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Interplay of Carbonic Anhydrase IX With Amino Acid and Acid/Base Transporters in the Hypoxic Tumor Microenvironment

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Solid tumors are challenged with a hypoxic and nutrient-deprived microenvironment. Hence, hypoxic tumor cells coordinatively increase the expression of nutrient transporters and pH regulators to adapt and meet their bioenergetic and biosynthetic demands. Carbonic Anhydrase IX (CAIX) is a membrane-bound enzyme that plays a vital role in pH regulation in the tumor microenvironment (TME). Numerous studies have established the importance of CAIX in mediating tumor progression and metastasis. To understand the mechanism of CAIX in mediating tumor progression, we performed an unbiased proteomic screen to identify the potential interactors of CAIX in the TME using the proximity-dependent biotin identification (BioID) technique. In this review, we focus on the interactors from this BioID screen that are crucial for nutrient and metabolite transport in the TME. We discuss the role of transport metabolon comprising CAIX and bicarbonate transporters in regulating intra- and extracellular pH of the tumor. We also discuss the role of amino acid transporters that are high confidence interactors of CAIX, in optimizing favorable metabolic state for tumor progression, and give our perspective on the coordinative interplay of CAIX with the amino acid transporters in the hypoxic TME.

Keywords: tumor microenvironment, hypoxia, carbonic anhydrase IX, amino acid transport, tumor metabolism

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INTRODUCTION

Tumor cells metabolize nutrients in an anabolic or catabolic mode to maintain their biosynthetic and bioenergetic demands, respectively. In a catabolic pathway, nutrients are broken down to generate energy for maintaining cellular integrity. Whereas, in an anabolic pathway, they are utilized to build new macromolecules such as nucleotides and amino acids that support cell growth and proliferation. Tumor cells can alter their metabolism in favor of either of these pathways based on their requirements, which is called metabolic reprogramming/rewiring (Ward and Thompson, 2012). Besides, tumor cells can utilize a diverse array of nutrients such as glucose, glutamine (Gln), essential amino acids, and fatty acids, thus offering them metabolic flexibility (DeNicola and Cantley, 2015). Both, metabolic reprogramming and flexibility give tumor cells the plasticity to adapt to any metabolic shifts and survive. Several cell-intrinsic and extrinsic factors influence metabolic reprogramming and flexibility in tumor cells (DeNicola and Cantley, 2015). One of the most important extrinsic factors is oxygen availability in the tumor microenvironment (TME)

(Nakazawa et al., 2016). Solid tumors are characterized by chaotic, immature vasculature that causes zones of varying oxygen tensions within the tumor. Depending on the proximity to blood vessels, tumors are comprised of poorly perfused, chronic hypoxic zones, and intermittently perfused, cycling hypoxic zones (Michiels et al., 2016). To survive the nutrient and oxygen deprivation caused by insufficient perfusion, tumor cells trigger hypoxia-inducible factor (HIF) signaling, which culminates in the stabilization and activation of the transcription factor, HIF1α or HIF2α, and alters the expression of several downstream targets to promote survival, tumor growth and progression (Xie and Simon, 2017). One of the HIF1α regulated proteins that play an important role in the hypoxic TME is the Carbonic Anhydrase IX (CAIX) (Wykoff et al., 2000; McDonald and Dedhar, 2014).

