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REVIEWS

Review

Glutamine as an Essential Amino Acid for KRas-Driven Cancer Cells

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Cancer cells consume glutamine, a nonessential amino acid (NEAA), at exceedingly high rates to fulfill their energetic and biosynthetic requirements for proliferation. Glutamine plays distinct roles from essential amino acids in cell cycle progression and in the activation of mammalian target of rapamycin (mTOR). Furthermore, the need of cancer cells for glutamine can be exploited therapeutically – especially those driven by KRas. In this review we explore several distinct cellular roles for glutamine that contribute to glutamine addiction in KRas-driven cancer cells and discuss opportunities for therapeutic intervention created by glutamine addiction.

Highlights

Glutamine activates mTORC1 by leucine-dependent and leucine-independent mechanisms.

There is a late-G_1 glutamine-dependent checkpoint that is dysregulated in KRas-driven cancer cells.

Blocking glutamine utilization in KRas-driven cancer cells causes S-phase arrest and sensitizes to rapamycin.

Aspartate generated by the glutamate-oxaloacetate transamination reaction promotes nucleotide biosynthesis and redox balance.

Glutamine Fulfills Many Biosynthetic Roles for Cancer Cells

Glutamine, although a non-essential amino acid (NEAA), plays an essential role in dividing cells. Glutamine is present at tenfold higher levels than other amino acids in tissue culture and is the most abundant amino acid in human sera [1]. Glutamine fulfills many biosynthetic roles in the cell, as it is utilized in amino acid, protein, lipid, and nucleotide biosynthesis and thus is essential for dividing cells. While cells are capable of synthesizing glutamine, this amino acid is considered a **'conditionally' essential amino acid (EAA)** (see Glossary). Under the demands of cell growth, the need for glutamine exceeds the cell's synthetic capability and cells require increased uptake of glutamine (Box 1) [2]. In particular, cancer cells undergoing proliferation require exogenous glutamine for their increased need for biosynthetic precursors derived from glutamine metabolism [1,3]. Furthermore, cancer cells commonly undergo aerobic glycolysis producing lactate, rather than the tricarboxylic acid (TCA) cycle precursor acetyl-CoA [4]. One of the most well-understood roles of glutamine is replenishing the TCA cycle through the **anaplerotic** production of the TCA cycle intermediate α-ketoglutarate (αKG) (Box 2 and Figure IA). Many cancer cells – notably those driven by Myc and KRas – have been metabolically reprogrammed to take up more glutamine and to shift from catabolic to anabolic utilization of glutamine [5–9]. The altered glutamine metabolism in Myc- and KRas-driven cancers has also created an addiction to glutamine for their survival [5–9].

While the anaplerotic metabolism of glutamine to the TCA cycle intermediate αKG is important for the maintenance of mitochondrial metabolism, there are several other critical roles for glutamine in dividing cancer cells. This review highlights emerging research that describes the utilization of glutamine for **cell cycle** progression, proliferation, and survival in KRas-driven cancer cells.

mTORC1 Activation by Glutamine

The **mammalian target of rapamycin** (**mTOR**) is a highly conserved serine/threonine kinase that is an important regulator of late G_1 cell cycle progression, where nutrient sufficiency is evaluated before the cell commits to replication of the genome and division [10–12]. Activation of mTOR complex 1 (mTORC1) is dependent on the presence of several nutrients and growth

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Box 1. Glutamine Transporters in Cancer

Glutamine, while a NEAA, becomes essential to cells proliferating at high rates, such as cancer cells [2]. For this reason, many cancer cells increase their expression of glutamine transporters to accommodate their increased need for glutamine. Glutamine enters the cell via the solute carrier family (SLC) 1A5 transporter (Figure 1). SLC1A5 is overexpressed in many cancer cells due to oncogenic Myc [8] and KRas [6]. Further, the tumor-suppressing retinoblastoma protein Rb has been shown to decrease SLC1A5 expression [74]. SLC1A5 requires the exchange of Na^+ for glutamine influx. However, the Na^+-dependent influx requires the concomitant Na^+-dependent efflux of another amino acid. This requirement leads to the coupling of the SLC1A5-dependent influx of glutamine with other transporters, such as SLC7A5 and SLC7A11 (Figure 1). SLC7A5, like SLC1A5, is overexpressed in many cancers and is highly correlated with increased cell proliferation and angiogenesis [75]. SLC7A5 expression is controlled by Myc [76] as well as hypoxia inducible factor 2α [77]. SLC7A5, which is an obligate exchanger, effluxes glutamine for influx of leucine (Figure 1). Together, SLC1A5 and SLC7A5 are responsible for the glutamine-dependent activation of mTOR (Figure 1), which acts to increase anabolism for cancer growth [15]. Accordingly, pharmacological inhibition of SLC1A5, which is considered a viable target for cancer [55], also blocks the functional effects of SLC7A5 for cancer survival (discussed further under 'mTORC1 Activation by Glutamine') [15]. SLC7A11, which exchanges glutamate (derived from glutamine) for the uptake of cystine (Figure 1), is also overexpressed in a Myc-dependent manner in cancers [78], promoting GSH synthesis, protection from oxidative stress, cell survival, and chemoresistance [79]. SLC6A14 can also serve as a candidate coupling transporter for SLC7A11 instead of SLC1A5 and is upregulated in a number of cancers [80]. SLC6A14 may be a potential cancer target, as it is essential for tumor growth but dispensable for normal development in mice [81]. SLC7A11 differs from other transporters in that the import of glutamine is dependent not on the transport of other amino acids but rather on a Na^+/Cl^- gradient. Both the SLC7A5 and SLC7A11 antiport systems require the formation of a heterodimer with SLC3A2 for their function. Similarly, SLC3A2 expression is induced by Myc, frequently expressed in cancer [78], and promotes tumorigenesis in Ras-driven cells by increasing extracellular matrix stiffness and the modulation of integrin signaling [82]. SLC38A5 is also involved in the uptake of glutamine into the cell. While the expression of this transporter has been linked to Myc [8], its role in cancer remains unclear [79]. Similarly, other transporters of glutamine exist in the cell, but their relationship to cancer has not been defined [79].

