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Review

GLS (Glutaminase)

José A. Campos-Sandoval, Mercedes Martin-Rufián, Javier Márquez

Department of Molecular Biology and Biochemistry, Faculty of Sciences, Campus de Teatinos, University of Málaga, 29071 Málaga, Spain, and Instituto de Investigación Biomédica de Málaga (IBIMA), 29010 Málaga, Spain; jacs@uma.es, mercherub@uma.es, marquez@uma.es

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Abstract

After metabolic reprogramming, many cancer cells become glutamine addicted, that is, they depend on a high consumption of this amino acid for their survival and proliferation. Glutaminase catalyzes the stoichiometric conversion of L-glutamine to L-glutamate and ammonium ions, the first step of glutaminolysis. GLS gene encodes two isoforms, known as kidney-type glutaminase (KGA) and glutaminase C (GAC). Upregulation of GLS is a common feature of many tumors and, in recent years, this enzyme and its interacting partners have attracted much attention as potential new targets for cancer therapy. Considerable effort is being devoted towards the development of small-molecule inhibitors of GLS.

Keywords
Glutaminase, glutamine addiction, anticancer therapy, BPTES, CB-839, compound 968, breast cancer, colorectal cancer, glioblastoma, hepatocellular carcinoma, leukemia, lung cancer, melanoma, ovarian cancer, prostate cancer.

Identity

Other names: GA; KGA; GAC; PAG
HGNC (Hugo): GLS (Glutaminase)
Location: 2q32.2

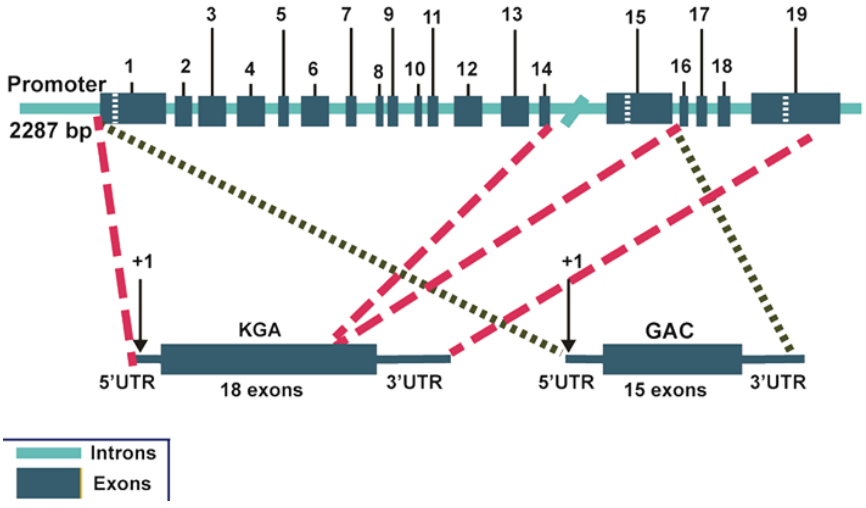

Figure 1. Genomic structure of human glutaminase GLS gene and alternative transcripts KGA and GAC. Introns are depicted as solid light blue lines and exons as numbered dark blue boxes. Dashed red or dotted black lines indicate the exons involved in the generation of KGA and GAC transcripts, respectively (Campos-Sandoval et al., 2015).

DNA/RNA

Description

Human GLS gene is located on chromosome 2 (Aledo et al., 2000). It is composed of 19 exons and spans approximately 82 kb (Porter et al., 2002). The gene resides on the forward strand. It starts at 190880821 and ends at 190965552 from pter (NCBI, GeneID 2744).

Transcription

Two different transcripts coding for functional proteins arise from this gene by a mechanism of alternative splicing: a long KGA transcript, first isolated from brain, which contains exons 1-14 and 16-19 of the GLS gene (Nagase et al., 1998; Holcomb et al., 2000; Porter et al., 2002), and a shorter GAC transcript, formed by the first 15 exons and originally described in human colon cancer cells (Elgadi et al., 1999). A third transcript has been found in heart and skeletal muscle that codes for a much shorter protein (GAM) with no measurable catalytic activity (Elgadi et al., 1999).

Several mechanisms regulate glutaminase expression. The first one to be described is based on the presence of AU-rich pH-responsive instability elements within the 3'-nontranslated region of GLS mRNA. These elements are implicated in the rapid turnover of mRNAs by exonucleolytic degradation. The onset of metabolic acidosis results in the increased binding activity of a RNA-binding protein CRYZ (identified as ζ-crystallin/NADPH quinone reductase) with high affinity for the pH-responsive elements, that selectively stabilizes GLS mRNA (Hansen et al., 1996; Tang and Curthoys, 2001). Upregulation of GAC by the long non-coding RNA (lncRNA) colon cancer-associated transcript 2 (CCAT2) has recently been reported in colon cancer. This lncRNA interacts with the cleavage factor I (CFIm) complex in an allele-specific manner and select the poly(A) site within 14th intron of GLS pre-mRNA, resulting in the preferential splicing of GAC isoform (Redis et al., 2016). MicroRNAs (miRNAs) are also implicated in GLS expression. Through downregulation of MIR23A / MIR23B, which target GLS mRNA, the oncogenic transcription factor MYC (v?myc myelocytomatosis viral oncogene homolog) indirectly relieves repression of GLS in lymphoma and prostate cancer cells (Gao et al., 2009).