CAIX – FUNCTION AND ROLE IN CANCER

Carbonic anhydrase IX is a dimeric, membrane-bound metabolic enzyme that belongs to the carbonic anhydrase (CA) family (Alterio et al., 2012). It plays a crucial role in pH regulation through the reversible hydration of carbon dioxide into bicarbonate and proton. CAIX comprises of extracellular facing proteoglycan (PG) and catalytic (CA) domains, a transmembrane (TM) domain, and an intracytoplasmic (IC) domain (Opavský et al., 1996). The presence of the PG domain is a unique feature of CAIX and is absent in other isozymes of the CA family. The dimerization of CAIX is mediated by the formation of a disulfide bond between the Cys-41 residue located on the CA domain (Alterio et al., 2009). Although CAIX expression is primarily driven under hypoxia through the HIF1α stabilization, the presence of extracellular lactate (Panisova et al., 2017) and glutamate (Glu) (Briggs et al., 2016) have also been shown to stabilize HIF1α and promote CAIX expression under normoxia. CAIX is predominantly expressed in solid tumors, with restricted expression in normal tissues (McDonald et al., 2012; Mboge et al., 2018) and, its expression can be correlated with poor prognosis (Chia et al., 2001; Loncaster et al., 2001; Klatte et al., 2009; Korkeila et al., 2009; Ilie et al., 2010) and response to therapy in solid tumors (Koukourakis et al., 2001; Generali et al., 2006; Tan et al., 2009; McIntyre et al., 2012). The role of CAIX in various steps of tumor progression and metastasis is well established in the past decade. Targeting CAIX, both, by genetic depletion and using small molecule inhibitors, has elucidated the importance of CAIX in tumor growth *in vivo* (Lou et al., 2011). In addition to its role in tumor growth, CAIX plays a crucial role in metastasis (Lou et al., 2011; Gieling et al., 2012; Chafe et al., 2015). Before the cancer cells metastasize to a distant site, they establish a conducive microenvironment for their survival, called the pre-metastatic niche. CAIX promotes granulocyte colony-stimulating factor (G-CSF) production by hypoxic breast cancer cells, which helps in the mobilization of granulocytic myeloid-derived suppressor cells to lung metastatic niche and primes for metastasis (Chafe et al., 2015). Furthermore, CAIX helps in the maintenance of stemness in cancer stem cells and favor metastasis (Lock et al., 2013; Gibadulinova et al., 2020; Peppicelli et al., 2020). While it is evident that CAIX is important in mediating various steps in tumor progression, the underlying mechanisms remain unclear. Considering the importance of CAIX in the hypoxic microenvironment, it is plausible that CAIX interacts with other proteins in tumor cells to mediate various functions. Hence, we recently conducted a comprehensive, unbiased study to identify the protein interactome of CAIX using the proximity-dependent biotinylation labeling technique called the BioID method (Roux et al., 2012). This study identified over 140 high confidence protein interactors of CAIX (Swayampakula et al., 2017). In this mini review, we will focus on the amino acid transporters (AATs) and acid/base transporters that were identified as high confidence interactors.

CAIX AND PH REGULATION

Active metabolism within tumor cells leads to the accumulation of acidic metabolic by-products, which, if unbuffered, will be lethal to the tumor cells. Therefore, tumor cells deploy several membrane acid/base transporters (pH regulators) to establish a favorable pH within the tumor cells (Neri and Supuran, 2011). Two major acidic metabolic by-products that are produced by tumor cells are CO_2 and lactic acid (Corbet and Feron, 2017). While CO_2 is predominantly produced as a by-product of aerobic respiration, lactic acid production is a result of anaerobic respiration or aerobic glycolysis in tumor cells. The CO_2 generated by tumor cells acts as a substrate for CAs, to produce bicarbonate and protons. Lactic acid, on the other hand, is extruded out of the cells by monocarboxylate transporters (MCT) as lactate and protons, or buffered intracellular by bicarbonate ions to produce CO_2 (Sun et al., 2020). The contribution of CO_2 and lactic acid in defining the intratumoral pH will depend on factors such as oxygenation and mitochondrial respiration in tumor cells. In deep hypoxic zones of a tumor, the mitochondrial respiration is impeded, and therefore, glycolysis becomes the primary state of metabolism (Corbet and Feron, 2017). Conversely, in moderately hypoxic zones of the tumor, the lactate that is released by surrounding anaerobic cells or Gln imported into cells can feed the TCA cycle and drive oxidative phosphorylation (Corbet and Feron, 2017; Faubert et al., 2017). Using tumor spheroids, Swietach et al. (2009, 2010) demonstrated that in spheroids of up to 300 um in size, CO_2 released by the mitochondria acts as a major substrate for CAIX activity rather than lactic acid accumulation. The source of CO_2 can either be from the tumor cells or can be provided to anaerobic regions by surrounding aerobic cells.