factors that promote a shift from catabolic to anabolic cell growth [13] and cell cycle progression from late G_1 into S phase [11,14]. mTORC1 activation stimulates protein, lipid, and nucleotide biosynthesis, all of which are dependent on the presence of glutamine [3,13]. Consistent with this role in the generation of metabolites needed for anabolic metabolism, glutamine has been reported to activate mTORC1 [15–17]. Intriguingly, glutamine has a unique mechanism for activating mTORC1 that is distinct from other amino acids. Canonically, Rag family GTPases respond to amino acids by recruiting mTORC1 to lysosomal membranes where it is activated in a manner that is dependent on the GTPase Rheb [18,19]. While Hall and colleagues have shown that αKG stimulates the GTP loading of RagB and the subsequent activation of mTORC1 [16], Guan and colleagues showed that glutamine is capable of activating mTORC1 in the absence of the Rag GTPases [17]. Intriguingly, the Rag-independent activation of mTORC1 by glutamine was dependent on ADP-ribosylation factor 1 (Arf1) [17]. Subsequently, our laboratory reported that the Rag-independent pathway required not only Arf1 but also phospholipase D (PLD) and the GTPase RalA [20], which is constitutively associated with PLD1 [21,22]. Arf family GTPases activate PLD1 after recruitment to a PLD–RalA complex on activation by Ras signaling cascades [23,24]. The activation of PLD results in the production of phosphatidic acid (PA), which binds to mTOR [25], promoting mTOR complex stability and activation [26]. Significantly, PLD is upregulated in Ras-driven cancers [27,28] in a RalA-, Arf-, Rheb-, and phosphatidylinositol 3-kinase-dependent manner [20,28,29]. These reports suggest multiple mechanisms for inhibition of PLD in Ras-driven cancers.

While Rag GTPases are not required for mTORC1 activation by glutamine, it remains unclear how mTORC1 is recruited to the lysosome in the absence of Rag GTPases [17]. Interestingly, Arf1, RalA, and PLD are all involved in vesicle trafficking [30–32]. Thus, it has been speculated that vesicle trafficking might be required in the absence of Rag GTPases to bring mTORC1 to the lysosome, where the Rheb GTPase is still required for mTORC1 activation by glutamine [20]. EAAs utilize RalA, PLD, and Rheb to activate mTORC1; however, the activation of

Glossary

Anaplerotic: the replenishment of metabolic intermediates that have been depleted for biosynthetic purposes. Anaplerosis most commonly refers to the process of replenishing TCA cycle metabolites, with the most prevalent example being that of glutamine regenerating the TCA cycle intermediate αKG.

Cell cycle: the series of events that is required for a cell to duplicate its cellular contents and divide into two daughter cells. The cell cycle is tightly regulated through several checkpoints to ensure that proper biosynthetic precursors are in place to prevent faulty progeny cells. In the gap phase, known as G_1, the cells have a high biosynthetic output to prepare for cell duplication. Cells without ample nutrients can either become arrested at this stage or enter quiescence, also known as G_0, in which the cell is not dividing. Cells that pass all of the checkpoints can continue on to S phase. S phase is the synthesis phase in which DNA is duplicated. Next is the growth phase, G_2, in which the cell undergoes rapid growth to prepare for cell division in the following phase. M phase is the mitotic phase in which the cell is divided into two daughter cells.

Conditionally essential amino acid (EAA): an amino acid that can be synthesized but under increased anabolic metabolism must be obtained from exogenous sources. This can occur in any rapidly proliferating or highly stressed cell, including, but not limited to, cancer cells. Proline, arginine, cysteine, and glycine can also be considered conditionally essential under certain physiological conditions.

Glutaminolysis: this term collectively defines the series of reactions that involve the breakdown of glutamine into the TCA cycle intermediate αKG. Glutamine is first deamidated by GLS to glutamate. Glutamate is then converted to αKG by GDH to generate αKG and free NH_4^+. Alternatively, glutamate can be converted to αKG via a transaminase reaction whereby there is concomitant conversion of oxaloacetate to aspartate.

Mammalian target of rapamycin (mTOR): a protein kinase found

mTORC1 by EAAs required Arf6 rather than Arf1 [29]. This suggests the utilization of different Arf GTPase isoforms for mTORC1 activation by EAA versus activation by glutamine. Nicklin *et al*. showed that glutamine activates mTORC1 through the SLC7A5/SLC3A2 antiport system, which brings glutamine out of the cell in exchange for the influx of leucine, a very potent Rag-dependent mTORC1 activator (Figure 1) [15]. Tumors with elevated levels of glutamine bypass the need to take up glutamine with SLC1A5 and are primed for mTOR activation via the efflux of glutamine and influx of leucine (Figure 1) [15].