The MTOR complex 1/ RPS6KB1 (mTORC1/S6K1 (mammalian target of rapamycin complex 1/ribosomal protein S6 kinase beta-1)) signaling pathway positively regulates GLS expression by enhancing the translation efficiency of Myc mRNA.

After inhibition of mTORC1 with rapamycin, an increase in miR-23a/b levels was observed (Csibi et al., 2014).

The activation of RELA (v-rel avian reticuloendotheliosis viral oncogene homolog A, also known as p65), a member of nuclear factor kappa B (NF-kB) family, also decreases miR-23a expression in leukemic cells, inducing GLS expression (Rathore et al., 2012).

Another transcription factor, JUN (v-jun avian sarcoma virus 17 oncogene homolog), when activated downstream of oncogenic Rho GTPase signaling, increases GLS expression in breast cancer cells by direct binding to its gene promoter (Lukey et al., 2016). After being activated by transforming growth factor (TGF)-β and Wnt (Wingless-type MMTV integration site family)-3a, the homeodomain transcription factor DLX2 (distal-less homeobox-2), involved in embryonic and tumor development, also upregulates GLS expression (Lee SY et al., 2016a).

Transcription of GLS gene is also activated in HIV-1 infected cells by interferon (IFN)-α through signal transducer and activator of transcription 1 (STAT1) phosphorylation, leading to glutamate overproduction (Zhao et al., 2012).

Retinoblastoma protein (RB1), a tumor suppressor that modulates cell cycle checkpoints, also regulates glutamine metabolism.

Deletion of RB family revealed an increase in GLS protein and activity (Reynolds et al., 2014).

Protein

Note

In recent years, considerable effort is being devoted towards the development of small-molecule inhibitors that target GLS and its interacting partners (Katt et al., 2017; Matés et al., 2018). Structural information of truncated or full recombinant human GLS complexed with substrate (glutamine), product (glutamate), the allosteric activator phosphate or inhibitors is now available (DelaBarre et al., 2011; Cassago et al., 2012; Thangavelu et al., 2012; Pasquali et al., 2017).

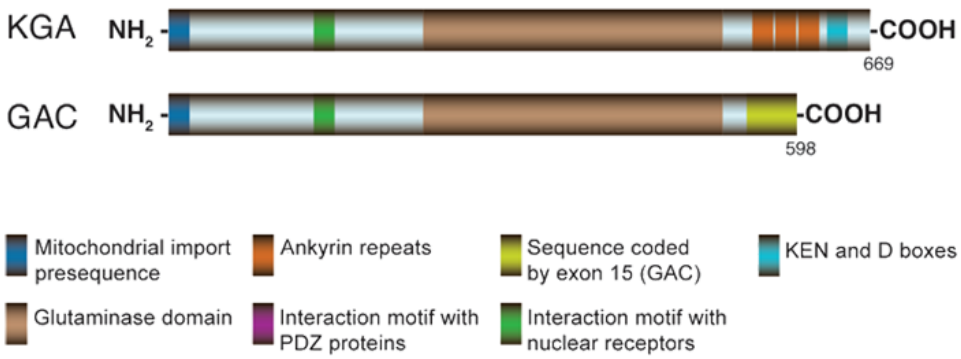

Figure 2. Schematic diagram of GLS isoforms showing the localization of predicted domains and motifs by sequence analysis (Márquez et al., 2016).

Description

Purification:

A 10000-fold purification of glutaminase (KGA) from pig kidney was achieved by Kvamme et al. (1970) using alternative solubilization and precipitacion steps, based on its property to aggregate in phosphate-borate buffers and to disaggregate in Tris-HCl buffers. KGA may appear as three different forms: a dimeric and inactive form in Tris-HCl buffer; a tetrameric and active form after combination of two dimers when dialyzed against phosphate buffer; and a polymeric form with mass higher that 2000 kDa in the presence of phosphate-borate buffer. These polymers consist of double-stranded helices (Kvamme at al., 1970; Olsen et al., 1970; Godfrey et al., 1977).