Carbonic anhydrase IX establishes a pH gradient of alkaline intracellular pH and acidic extracellular pH in tumor cells that helps in survival and tumor growth (Chiche et al., 2009). Maintenance of intracellular pH by CAIX is critical to support glycolysis and help cancer cells to adapt under hypoxia (Benej et al., 2020). Numerous studies have shown that CAs associate with acid/base transporters to form a temporary complex called transport metabolon (Deitmer and Becker, 2013). CAs form a transport metabolon with MCTs to effectively shuttle the protons from and to MCT, and enhance its activity (Klier et al.,

2014). In CAIX, the 18 Glu and 8 Asp residues in the PG domain have been proposed to act as proton antenna or proton collectors (Ames et al., 2018), whereas the His200 in the catalytic domain facilitates the proton shuttle from the catalytic center into surrounding space, and support MCT activity (Jamali et al., 2015). Another important transport metabolon in the context of CAIX is the bicarbonate metabolon that involves the association of CAIX with bicarbonate transporters. CAIX co-localizes and functionally cooperates with the bicarbonate transporter, NbCe1 (SLC4A4), in the invadopodia to achieve an alkaline pH that promotes invadopodia formation (Debreova et al., 2019). Additionally, CAIX interacts with matrix metalloproteinase 14 (MMP14) in invadopodia. MMP14 is a proteolytic enzyme that degrades the extracellular matrix (ECM) and its activation is important for invadopodial function. The association of CAIX with MMP14 provides protons for MMP14 activation and therefore helps in the invadopodial function (Swayampakula et al., 2017). The increased MMP14 activity, coupled with the intracellular alkalinization within the invadopodia, aids in invadopodia elongation and therefore in tumor cell invasion.

The sodium-bicarbonate transporter, NBCn1 (also known as SLC4A7) is a high confidence interactor of CAIX that emerged in the BioID study. Genome-wide association studies have shown NBCn1 to be a causative gene in breast cancer (Ahmed et al., 2009). NBCn1 functions as an acid extruder and creates a favorable pH gradient in tumors (Boedtkjer et al., 2013; Lee et al., 2016). Furthermore, loss of function studies by the genetic depletion of NBCn1 has elucidated its role in tumor growth (Lee et al., 2016) and cell cycle progression (Flinck et al., 2018). Considering the importance of this bicarbonate transporter in regulating pH in tumor cells, it may mediate an important function by forming a bicarbonate metabolon with CAIX. However, to this date, the role of this interaction remains uninvestigated.

CAIX AND AMINO ACID TRANSPORT

Hypoxic zones in the tumor have a restricted supply of nutrients and therefore continually adapt to metabolize various nutrients to maintain their biologic functions (Samanta and Semenza, 2018). Amino acids are a major source of carbon and nitrogen for the biosynthesis of various macromolecules (**Figure 1**). In this section, we will discuss three AATs that were identified as potential interactors of CAIX from the BioID screen (**Table 1**). We will describe the role and regulation of these transporters in cancer, and then discuss how these transporters may work coordinatively with CAIX in the hypoxic TME.