The work of Hall and colleagues [16] implicated **glutaminolysis** and the production of αKG with mTORC1 activation in the response to glutamine via the Rag GTPases. Furthermore, αKG, like glutamine, required Arf1 and PLD to activate mTORC1, and while Hall and colleagues showed that αKG utilizes the Rags, Bernfeld *et al*. showed that αKG did not require the Rag GTPases (Figure 1) [20]. Ablation of either glutamate-oxaloacetate transaminase (GOT) or glutamate dehydrogenase (GDH) to abolish αKG production blocked mTORC1 activation by glutamine [20]. Aspartate, which is produced along with αKG by GOT, was incapable of rescuing mTORC1 activity following glutamine deprivation [20]. This was somewhat unexpected in KRas-driven cancer cells, which have previously been shown to depend more on GOT for the production of aspartate for nucleotide biosynthesis [5,33,34]. Thus, the key factor for the activation of mTORC1 in response to glutaminolysis is αKG, not aspartate. How αKG activates Arf and PLD to stimulate mTORC1 is unknown.

Glutamine Is Required for G_1 Cell Cycle Progression

The G_1 phase of the mammalian cell cycle can be divided into two parts – early G_1 and late G_1 – that are separated by a growth factor (GF)-dependent restriction point (R) where cells coming from mitosis determine whether to exit the cell cycle or continue to divide (Figure 2). During late G_1, there are several metabolic checkpoints where cells monitor whether there are sufficient nutrients to replicate the genome and double the mass of a cell prior to cell division [10]. In addition to the metabolic checkpoints, there is a checkpoint mediated by mTORC1 that senses both nutrient and GF input [12,35]. The mTORC1 checkpoint is mediated by transforming growth factor (TGF)β and has also been referred to as R [36]. We have referred to the late-G_1 R as R2 [37] and the original R characterized by Pardee and Zetterberg [38,39] as R1 (Figure 2A). One of the critical nutrients being sensed in late G_1 is glutamine, which serves as a source of both carbon and nitrogen and is essential for cell growth and proliferation. While resting cells can synthesize glutamine, proliferating cells need exogenously supplied glutamine. Hence, glutamine is considered a 'conditionally essential' amino acid. Consistent with this distinction,

across most eukaryotic organisms that acts as a master regulator of cellular growth. It is responsive to several nutrient inputs, such as amino acids, growth factors, glucose, energy status, lipids, and nucleotides, to turn on anabolic pathways and turn off catabolic processes such as autophagy. mTOR regulates cellular metabolism by phosphorylating several downstream effectors to relay the signal that there are sufficient nutrients to synthesize more complex molecules needed for cellular proliferation. Many cancers have dysregulated mTOR signaling to allow increased cell growth to sustain cell survival and suppress apoptosis.

Synthetic lethality: occurs when a defect in two or more genes simultaneously results in cell death whereas perturbation of only one of those genes does not affect viability. Synthetic lethality can occur through mutations, inhibitors, or a combination of both. Synthetic lethal interactions are of interest in cancer in that they can reveal liabilities that can be exploited therapeutically.

Transamination: a chemical reaction catalyzed by transaminases in which an amino group is transferred from an amino acid to a ketoacid, creating a new amino acid (see glutaminolysis, which involves a transamination reaction under many circumstances).

Box 2. Cellular Fate of Glutamine

While glutamine accounts for nearly 5% of all amino acid residues found in the human proteome, cancer cells take up far more glutamine than is needed for protein synthesis, suggesting additional cellular fates of glutamine beyond protein synthesis [1]. Glutamine can enter into the mitochondria and be metabolized to glutamate via GLS (Figure IA). Glutamate can then be converted into αKG via GDH, which produces ammonia that can then serve as a nitrogen source (Figure IA). Glutamate can also be converted to αKG by mitochondrial GOT2, which produces aspartate from oxaloacetate in the mitochondria (Figure IA,C). Glutamine-derived αKG replenishes the TCA cycle and becomes sequentially oxidized resulting in both substrate-level ATP production and NADH for reducing power that can drive the electron transport chain. The metabolism of αKG can also lead to the generation of pyruvate and NADPH from the TCA cycle intermediate malate (Figure IC). αKG can also be utilized in the reverse direction of the TCA cycle whereby αKG is reductively carboxylated to generate citrate [83–85], which can exit the mitochondria and generate acetyl-CoA for *de novo* fatty acid synthesis in the cytosol (Figure IA). Reductive carboxylation of glutamine-derived αKG to citrate allows the production of lipids and glucose to be used for other biosynthetic pathways and lactate production [3]. Glutamine can also be converted into glutamate in the cytosol, via phosphoribosyl pyrophosphate amidotransferase (PPAT), an enzyme that extracts the amide nitrogen of glutamine as a nitrogen source for nucleotide biosynthesis [86] (Figure IB). Glutamine-derived ammonia production has been associated with increased autophagy and protection from tumor necrosis factor-dependent cell death [87]. Glutamine-derived glutamate can also be utilized in the synthesis of GSH [88], assisting in redox balance in the cell, or can be converted into αKG via transaminases, such as GOT1, producing aspartate, or other NEAAs to be utilized in protein synthesis (Figure IB). Aspartate derived from the transamination reaction between glutamate and oxaloacetate can also be used in nucleotide metabolism [34,59,60] (Figure IB,C). Thus, glutaminolysis is highly versatile and can result in the production of a variety of metabolic products depending on need.