Structure:

KGA transcript (ORF: 2010 nt) codes a 669-residues protein, with a predicted molecular mass of 73.5 kDa. GAC transcript (ORF: 1797 nt) codes a 598-residues protein, with a predicted molecular mass of 65.5 kDa. Both precursors are identical in almost all their primary structure (exons 1-14, residues 1-550), except in the C-terminal region (residues 551-669 for KGA; 551-598 for GAC). They are composed of a N-terminal domain folded into an EF-hand-like four-helix bundle (Pasquali et al., 2017) and a central glutaminase domain from residues 220 to 530, which belongs to the beta lactamase/transpeptidase-like superfamily and contains the catalytic active site (Thangavelu et al., 2012). In addition, the C-terminal region of KGA possesses three ankyrin repeats, also present in GLS2 proteins but not in the GAC isoform (Pasquali et al., 2017), and KEN (Lys-Glu-Asn) and D (destruction) boxes. The ankyrin repeats are protein-protein interaction modules of 33 residues that have been found in many important proteins such as transcriptional factors, cell cycle regulators, cytoskeletal organizers, etc. (Sedgwick and Smerdon, 1999; Mosavi et al., 2004). Both KEN and D boxes are recognized by the ubiquitin ligase APC/C-Cdh1 (anaphase-promoting complex/cyclosome-Cdh1) which targets KGA for degradation by the proteasome (Colombo et al., 2011). The GLS precursor also has a putative N-terminal mitochondrial targeting sequence of 16 residues (Shapiro et al., 1991). The Curthoys laboratory, in a series of studies on the biosynthesis and processing of the rat KGA, found that the 74 kDa precursor is processed in the mitochondria by a matrix processing peptidase to yield two subunits of 66 (cleavage site at residue 72) and 68 kDa with a 3:1 stoichiometry (Perera et al., 1990; Srinivasan et al., 1995). For the human GLS, only a 66 kDa protein has been detected when expressed as a recombinant protein using the baculovirus expression system (Holcomb et al., 2000). As previously described for GLS2 (Olalla et al., 2002), GLS proteins show a LXXLL motif or nuclear receptor box from Leu144 to Leu148 (Cassago et al., 2012). In recent years, several groups have revealed the structure of GLS based on X-ray crystallography. In the asymmetric unit, GLS is organized as a tetramer where the monomers interact through two sets of interfaces at the glutaminase domains. This domain is composed of two subdomains: an α/β subdomain that contains five-stranded anti-parallel β-sheet surrounded by several α-helices, and an α-helical subdomain with seven α-helices. Two GLS monomers are joined by a long interface of their respective α-helical subdomains to form an inactive dimer. Two opposing dimers then associate by a short interface involving two pairs of equivalent anti-parallel α-helices (α-13) at the α /β subdomains, which come into contact by hydrophobic interactions. (DeLaBarre et al., 2011; Cassago et al., 2012; Thangavelu et al, 2012). When a phenylalanine residue (Phe394 in the mouse GAC) in the short dimer interface was mutated to a serine one, GAC persisted as an inactive dimer even at high concentrations of phosphate (Cassago et al., 2012). The presence of salt bridges between Asp391 and Lys401 at both ends of the pairing helices further

stabilizes the quaternary structure. The replacement of Asp391 by a lysine residue had a similar effect to that caused by the phenylalanine substitution (Li et al., 2016).

Kinetic properties:

Both GLS isoforms require a polyvalent anion to be active. A decade after Krebs (1935) proposed the existence of two different types of glutaminase, one of them (GLS) strongly inhibited by the product glutamic acid, Carter and Greenstein (1947) observed an accelerated deamidation of glutamine in aqueous rat-liver extracts in the presence of phosphate, arsenate or sulfate and suggested that this effect could be due to an augmentation of the activity of glutaminase enzyme. Errera and Greenstein (1949) characterized it as phosphate-activated glutaminase. It was later found that phosphate induced the association of catalytically inactive dimers into active tetramers, whereas glutamate caused inhibition of glutaminase by reversing this process (Godfrey et al., 1977; Morehouse and Curthoys, 1981). At a concentration of phosphate of 100 mM, tetramerization of glutaminase is produced and the enzyme reaches its maximum activity. The activation by phosphate is sigmoidal, with a $K_{0.5}$ of 25 mM and a Hill index of 1.5. When phosphate concentration is increased, both the inhibition by glutamate -which is competitive with respect to glutamine- and the K_M for glutamine decrease (Haser et al., 1985). Although the activity of the purified enzyme is completely dependent on added phosphate, it was not known if the high concentration of phosphate required for the in vitro reaction could also occur in the intracellular site of glutaminase action or whether phosphate was the substitute of an unknown natural activator (Sayre and Roberts, 1958). In addition to phosphate, a wide variety of physiological compounds (tricarboxylic acids, nucleotide triphosphates, acyl-CoA derivatives) was found that could act as activators (or inhibitors) of glutaminase (O'Donovan and Lotspeich, 1966; Weil-Malherbe, 1969; Kvamme and Torgner, 1975). However, recent X-ray analysis has provided support for the candidature of phosphate as a relevant in vivo effector: crystal structure of glutaminase shows that each GAC monomer encloses a single phosphate ion inside its active site. It has been proposed that, after binding of phosphate, a flexible activation loop located near the short dimer interface undergoes a major conformational change that stabilizes the active site and promotes catalytic turnover (Thangavelu et al., 2012; Li et al., 2016, Stalnecker et al., 2017).