ASCT2

The Alanine Serine Cysteine Transporter 2 (ASCT2) aka SLC1A5, is a plasma membrane amino acid transporter that mediates sodium-dependent antiport of neutral amino acids. Despite what the name suggests, ASCT2 preferentially transports glutamine, while cysteine acts as a modulator of the transport (Utsunomiya-Tate et al., 1996; Scalise et al., 2018). ASCT2 is a trimeric protein comprising a scaffold domain that enables the interaction between protomers, and a transport domain that helps in the amino acid transport (Garaeva et al., 2018). As one of the major glutamine transporters in cells, ASCT2 is ubiquitously expressed across various tissues in the body and plays a crucial role in mediating cellular functions such as hematopoietic stem cell differentiation (Oburoglu et al., 2014) and T-cell activation (Nakaya et al., 2014; Poffenberger et al., 2014). Increased expression of ASCT2 is observed in several cancer types and is associated with poor prognosis (Witte et al., 2002; Shimizu et al., 2014; Kaira et al., 2015b; Liu et al., 2015; Sun et al., 2016; Bernhardt et al., 2017). The upregulated cellular expression of ASCT2 in cancer is mediated by oncogenic signals such as Kirsten rat sarcoma (K-Ras) (Toda et al., 2017) and myelocytomatosis (N-Myc) (Ren et al., 2015). K-Ras plays an important role in mediating various growth signaling pathways in cells. Mutation in K-Ras is shown to upregulate the expression of ASCT2 and promote cell proliferation in colorectal cancer (Toda et al., 2017). N-Myc, on the other hand, is a transcription factor that drives the expression of genes involved in cell proliferation. Ren et al. (2015) showed N-Myc to upregulate ASCT2 expression by directly binding to its promoter region. In addition to oncogenic signals, cellular stress such as amino acid starvation can also upregulate ASCT2 expression. Under amino acid deprivation, a stress response transcription factor called activating transcription factor 4 (ATF4) binds to ASCT2 promoter and increases ASCT2 expression (Ren et al., 2015). Functional studies using *in vitro* and *in vivo* models have shown ASCT2 inhibition to effectively reduce tumor growth in various types of cancer (van Geldermalsen et al., 2016; Marshall et al., 2017; Ye et al., 2018) by attenuating the mechanistic Target of Raptor (mTOR) signaling pathway (**Figure 1**; Wang et al., 2014, 2015). Furthermore, ASCT2 has been shown to facilitate Gln uptake in cancer stem cells and promote tumor growth in pancreatic ductal adenocarcinoma (PDAC) (Wang V.M. et al., 2019). Based on this evidence, it can be concluded that ASCT2 plays an important role in tumorigenesis and is an attractive candidate to target cancer. Over the years, several drug candidates to target ASCT2 have been discovered. However, identifying drugs that selectively target ASCT2 has been a challenge due to limited structural studies until recently (Jiang et al., 2020). The recent development of an antagonist, V9302 by Schulte et al. (2018) has shown promise in targeting ASCT2 (Scopelliti et al., 2018).

SNAT2

Sodium coupled neutral amino acid transporter 2 (SNAT2) aka SLC38A2, mediates uniport of neutral amino acids including glutamine in a sodium-dependent manner (Mackenzie and Erickson, 2004). It comprises of 11 hydrophobic membrane-spanning domains with an extracellular C-terminus and an intracellular N-terminus (Ge et al., 2018). SNAT2 expression is regulated by extracellular amino acid. Under amino acid deprivation, the global translation is halted, with a concomitant increase in the ATF4 translation. The binding of ATF4 to the amino acid response element (AARE) on the SNAT2 promoter increases SNAT2 expression (Palii et al., 2006). Conversely, SNAT2 can sense the presence of amino acids such as Tyr and Gln, and inhibit its expression (Hyde et al., 2007; Hundal