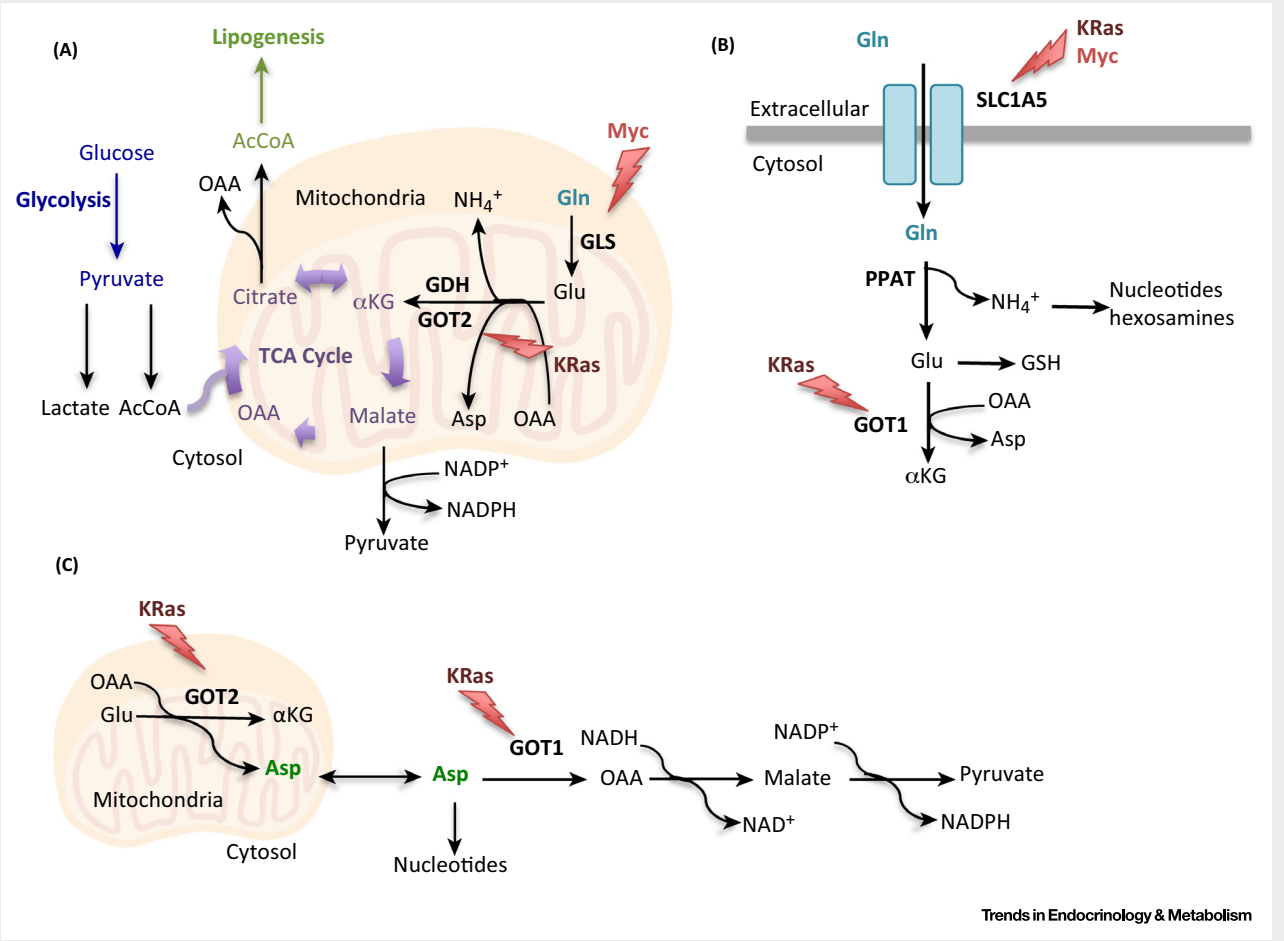

Figure I. Glutamine (Gln) Fulfills Many Biosynthetic Roles. (A) In the mitochondria, Gln can be metabolized to glutamate (Glu) by glutaminase (GLS). The production of Glu by GLS is enhanced by Myc overexpression. Glu can be converted into α-ketoglutarate (αKG) via glutamate dehydrogenase (GDH) or via glutamate-oxaloacetate transaminase (GOT)2, which concurrently produces aspartate (Asp) from oxaloacetate (OAA). αKG utilization through the tricarboxylic acid (TCA) cycle can generate malate, which can be used as an NADPH source for biosynthetic processes. The TCA cycle intermediate citrate can exit the mitochondria and be used to generate acetyl-CoA (AcCoA) for lipogenesis in the cytosol. (B) Gln enters the cell through SLC1A5. KRas- and Myc-driven cells enhance their uptake of Gln by increasing their expression of SLC1A5. Once in the cytosol, Gln can be converted into Glu via amidotransferases involved in nucleotide biosynthesis, such as phosphoribosyl pyrophosphate amidotransferase (PPAT), producing nitrogen needed for nucleotide and hexosamine synthesis. Cytosolic Glu can be used to maintain cellular redox balance via the production of glutathione (GSH), and Glu contributes to nonessential amino acid (NEAA) production via GOT1 production of αKG. KRas-cells increase their utilization of Gln and their cytosolic production of Asp and αKG by increasing expression of GOT1. (C) The GOT2 reaction produces Asp in the mitochondria, which can be exported into the cytosol through the malate-aspartate shuttle. This allows the cytosolic production of malate and pyruvate, concomitantly producing the electron acceptor NAD^+ and reducing power in the form of NADPH, which is required for biosynthetic pathways.