Interacting partners:

The first binding partner for KGA to be described was the caytaxin ATCAY (or BNIP-H for BNIP-2 homology), a protein exclusively expressed in neural tissues and encoded by a gene linked to Cayman ataxia and dystonia. This protein interacts with KGA through a conserved protein-protein interaction domain known as the BNIP-2 and Cdc42GAP homology (BCH) domain. Caytaxin regulates the intracellular localization and activity of KGA: it relocalized KGA from cell body to neurite terminals and reduced glutamate levels by inhibiting its activity (Buschdorf et al., 2006). Subsequently, another member of the family of BCH-domain containing proteins, PRUNE2 (prune homolog 2 with BCH domain, also known as BMCC1s), has been shown to directly interact with KGA. This protein, associated to microtubules and intermediate filaments in astrocytes and neurons, may influence import of KGA to mitochondria: overexpression of PRUNE2 in mouse neurons led to an accumulation of KGA within the cytoplasm (Boulay et al., 2013). Very recently, GLS has been reported to interact with peroxisome proliferator-activated receptor gamma (PPARG) in the nuclei of prostate cancer cells, and this interaction decreased the nuclear receptor activity (de Guzzi Cassago et al., 2018).

Post-translational modifications:

Aside from the regulation of GLS gene expression, several post-translational modifications affect glutaminase activity, with implications for cancer cells metabolism. Most of the identified modifications correspond to phosphorylation of serine, tyrosine and threonine residues (see Ascenção et al., 2018 for references), although the effect of specific modified residues on GLS activity has only recently been studied. Thangavelu et al. (2012) revealed that GLS activity in human breast adenocarcinoma cells was dependent on phosphorylation by epidermal growth factor (EGF) via the RAF/MEK/ERK signaling module and was completely abolished after treatment with MEK inhibitors or co-expression with protein phosphatase PP2A. Han et al. (2018) have recently found that GLS activity is drastically reduced when one of the ten identified phosphorylation sites, Ser314 at the glutaminase domain, is mutated to alanine. A remarkable reduction in Ser314 phosphorilation and GLS activity occurs when cell lysates of several tumor types are treated with alkaline phosphatase. This phosphorylation is regulated by the NF-kB-PKCε (PRKCE protein kinase C-epsilon) axis. After treatment with a NF-kB inhibitor, GLS activity could be restored with the overexpression of PKCε (Han et al., 2018). In contrast, phosphorylation of Ser95 at the N-terminal domain inhibits GLS activity, but its mechanism of deregulation in cancer cells is still unknown (Ascenção et al., 2018). Another post-translational modification that affects GLS activity is succinylation. The mitochondrial protein sirtuin 5 (SIRT5), which removes short-chain acyl moieties from lysine residues, regulates GLS activity by

reducing its level of succinylation (Polleta et al., 2015). Acetylation has also been speculated to regulate GLS activity. Several lysine residues that occur at the α-helix-13 has been identified as sites of this modification and their acetylation could prevent the formation of active tetramers (McDonald et al., 2015). As already mentioned above, KGA is also modified by ubiquitination at its C-terminal region (Colombo et al., 2011).

Drug inhibitors:

The first glutaminase inhibitors to be discovered several decades ago were L-glutamine analogs isolated from Streptomyces, such as azaserine, diazo-O-norleucine (DON) and acivicin. They act as irreversible competitive inhibitors for GLS and other glutamine-utilizing enzymes: DON binds covalently to residue Ser286 at the GLS active site (Thangavelu et al., 2014). Although they show a significant inhibitory effect on several types of tumors in preclinical tests, their pronounced toxicity has compromised the therapeutical potential of these compounds (Ahluwalia et al., 1990). The bisthiadiazole derivatives form the second important group of inhibitors that was described the past few years, the prototype being BPTES (Robinson et al., 2007). A more potent derivative of BPTES, CB-839 (Gross et al., 2014), is being tested in clinical trials (more information at http://www.clinicaltrials.gov database). These non-competitive allosteric inhibitors bind to the interface between two dimers, stabilizing an inactive tetrameric form of GLS (DeLaBarre et al., 2011; Thangavelu et al., 2012). The newest molecule in this group is the compound UPGL00004, with similar binding affinity as CB-839 but with better microsomal stability (Huang et al., 2018). The third group of inhibitors is represented by compound 968, a benzophenanthridinone that binds to a pocket at the interface between two GLS monomers. Unlike BPTES, compound 968 preferentially binds to the monomeric, inactive form of GLS, and is unable to inhibit GLS which has been pre-activated with phosphate (Wang et al., 2010; Stalnecker et al., 2017). Other new GLS inhibitors recently described are physapubescin K, (Cheng et al., 2017; Wu et al., 2017), zaprinast (Elhammali et al., 2014) and brachyantheraoside A8 (Li et al., 2017). One essential point of GLS inhibition is that these novel compounds lack the high degree of toxicity observed with glutamine mimetics (Matés et al., 2018). For an overview of glutaminase inhibitors, see: Katt et al., 2017; Song et al., 2018; Wu et al., 2018; Xu et al, 2018.