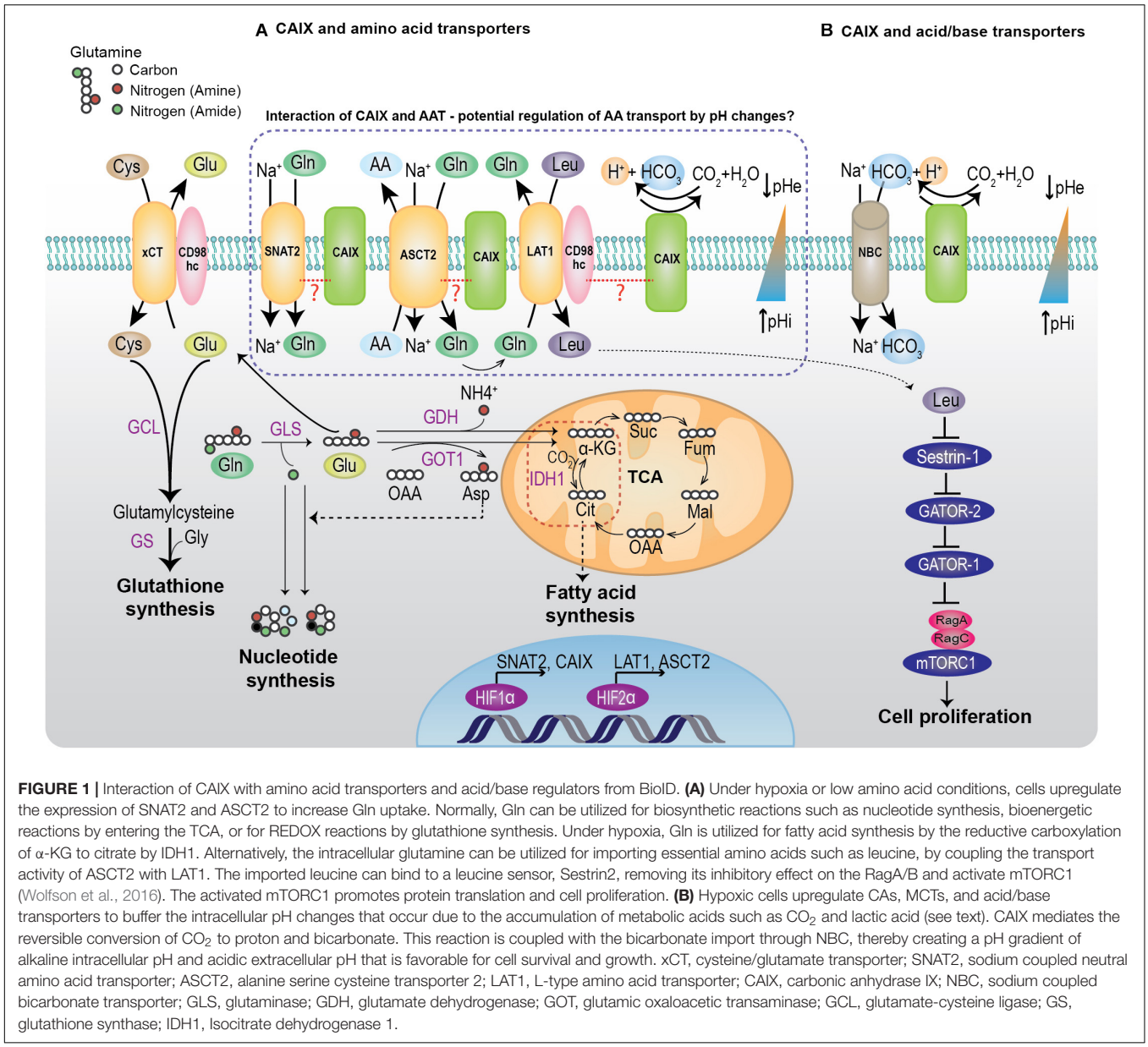

FIGURE 1 | Interaction of CAIX with amino acid transporters and acid/base regulators from BioID. **(A)** Under hypoxia or low amino acid conditions, cells upregulate the expression of SNAT2 and ASCT2 to increase Gln uptake. Normally, Gln can be utilized for biosynthetic reactions such as nucleotide synthesis, bioenergetic reactions by entering the TCA, or for REDOX reactions by glutathione synthesis. Under hypoxia, Gln is utilized for fatty acid synthesis by the reductive carboxylation of α-KG to citrate by IDH1. Alternatively, the intracellular glutamine can be utilized for importing essential amino acids such as leucine, by coupling the transport activity of ASCT2 with LAT1. The imported leucine can bind to a leucine sensor, Sestrin2, removing its inhibitory effect on the RagA/B and activate mTORC1 (Wolfson et al., 2016). The activated mTORC1 promotes protein translation and cell proliferation. **(B)** Hypoxic cells upregulate CAs, MCTs, and acid/base transporters to buffer the intracellular pH changes that occur due to the accumulation of metabolic acids such as CO_2 and lactic acid (see text). CAIX mediates the reversible conversion of CO_2 to proton and bicarbonate. This reaction is coupled with the bicarbonate import through NBC, thereby creating a pH gradient of alkaline intracellular pH and acidic extracellular pH that is favorable for cell survival and growth. xCT, cysteine/glutamate transporter; SNAT2, sodium coupled neutral amino acid transporter; ASCT2, alanine serine cysteine transporter 2; LAT1, L-type amino acid transporter; CAIX, carbonic anhydrase IX; NBC, sodium coupled bicarbonate transporter; GLS, glutaminase; GDH, glutamate dehydrogenase; GOT, glutamic oxaloacetic transaminase; GCL, glutamate-cysteine ligase; GS, glutathione synthase; IDH1, Isocitrate dehydrogenase 1.

TABLE 1 | List of potential CAIX interactors with role in nutrient or metabolite transport function from the BioID.

Functional class	Gene symbol	Gene name	Biological role