there is a temporally unique late-G_1 glutamine checkpoint that can be distinguished from an EAA checkpoint [11], indicating that it represents a separate nutritional input for determining cell cycle progression into S phase. This finding underscores the importance of glutamine to a dividing cell, despite its nonessential classification. Exogenously supplied glutamine is first deamidated to glutamate and then deaminated to αKG (Box 2 and Figure IA–C). The latter step can occur via a dehydrogenase reaction to generate αKG and ammonia or via a **transamination** reaction between glutamate and oxaloacetate to generate αKG and aspartate. Of significance, KRas-driven cancer cells are dependent on the transamination reaction and the aspartate that is generated, which is needed to maintain redox balance [5] and nucleotide

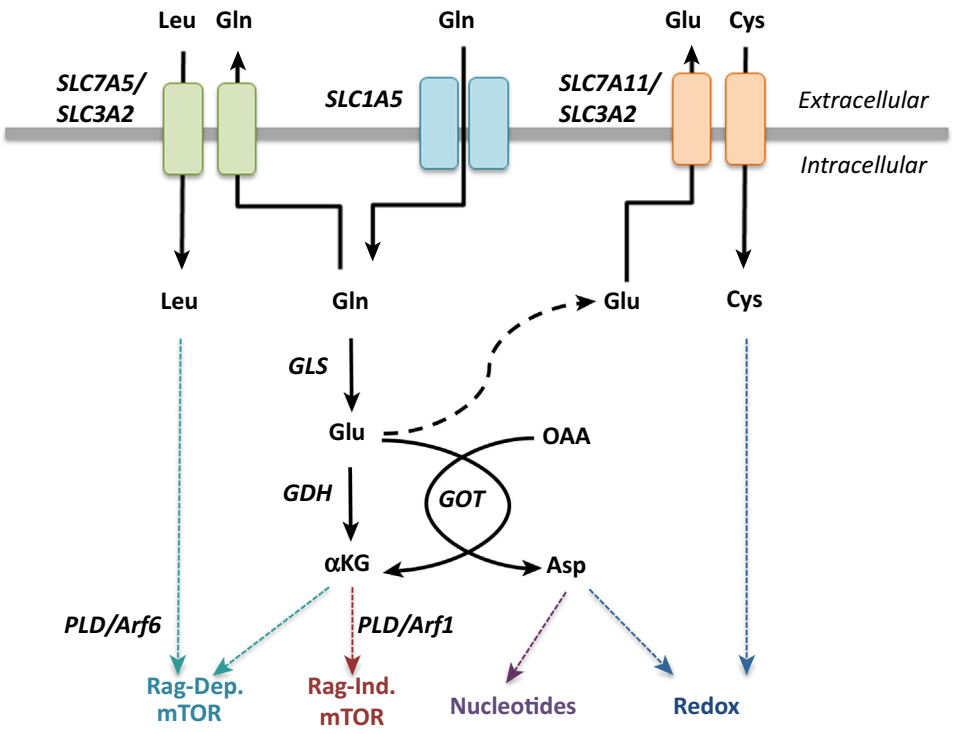

Figure 1. The Products of Glutamine (Gln) Metabolism and Transport Play Diverse Roles in Cellular Metabolism. Following Gln uptake into the cell via SLC1A5, Gln can be exported out of the cell in an antiport system comprising SLC7A5/SLC3A2. Gln is exchanged for leucine (Leu), an essential amino acid (EAA) and potent activator of mammalian target of rapamycin (mTOR) via the canonical amino acid (AA)-sensing Rag GTPase machinery (Rag-Dep. mTOR). Gln has also been shown to be an activator of mTOR signaling via the vesicle trafficking enzymes phospholipase D (PLD) and ADP-ribosylation factor 1 (Arf1), and dependent on the metabolism of Gln to α-ketoglutarate (αKG). This mechanism is independent of the Rag GTPases (Rag-Ind. mTOR). The metabolism of Gln to αKG involves the intermediate production of glutamate (Glu), which is exported through the SLC7A11/SLC3A2 (xCT) transporter in exchange for cystine (Cys), which is required for glutathione (GSH) production to sustain redox balance. The production of αKG via the glutamate-oxaloacetate transaminase (GOT) reaction produces aspartate (Asp), required for nucleotide synthesis and redox balance via the production of NADPH.

biosynthesis [34] (Box 2, Figure IC, and Figure 1). Intriguingly, EAAs, glutamine, and lipids also activate mTORC1 [16–18,40], making the mTORC1-mediated checkpoint appear redundant. However, mTORC1 is also responsive to GFs and has been suggested to be an integrator of GF and nutrient input [12,35]; thus, the apparent redundancy of glutamine checkpoints in late G_1 underscores the importance of glutamine for progression into S phase and the integration of glutamine input with GF instructions.