Expression

The rat KGA isoform has been found in all nonhepatic tissues with glutaminase activity, including fetal liver (Smith and Watford, 1990; Curthoys and Watford, 1995). In human tissues, the KGA isoform is expressed in kidney, brain, heart, lung and pancreas, but not in skeletal muscle or liver (Aledo et al., 2000). The human GAC isoform, first described in a cancer cell line, is found principally in cardiac muscle and pancreas, at lower levels in lung, kidney and placenta, and is not detected in brain or liver. (Elgadi et al., 1999). GLS proteins are also overexpressed in a wide variety of tumors compared to normal tissues, with GAC being the predominant isoform in many of them, such as breast carcinoma (Elgadi et al., 1999), prostate cancer (Gao et al., 2009), non-small cell lung cancer (van den Heuvel et al., 2012), leukemia (Pérez-Gómez et al., 2005; Matre et al., 2016), glioma (Szeliga et al., 2008; Tanaka et al., 2015), among others.

Localisation

Errera and Greenstein (1949) proposed for the first time a mitochondrial location for glutaminase in liver, and subsequently different authors demonstrated experimentally this location in both liver and other organs such as kidney, brain and intestine (Klingman and Handler, 1958; Guha, 1961; Katunuma et al., 1967; Pinkus and Windmueller, 1977). However, some authors have described a non-mitochondrial cytoplasmic pool of glutaminase (Aoki et al., 1991). In a recent study with several tumor cell lines, Cassago et al. (2012) have reported a cytosolic localization for KGA, with only GAC located in mitochondria. These findings suggest that the intracellular localization of GLS may depend on other factors other than the mitochondrial targeting sequence at its N-terminus. The sub-mitochondrial location of glutaminase has been studied extensively, but it is still a controversial issue. Although glutaminase seems to be associated with the inner mitochondrial membrane (IMM), different orientations of the enzyme have been proposed: to both sides of the IMM (Kvamme and Olsen, 1979), the intermembrane space (c-side) (Kvamme et al., 1991; Roberg et al., 1995), or the matrix space (m-side) (Shapiro et al., 1985; Aledo et al., 1997).

Function

GLS (E.C. 3.5.1.2.) catalyzes the hydrolytic deamidation of L-glutamine to form L-glutamate and ammonium, the first step of glutaminolysis. This enzyme fulfills essential tasks related to tissue-specific function. In kidney, glutaminase reaction is the initial step in renal ammoniagenesis. As mentioned above, GLS is induced through its mRNA stabilization in response to metabolic acidosis. This results in an increased consumption of glutamine extracted from plasma in the proximal convolute tubules and the excretion of the generated ammonium ions in the urine. The further

metabolization of α-ketoglutarate (α-KG) yields bicarbonate ions that are transported into blood to restore the acid-base balance (Curthoys, 2001). In brain, GLS participates in the synthesis and recycling of glutamate, the principal excitatory neurotransmitter, through the astrocytic-neuronal glutamate-glutamine cycle (Nicklas et al., 1987; Daikhin and Yudkoff, 2000). Knockout mice for the Gls gene die shortly after birth due to impaired glutamatergic synaptic function, stressing the importance of this enzyme in glutamatergic transmission (Masson et al., 2006). Moreover, it has been shown that small interfering RNA (siRNA) silencing of GLS in neural progenitor cells impaired their differentiation, proliferation and survival, suggesting a critical role of GLS in neurogenesis (Wang et al., 2014).

Besides its role in normal tissues, glutaminase is highly expressed in tumors, where its activity has been correlated with malignancy (Knox et al., 1967; Linder-Horowitz et al., 1969; Kovacevik and McGivan, 1983). Many cancer cells develop what has been called "glutamine addiction", a term now widely used to reflect the strong dependence for this essential nitrogen substrate after metabolic reprogramming (Wise et al., 2008). Silencing GLS expression by genetic knockdown or inhibiting its activity with drug inhibitors reveal the critical role of GLS in this addiction. Thus, the specific inhibition of GLS by antisense mRNA slowed tumor cell growth and induced phenotypic changes that made these cells vulnerable to the host's immune system (Lobo et al., 2000; Segura et al., 2001). In addition, GLS enables cancer cells to counteract ROS. Glutamate is the precursor of glutathione (GSH) and a source or reducing equivalents (Hensley et al., 2013). Based on the abundant published data on recent years on the role of glutaminase in cancer, this enzyme has become a potential drug target for therapeutic intervention (Matés et al., 2018).