Amino acid transporters	SLC3A2 or CD98hc	Solute carrier family 3 member 2 or Cluster differentiation 98 heavy chain	Amino acid transport heavy chain subunit. Forms a complex with light chain subunit to create functional heteromeric amino acid transporter
	SLC7A5 or LAT1	Solute carrier family 7 member 5 or L-type amino acid transporter 1	Sodium independent transport of large neutral amino acids such as Leu, Ile, Val, His, Met, Trp, and Phe Tyr (Kanai et al., 1998)
	SLC1A5 or ASCT2	Solute carrier family 1 member 5, ASCT2 or Alanine Serine Cysteine transporter 2	Sodium-dependent transport of neutral amino acids such as Glu, Gln, Ala, Ser, Thr, Val, and Gly (Utsunomiya-Tate et al., 1996)
	SLC38A2 or SNAT2	Solute carrier family 38 member 2 or Sodium coupled neutral amino acid transporter 2	Sodium-dependent transport of neutral amino acids such as Ala, Gln, Ser, Met, Asn, Cys, Gly, His, and Pro (Mackenzie and Erickson, 2004)
Acid/base transporter	SLC4A7 or NBCn1	Solute carrier family 4 member 7 or Sodium bicarbonate cotransporter 3	Sodium bicarbonate cotransport

and Taylor, 2009). Besides extracellular amino acid, SNAT2 expression is regulated by endoplasmic reticulum stress (ERS). In breast cancer cells, paclitaxel-induced ERS causes a ubiquitin ligase, RNFα to associate with SNAT2 and ASCT2, and cause their degradation. This leads to decreased Gln uptake, decreased proliferation, and increased cell death in the tumor cells (Jeon et al., 2015; Moses and Neckers, 2015). Studies from Broer's group have elucidated that SNAT2 expression increases upon the disruption of ASCT2 transporter activity (Broer et al., 2016, 2019), thereby classifying SNAT2 as a rescue transporter. In addition to its role as a Gln transporter, SNAT2 has shown to play an important role in transporting Ala into PDAC cells from the surrounding pancreatic stellate cells (Parker et al., 2020). Furthermore, genetic depletion of SNAT2 in PDAC cells decreases the Ala uptake and reduce tumor growth *in vivo* (Parker et al., 2020). These studies highlight the importance of SNAT2 in cancer, however, the clinical relevance of this transporter in cancers remains unexplored.

