of an astrocytoma. Thus, advances in genetics and metabolic dysfunction may be reaching toward critical thresholds in the treatment of this difficult disease (37, 79, 80).

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