Implicated in

Breast cancer

Breast cancer exhibits subtype-specific phenotype of glutamine dependence. Triple negative breast cancer (TNBC) showed higher GLS expression than ERBB2 (Erb-B2 receptor tyrosine kinase 2)-positive or luminal subtypes (Kung et al., 2011; Kim et al., 2013; Lampa et al., 2017). In a study with a panel of breast cancer cell lines, GLS inhibitor CB-839 had an anti-proliferative effect on TNBC cells but not on estrogen receptor (ER)-positive or ERBB2-positive cell lines. This effect was associated with a significant decrease in both glutamine consumption and levels of tricarboxylic acid (TCA) cycle intermediates. Sensitivity of TNBC cells to CB-839 was correlated with an elevated expression of GAC but not KGA (Gross et al., 2014). The new GLS inhibitor UPGL00004 had a similar effect: it potently inhibited growth in TNBC but not in receptor-positive cells (Huang et al., 2018). A higher activity of MYC in cells of this subtype could maintain expression of GLS (Kung et al., 2011). Another effect of GLS inhibition with CB-839 was a decrease in MTOR activity. Both GLS and mTOR inhibition synergized in TNBC cells (Lampa et al., 2017). GLS expression is also upregulated by ERBB2 activation. When non-tumorigenic breast epithelial cells were transformed with a constitutive active form of ERBB2, GLS mRNA and protein levels were increased through activation of NF-kB signaling (Qie et al., 2014). In a similar model, knockdown of ephrin-A1, a ligand of EPH receptor A2 (EPHA2), enhanced GLS activity through increased levels of Rho GTPase and addition of α-KG restored proliferation inhibited by compound 968 (Youngblood et al., 2016). Activation of JUN downstream of Rho GTPase signaling led to enhanced glutaminase activity (Lukey et al., 2016). An inverse correlation between the expression of glutamine synthetase (GS, encoded by GLUL) and GLS has been observed in luminal and TNBC cells. Moreover, GLUL repression led to an increase of GLS mRNA levels, and ectopic GDH over-expression reduced those levels (Kung et al., 2011).

Colorectal cancer

GLS expression is upregulated in colorectal cancer (CRC) tissues compared to adjacent normal tissues (Huang et al., 2014). In recent years, several mechanisms have been described to regulate GLS expression in colorectal cancer. Supranutritional doses of selenite induced GLS ubiquitination by APC/C-CDH1, leading to suppression of glutaminolysis and increasing apoptosis in CRC cells (Zhao et al., 2017). Heat shock factor 1 (HSF1) participates in malignant transformation in many types of tumors. In CRC, high levels of HSF1 correlates with poor survival. Through inhibition of MIR137 which targets GLS, HSF1 stimulates GLS expression, resulting in enhanced glutaminolysis and MTOR activation (Li et al., 2018). MYCN downstream regulated gene 2 (NDRG2), a tumor suppressor, has been shown to regulate GLS expression. When overexpressed in CRC cells, NDRG2 inhibited glucolysis and glutaminolysis via repression of MYC. (Xu et al., 2015). GLS expression seems to be also regulated by TP53. Costunolide, a natural sesquiterpene lactone with antitumoral activity, stimulated the nuclear translocation of TP53 and downregulated GLS mRNA and protein levels, resulting in lower intracellular levels of glutamate and α-KG. Treatment with a TP53 inhibitor rescued GLS expression, indicating that activation of TP53 was

required for costunolide to downregulate GLS expression in CRC cells (Hu et al., 2018).

Glioblastoma

The effect of glutaminase inhibition on glioblastoma (GBM) have been addressed in isocitrate dehydrogenase (IDH1 or IDH2) wild type or mutant cell lines. GBM cells that harbour IDH mutations acumulate 2-hydroxyglutarate (2-HG), an oncometabolite primarily derived from glutamine (Dang et al., 2009). When compared to IDH wild type cells, IDH mutant cells treated with the GLS inhibitor BPTES showed a slower growth rate that could be restored by adding exogenous α-KG (Seltzer et al., 2010). 2-HG inhibits the synthesis of glutamate by α-KG-dependent branched-chain amino acid aminotransferases BCAT1 and BCAT2 and, as a consequence, dependence of these cells on glutaminase for glutamate and GSH biosynthesis increases. Inhibition of GLS with CB-839 made IDH mutant cells more susceptible to reactive oxygen species (ROS)-induced cell death and radiation. Based on these findings, a combination of CB-839, the alkylating agent temozolomide and radiation is now being tested in patients with IDH1 mutant gliomas (McBrayer et al., 2018). ROS generation by treatment with oxidizing agents synergized with GLS silencing or GLS2 overexpression to suppress malignant properties of GBM cells (Martèn-Rufián et al., 2014). In IDH wild type GBM cells, pharmacological NOTCH pathway blockade reduced the levels of GLS mRNA, which was reflected in reduced intracellular glutamate concentration (Kahlert et al., 2016). MiRNAs also play an important role in glutamine metabolism of glioblastoma. Thus, MIR153 has been found to be downregulated in glioblastoma and its overexpression inhibits cell growth and promotes apoptosis by directly targeting GLS expression (Liu et al., 2017). Compensatory anaplerotic mechanisms have also been described in GBM. In those that use glutamine as the preferred anaplerotic precursor, inhibition of GLS is accompanied by augmented activity of pyruvate carboxylase to stimulate glycolytic metabolism (Cheng et al., 2011). Conversely, glioblastoma cells respond to deficit in glycolysis after mTOR inhibition by increasing glutamine metabolism through elevated GLS expression. Combination of MTOR and GLS inhibition caused suppression of tumor growth in vivo (Tanaka et al., 2015).