LAT1

The L-type amino acid transporter (LAT1) aka SLC7A5, is a heterodimeric amino acid transporter that mediates a sodium independent antiport of neutral essential amino acids (Kanai et al., 1998; Wagner et al., 2001). It comprises a heavy chain subunit called cluster differentiation 98 (CD98hc) that associates with a light chain subunit such as LAT1. The heavy chain consists of an extracellular C-terminus, a transmembrane helix, and an intracellular NH2 terminus. The light chain, on the other hand, has 12 transmembrane domains with both COOH and the NH2 termini facing the intracellular space (Yan et al., 2019). While LAT1 is expressed across various tissues, it is highly expressed at the blood-brain barrier and functions in amino acid transport to the brain (Boado et al., 1999). The importance of LAT1 in tumor growth was elucidated by Cormerais et al., using knock out models for LAT1 and CD98hc. This study showed the genetic disruption of LAT1 to decrease leucine uptake and inhibit mTORC1, causing decreased tumor growth *in vivo*. N-Myc upregulates LAT1 expression by directly binding to the promoter region of LAT1. The resulting amino acid uptake promotes the sustained translation of Myc, thereby working in a feed-forward loop that helps in tumorigenesis (Yue et al., 2017). LAT1 expression is associated with poor prognosis (Kaira et al., 2015a; Shimizu et al., 2015) and resistance to chemotherapy in solid tumors (Altan et al., 2018). Downregulation of LAT1 has been shown to decrease cell growth (Marshall et al., 2016) invasion, and migration (Janpipatkul et al., 2014). Targeting LAT1 using a small molecule inhibitor, JPH203 has shown success in pre-clinical trials and was recently evaluated in clinical phase trial 1. Although the sample size was low in this clinical trial, the drug was well tolerated and showed promise in targeting LAT1 in patients with advanced solid tumors (Okano et al., 2018).

Potential Role of CAIX in Amino Acid Transport Regulation

As discussed above, AATs play important roles in promoting tumor progression, and their interaction with CAIX suggests an important mode of functional regulation that requires further investigation.

It is well-known that Gln is an important amino acid in cancer metabolism and several cancer types rely on Gln, which is called Gln addiction (Wise and Thompson, 2010). In hypoxic tumor cells, Gln is channeled for lipid biosynthesis to support cell proliferation (**Figure 1**). This process is mediated by the reductive carboxylation of α-ketoglutarate (α-KG) to citrate, and the subsequent entry of citrate into lipogenesis (Metallo et al., 2011). Furthermore, it is shown that Gln carbon and nitrogen are efficiently metabolized to support lipid biosynthesis under hypoxia (Wang Y. et al., 2019). The glutamine transporter SNAT2 is upregulated under hypoxia (Morotti et al., 2019) and support glutamine uptake in cancer cells. Interestingly, studies have shown that SNAT2 compensates for the loss of function of ASCT2 (Broer et al., 2019). Moreover, ASCT2 and LAT1 function as obligatory transporters, in which, the influx through one transporter is coupled to the efflux through the second transporter (Nicklin et al., 2009). These data suggest that these three AATs work cooperatively in the TME to promote cancer progression (**Figure 1**). To our knowledge, there is no existing evidence in the literature on the functional role of CAIX in AA transport, although the identification of potential interactions between CAIX and these AATs suggests that reciprocal functional regulations may be important hallmarks for tumor progression. Considering the pivotal role of CAIX in pH regulation, it is probable that the pH gradient mediated by CAIX in the tumor could influence the function of these AATs. In fact, the transport function of these AATs are influenced by pH, however, the effects are different. The amino acid transport by SNAT2 is sensitive to extracellular pH, where increased extracellular protons compete with sodium ions and impede the SNAT2 activity. This pH sensitivity of SNAT2 is shown to be mediated by the presence of His residues at the C-terminus (Baird et al., 2006). In contrast, glutamine transport by ASCT2 is not hugely influenced by pH (Utsunomiya-Tate et al., 1996). However, ASCT2 also mediates Glu antiport, and this is highly pH-dependent (Utsunomiya-Tate et al., 1996). At a pH gradient of low extracellular pH (6.0) and high intracellular pH (7.0), the Glu influx increased in proteoliposomes containing ASCT2 (Scalise et al., 2020). In addition to altering the transport by ASCT2, changes in pH has shown to impact the expression of ASCT2. Under chronic acidosis, ASCT2 is upregulated by HIF2α and cause a shift in the cancer cell metabolism to favor reductive glutamine metabolism (Corbet et al., 2014). These studies suggest that the pH gradient across the tumor could influence the function of AATs, however, whether CAIX's pH regulatory role influences the coordinative interplay of these AATs remains a topic of future investigation. Investigating the effect of loss of function of CAIX on amino acid transport and metabolism could reveal the importance of CAIX's interaction with the AATs. Such studies could be of importance in highly aggressive cancers like pancreatic cancer that have complex metabolic network (Sperb et al., 2020) and are difficult to treat. CAIX expression (Strapcova et al., 2020) and its function in altering tumoral pH (Cruz-Monserrate et al., 2014) are shown to be important in the early events of pancreatic carcinogenesis. Furthermore, ASCT2 and SNAT2 play an important role in