KRas-Driven Cancer Cells Override the Late-G_1 Glutamine Checkpoint

One of the hallmarks of cancer is the shift from catabolic to anabolic metabolism to promote cell growth and proliferation [41,42]. A consequence of activated KRas in cancer cells is the ability to override the late G_1 glutamine-dependent checkpoint (Figure 1A,B) [33]. Significantly, KRas-driven cancer cells deprived of glutamine arrested in S or G_2/M [33]. The S/G_2/M-phase arrest seen in the KRas-driven cancer cells was independent of tissue type and KRas mutation site [33]. Chiaradonna and colleagues observed similar results, in that KRas-transformed NIH3T3 cells accumulated in S phase on glutamine deprivation whereas nontransformed NIH3T3

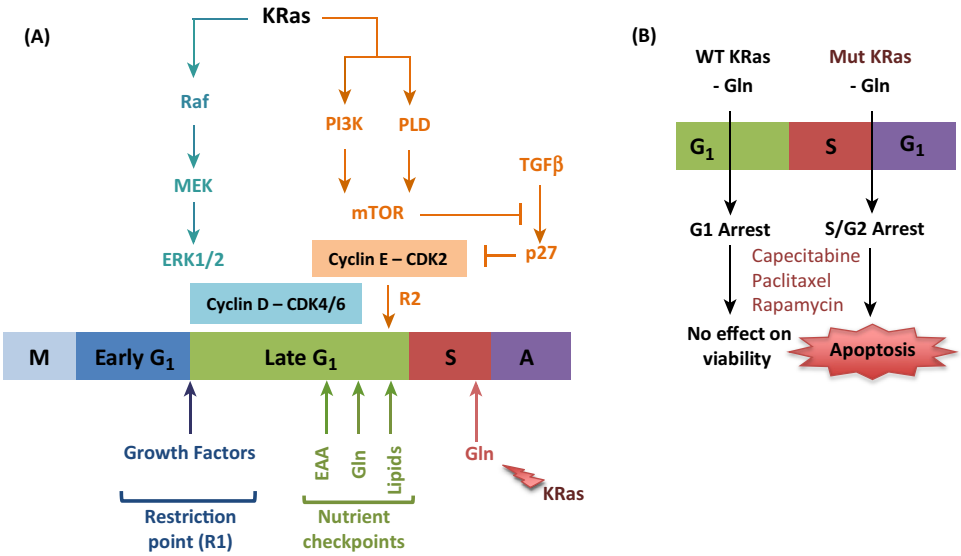

Figure 2. Cell Cycle Progression in Response to Nutrients and Ras Signaling. (A) The cell cycle is tightly controlled by a series of checkpoints to control progression through G_1 and into S phase. Growth factors mediate progression through the mid-G_1 restriction point (R1), whereas nutrients and mammalian target of rapamycin (mTOR) complex 1 (mTORC1) regulate passage through the late-G_1 checkpoints. KRas promotes passage through early and late G_1 through separate signaling cascades, where Raf/MEK/ERK activates cyclin D-dependent passage through R1, and mTOR signaling promotes cyclin E–cyclin dependent kinase2 (CDK2) activity later in G_1 by suppressing transforming growth factor (TGF)β signals that inhibit cyclin E–CDK2. While Gln typically mediates a late-G_1 nutrient checkpoint, KRas-driven cancer cells override this checkpoint and instead arrest in S phase on glutamine (Gln) deprivation. Activation of both of the KRas pathways is required to override the Gln-dependent G_1 checkpoint. (B) While wild-type (WT) KRas cells arrest in G_1 on Gln deprivation, mutant (Mut) KRas cells will instead arrest in S or G_2 phase in the absence of Gln. This creates a synthetic lethal phenotype for Mut KRas cells when treated with S-phase-specific cytotoxic drugs such as capecitabine, paclitaxel, or rapamycin. Conversely, these drugs do not affect WT KRas cells in the absence of Gln, as these cells are stalled at G_1.

fibroblasts did not [43]. Progression through the mid-G_1 R1 requires only GFs, whereas both nutrients and GFs regulate passage through the late-G_1 mTORC1 checkpoint (R2) (Figure 2A) [10]. Interestingly, KRas promotes passage through both of these regulatory checkpoints through separate signaling cascades (Figure 2A) [44]. KRas activates Raf/MEK/ERK signaling, activating cyclin D-dependent passage through the GF-mediated R1 [45], whereas passage through the mTORC1 checkpoint (R2) is dependent on cyclin E, downstream of the phosphatidylinositol 3-kinase/mTOR pathways (Figure 2A) [11,14,46]. Activation of both of these KRas downstream signals was required to override the glutamine-dependent G_1 checkpoint and restoration of G_1-arrest following glutamine deprivation was achieved by inhibition of both MEK and mTOR simultaneously [33]. Likewise, Chiaradonna and colleagues reported that KRas-transformed cells have elevated expression of both cyclin D and E to promote entrance into S phase under glutamine deprivation [43]. While KRas-dependent activation of pathways that target both cyclin D and cyclin E are required for bypass of the late-G_1 glutamine checkpoint, ectopic expression of the KRas G12V mutation into nontransformed fibroblasts was not sufficient to induce S-phase arrest in response to glutamine deprivation [33]. Moreover, expression of KRas by itself in primary cells leads to apoptosis or senescence [47]. If mutant Ras was introduced with tumor-promoting phorbol ester, a transformed phenotype was observed [48], and this combination also led to S-phase arrest upon glutamine deprivation,

indicating that multiple genetic alterations are required to promote bypass of the late-G_1 glutamine checkpoint, which importantly results in S-phase arrest where cells are uniquely vulnerable to apoptotic insult [33].