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most aggressive cancers. A high correlation between growth rate and dedifferentiation of rat hepatomas and GLS activity was already described 50 years ago (Linder-Horowitz et al., 1969). In a recent study, a large set of clinical samples of HCC and normal liver was analysed and GLS was found to be highly expressed in tumor samples, whereas GLS2 was preferentially expressed in normal cells. During malignant progression, a switch from GLS2 to GLS was observed. High GLS and/or low GLS2 expression in HCC determined poor prognosis in patients with this tumor (Yu et al., 2015). Several mechanisms that regulate GLS expression in HCC have been described. Wnt/β-catenin pathway, associated to HCC stemness, promotes GLS expression. After treatment with an agonist of Wnt/β-catenin pathway, both GLS mRNA and protein increased. In addition, the increase in ROS caused by treatment with compound 968 decreased the amount of nuclear β-catenin (CTNNB1) and subsequently inhibited expression of target genes implicated in HCC stemness (Li et al., 2019). RELA also controls GLS expression in HCC and its inactivation reduced GLS mRNA levels. Dysregulation of RELA and GLS correlated with poor prognosis in HCC patients (Dong et al., 2018). HCC cells with a mesenchymal phenotype showed higher levels of TGF-β than their epithelial counterparts, and this resulted in increased GLS expression and glutamine anaplerosis (Soukupova et al., 2017). A lncRNA that participates in HCC progression, HOXA distal transcript antisense RNA (HOTTIP), is negatively regulated by MIR192 and MIR204 and this regulation modulates GLS expression. Inhibition of HOTTIP by siRNA or by ectopic expression of miR-192/-204 suppressed GLS expression and hence HCC proliferation (Ge et al., 2015). A therapeutic strategy combining GLS inhibition with ROS induction has been tested in HCC cells. Treatment with compound 968 and dihydroartemisinin (an anti-cancer drug that enhances ROS production) synergistically sensitized HCC cells to ROS-induced cytotoxicity resulting in apoptosis (Wang et al., 2016).

Leukemia

In a study with leukemia cells from medullar blood of human patients, both GLS and GLS2 isoforms were expressed, although GLS accounted for the majority of glutaminase activity in these cells. Furthermore, GLS expression was positively correlated with the rate of proliferation (Pérez-Gómez et al., 2005). Acute myeloid leukemia (AML) cells that harbour IDH mutations are glutamine addicted and their growth could be suppressed by treatment with BPTES (Emadi et al., 2014). Analysis of AML cell lines showed high levels of GAC, while KGA was expressed at low level. Inhibition of GLS with BPTES or CB-839 caused reduction of key metabolites downstream of glutamate, including TCA cycle intermediates and GSH. CB-839 exposure also reduced levels of oncometabolite 2-HG and this reduction associated

with signs of differentiation (Matre et al., 2016). Combined therapy with GLS inhibitors dramatically reduced proliferation in AML models. Thus, GAC inhibition by CB-839 sinergized with BCL2 (B-Cell Leukemia/Lymphoma 2) (Jacque et al., 2015) or FLT3 (FMS-like tyrosine kinase 3) inhibition (Gregory et al., 2018) to induce loss of viability through apoptotic cell death. Synergistic antileukemic effects were also observed after treatment with BPTES in combination with NOTCH1 inhibition (Herranz et al., 2015). MiRNAs have also been implicated in glutamine metabolism in leukemic cells. Thus, an increase in expression and translocation of RELA to the nucleus was observed in leukemic Jurkat T cells growing in medium with glutamine, which caused a higher NF-kB activity. Activation of RELA reduced MIR23A expression, leading to a higher expression of GLS. Overexpression of MIR23A in these cells impaired glutamine metabolism and caused mitochondrial dysfunction and cell death (Rathore et al., 2012).