importing AA in pancreatic cancer, as described earlier in this section. Therefore, it is plausible that CAIX and AATs coordinate their functions to support tumor metabolism and promote pancreatic cancer progression.

One of the interesting findings of the BioID CAIX interactome (Swayampakula et al., 2017) was the potential interaction of LAT1 and CD98hc with CAIX. Modulation of LAT1 function and transport of neutral amino acids by CAIX, perhaps in the context of complexes with integrins, which also associate with CD98hc (Fenczik et al., 2001; Feral et al., 2005), may have significant effects on cellular growth through the regulation of protein translation by mTORC1. While this possibility needs to be investigated in further detail, it is interesting that inhibition of CAIX modulates mTORC1 signaling in breast cancer cells grown in 3D cultures (Lock et al., 2013).

CONCLUSION

Since the seminal findings of Otto Warburg on altered metabolism in cancer, the concept of metabolic reprogramming in cancer has evolved and led to a better understanding of the complex nature of cancer metabolism (Martinez-Outschoorn et al., 2017). Research on the role of metabolite transport has progressed tremendously and unraveled the importance of numerous nutrient and acid/base transporters in the TME (Ganapathy et al., 2009; Bhutia et al., 2015; Becker and Deitmer, 2020). Understanding the interaction of these metabolic proteins in the TME would be beneficial in identifying novel targets for effective therapy. Based on our interactome study, we identified the potential interaction of nutrient transporters and acid/base transporters with CAIX in the hypoxic TME. CAIX is an important pH regulatory protein in the TME that mediates tumor progression in several solid tumors. The CAIX/CAXII specific small-molecule inhibitor, SLC-0111 (Pacchiano et al., 2011; Supuran, 2018) has shown promising effect on suppressing tumor growth and metastasis by itself and in combination with conventional chemotherapeutic drugs (Boyd et al., 2017; McDonald et al., 2019) or immune checkpoint inhibitors (Chafe et al., 2019). Currently, SLC-0111 has completed the Phase-I clinical trial and progressed into a Phase-Ib trial (ClinicalTrials.gov Identifier: NCT03450018) in combination with gemcitabine in CAIX-positive pancreatic cancer patients (McDonald et al., 2020).Furthermore, recent studies have shown that the metabolic plasticity in solid tumors offers adaptation and resistance to single therapy strategies by initiating compensatory mechanisms, however, this is effectively overcome by combinatorial therapy (Biancur et al., 2017; Momcilovic et al., 2018). Therefore, investigating the interaction of CAIX with these nutrient transporters might open new avenues of co-targeting strategies for the treatment of solid tumors.

AUTHOR CONTRIBUTIONS

GV and SD conceived and designed the manuscript and revised and approved the final manuscript. GV drafted the manuscript. Both authors contributed to the article and approved the submitted version.

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