Glutamine Deprivation Creates a Synthetic Lethality in KRas-Driven Cancer Cells for Compounds that Target Cells in S Phase

The differential cell cycle arrest observed in KRas-driven cancer cells in response to glutamine deprivation creates an opportunity for therapeutic intervention. Saqcena *et al*. utilized capecitabine, which is converted to 5-fluorouracil, causing DNA damage and apoptosis in S-phase-arrested cells, and paclitaxel, which stabilizes microtubules during mitosis, causing apoptosis in G_2/M-arrested cells [33]. Treatment of glutamine-deprived cancer cells with wild-type KRas with either of these drugs did not significantly induce cell death (Figure 2B) [33]. By contrast, treatment of glutamine-deprived mutant KRas cancer cells with either of these drugs significantly increased the amount of nonviable cells (Figure 2B) [33]. Of significance, rapamycin also kills cells in S phase [49] and was effective in killing KRas-driven cancer cells subjected to glutamine deprivation (Figure 2B) [50]. Thus, glutamine deprivation creates a **synthetic lethality** in KRas-driven cancer cells for compounds that target cells in S phase. While glutamine deprivation in an animal is not a viable therapeutic option, it is possible to block glutamine utilization. Kimmelman and colleagues reported that KRas-driven cancers generate αKG primarily from glutamine via a transamination reaction between glutamine-derived glutamate and oxaloacetate to generate αKG and aspartate rather than the canonical GDH-catalyzed deamination reaction [5,51] (Box 1 and Figure IA,B). The transamination reaction can be inhibited by aminooxyacetate (AOA), which inhibits GOT and blocks the generation of both αKG and aspartate. Importantly, AOA mimicked the effect of glutamine deprivation and created a synthetic lethality for capecitabine, paclitaxel, and rapamycin (Figures 1 B and 2) [33,50]. Since normal cells generally use the GDH reaction, the effects of AOA may have some specificity for KRas-driven cancer cells that preferentially utilize the transamination reaction [5]. AOA has been used in mouse xenograft studies without apparent side effects [52] and may therefore be a viable strategy in humans. Further, a recent study discussed a medicinal chemistry-based method to optimize GOT inhibitors for use in pancreatic ductal adenocarcinoma due to their high dependency on GOT1 [53]. Other enzymes of glutaminolysis, such as glutaminase (GLS) or GDH, may prove to be similarly effective targets of anticancer therapeutics, which has been reviewed extensively by others [54,55].

Proliferating Cells Require Glutamine-Dependent Aspartate Synthesis for Nucleotide Biosynthesis

Two studies have found that the S-phase arrest seen in KRas-driven cells following glutamine deprivation could be rescued with the addition of exogenous nucleotides, suggesting that the S-phase arrest induced by glutamine deprivation is the result of an insufficient amount of nucleotides to complete DNA replication in S phase (Figure 3) [34,43]. This lack of nucleotides results in single-strand DNA breaks during DNA synthesis, which initiates the ataxia telangiectasia and Rad3-related (ATR)-mediated DNA damage pathway leading to S-phase arrest (Figure 3) [34]. The transamination reaction between glutamate and oxaloacetate to generate αKG and aspartate is preferentially used for glutaminolysis in KRas-driven cancer cells [5,56]. Inhibition of this transamination reaction blocks the production of aspartate, and importantly, aspartate is necessary for nucleotide biosynthesis [57]. Consistent with this, aspartate, but not αKG, was able to rescue the S-phase arrest caused by either glutamine deprivation or AOA treatment [34]. A recent report further supported this by demonstrating that cytosolic aspartate is critical for cancer cell survival and that loss of the mitochondrial aspartate exporter sensitizes