Lung cancer

GAC is the predominant GLS isoform in glutamine-dependent non-small cell lung cancer (NSCLC). The increased GAC:KGA ratio observed in these cells, compared with normal lung tissue, was due to a significant decrease of KGA expression in tumoral cells. GAC knockdown in glutamine-dependent cells strongly reduced cell growth, whereas the effect of KGA knockdown was much less pronounced, suggesting that GAC is the more essential GLS isoform in these cells (van den Heuvel et al., 2012). GAC protein levels were similar between tumor and normal tissues: the high glutaminase activity in NSCLC cells resulted from GAC phosphorylation (Han et al., 2018). However, other authors have found that GLS expression is significantly higher in NSCLC than in cells from normal lung tissues (Lee JS et al., 2016b). A 2-fold increase in GAC levels and a reduction in KGA and pyruvate carboxylase levels were observed in NSCLC cells that underwent ephitelial to mesenchymal transition induced by TGF-β, making these cells more susceptible to GLS inhibition, as shown by impaired TCA cycle anaplerosis and increased sensitivity to oxidative stress after treatment with BPTES (Ulanet et al., 2014). Synergic treatments that combine inhibition of GLS and other drug targets have been described in recent studies. Thus, treatment with GLS inhibitor compound 968 sensitized lung cancer cells to apigenin-mediated apoptosis (Lee YM et al., 2016c). Dual treatment with GLS inhibitor BPTES and thymidylate synthase inhibitor 5-fluorouracil resulted in the reversal of NSCLC in a preclinical xenograft model (Lee JS et al., 2016b). In epidermal growth factor receptor (EGFR) mutant lung cancer cells, targeted inhibition of EGFR signaling and GLS with erlotinib and CB-839, respectively, induced a reduction in glucose and glutamine transporters levels and led to tumor regression in xenograft models (Momcilovic et al., 2017). Unfortunately, acquired resistance is developed by patients with NSCLC after several months of treatment with erlotinib. Compound 968, when combined with erlotinib, inhibited the proliferation of erlotinib-resistant NSCLC cells by decreasing the expression of EGFR (Xie et al., 2016). The adquired resistance of lung squamous cell carcinomas to MTOR inhibition via enhanced glutaminolysis was overcome by treatment with CB-839 (Momcilovic et al., 2018).

Melanoma

Two miRNAs targeting GLS have been found to be downregulated in melanoma cells and their low levels associated with poor prognosis. When overexpressed, both miRNAs (MIR137 and MIR203) suppressed glutamine catabolism and proliferation of melanoma cells (Chang et al., 2016; Luan et al., 2018). MiRNA203 also sensitized resistant melanoma cells to temozolomide (Chang et al., 2016).

Ovarian cancer

Patients with ovarian cancer who initially respond to platinum and taxane-based chemotherapy will develop drug resistance. Compared to sensitive cells, cisplatin-resistant cells showed glutamine dependence, associated with the upregulated expression of glutamine transporter SLC1A5 (ASCT2) and GLS mediated by higher levels of MYC. GLS overexpression in cisplatin-sensitive cells resulted in increased resistance to this agent. Conversely, knockdown of GLS re-sensitized resistant cells to cisplatin. Combination of BPTES and cisplatin synergistically increased apoptosis (Hudson et al., 2016). A similar effect on cell proliferation was obtained after treatment of ovarian cancer cells with paclitaxel and compound 968. This GLS inhibitor induced cell cycle arrest and apoptosis, and sensitized cells to paclitaxel (Yuan et al, 2016). Moreover, knockdown of both GLS isoforms by siRNA sensitized cells to chemotherapy more effectively than depletion of GAC or KGA alone (Masamha and LaFontaine, 2018). Another proposed therapeutic strategy combined inhibition of PI3K/Akt/mTOR axis (frequently activated in ovarian cancer cells) and GLS with PP242 (an inhibitor of mTORC1 and mTORC2) and CB-839, respectively. GLS inhibition resulted in a dramatic induction of PP242-mediated cell death not only by a reduced glutamine anaplerosis, but also by a decreased level of phosphorylated STAT3 (signal transducer and activator of transcription 3) (Guo et al., 2016).

Prostate cancer

GLS is highly expressed in prostate cancers and positively correlates with tumor state and progression. In glutamine addicted prostate cancer cells, GLS regulates glucose uptake through inhibition of thioredoxin-interacting protein (TXNIP) expression, a potent negative regulator of aerobic glycolisis and glucose uptake. When GLS activity was suppressed by siRNA or BPTES, TXNIP was remarkably induced and the invasive capacity of these cells significantly reduced (Pan et al., 2015). It was in these tumors that MYC, which contributes to their initiation and progression (Koh et al., 2010), was described to upregulate GLS expression through suppression of MIR-23a/b (Gao et al., 2009). Recent results show that GLS is highly expressed in large extracellular vesicles (LEVs) extruded from prostate cancer cells (Minciacchi et al., 2015). Release of these vesicles is dependent on glutamine metabolism. Thus, a significant reduction in the production of LEVs is observed when these cells are treated with BPTES (Dorai et al., 2018).

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