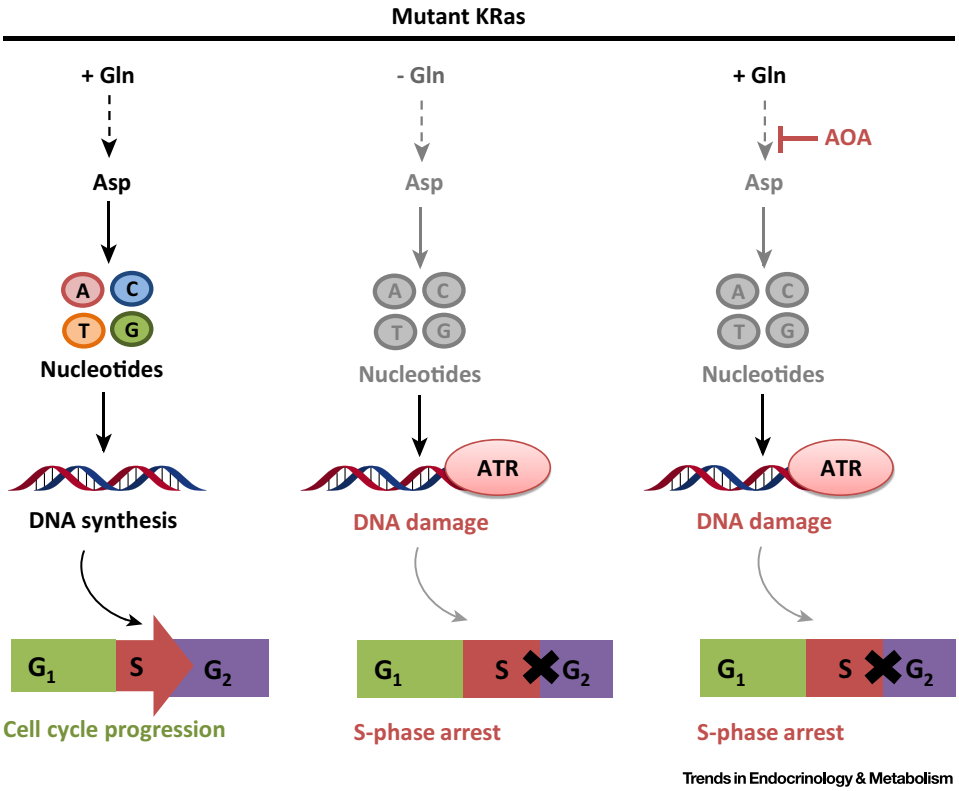

Figure 3. Mutant KRas Cells Require Glutamine (Gln)-Derived Nucleotides for S-Phase Progression. Gln (+ Gln, left panel) is used for the production of aspartate (Asp), both of which are required for nucleotide synthesis and, subsequently, the synthesis of DNA required for S-phase progression. In the absence of Gln (– Gln, center panel), aspartate is depleted, leading to a decrease in nucleotides resulting in single-strand breaks in DNA, induction of the ATR-mediated DNA damage response, and S-phase arrest. Depriving patients of Gln is not a viable therapeutic option; however, the transaminase inhibitor aminooxyacetate (AOA) can be used to inhibit the production of Asp from Gln (+ Gln with AOA, right panel), leading to the same downstream effects as Gln deprivation as seen in Figure 2B. S-phase-arrested Mut KRas cells can be treated with S-phase-cytotoxic drugs to cause cell death.

tumors to GLS inhibition [58]. It was also reported previously that cell cycle arrest caused by inhibition of the electron transport chain could be overcome with aspartate [59–61]. These studies revealed the importance of the generation of cytosolic aspartate in cancer and proliferating cells. αKG, the other product of the transaminase reaction, is needed for the induction of mTORC1 in response to glutamine in KRas-driven cancer cells [20]. Thus, there appear to be distinct pathways for the utilization of downstream metabolites of glutaminolysis for different cellular purposes in KRas-driven cancer cells.

KRas-Driven Cancer Cells Require Glutaminolysis for Redox Balance

Work by Kimmelman and colleagues showed that aspartate plays another critical role in KRas-driven pancreatic cancer cell growth [5]. This work demonstrated that the GDH inhibitor epigallocatechin gallate did not affect cell growth whereas AOA strongly reduced the growth of pancreatic ductal adenocarcinoma cells [5]. These results were surprising and indicated that KRas-driven cancer cells metabolize glutamine differently than through the canonical GDH pathway to produce αKG and further implicate aspartate as being critical for cell growth and proliferation. This study went on to show that the aspartate promoted redox balance, whereby

the aspartate generated by mitochondrial GOT exits the mitochondria where it is converted to oxaloacetate by cytosolic GOT. Oxaloacetate is then reduced by cytosolic NADH to generate malate, which can then be oxidatively decarboxylated to pyruvate with the generation of NADPH from $NADP^+$ by malic enzyme 1, the net effect being the transfer of electrons from NADH, which provides electrons for the electron transport chain, to $NADP^+$ to generate NADPH, which is used for anabolic reactions needed by proliferating cells. Cells undergoing high rates of proliferation, like tumor cells, encounter high levels of oxidative stress that require elevated NADPH:$NADP^+$ ratios for cell survival [62]. Consequently, the cytosolic GOT pathway represents a significant source of NADPH for KRas-driven cancer cells and is required to maintain cellular redox homeostasis [5,51] (Box 2 and Figure IA,B).

Glutamine also contributes to the cell's antioxidant defenses through its metabolism to glutamate, which is used for the *de novo* production of glutathione (GSH) (Box 2 and Figure IB). GSH is a tripeptide comprising glutamate, cysteine, and glycine and is the primary nonenzymatic antioxidant in the cell. Glutamine-derived glutamate also contributes to GSH synthesis by stimulating the uptake of cystine and efflux of glutamate through the xCT antiport system (SLC7A11/SLC3A2) (Figure 1) [63]. Cystine, the oxidized dipeptide of cysteine, is converted into cysteine in the cell for its subsequent incorporation into GSH. Accordingly, glutamine deprivation has been associated with decreased uptake of cystine through xCT [64,65] and decreased intracellular GSH levels [7]. Thus, the impact of glutamine on redox balance involves both the generation of aspartate in the transamination reaction between αKG and oxaloacetate and the generation of glutamate to import cystine for incorporation into GSH. Additionally, the xCT antiporter system has been reported to be responsible for regulating glutamine dependency in cancer [64].

Reprogramming Glutamine Metabolism *In Vivo*

An important consideration moving forward is the universality of the metabolic reprogramming of KRas-driven tumors. Studies have assessed various lung [66] and breast [67] cancer cell lines and found considerable variation in their dependency on glutamine. Non-small cell lung cancers with high GLS1 expression were highly dependent on glutamine, whereas other non-small cell lung cancer cell lines showed almost complete glutamine independence [66]. Likewise, whereas basal-type breast cancers tended to be more dependent on exogenous glutamine, luminal breast cancers showed increased synthesis of glutamine via glutamine synthetase and were more glutamine independent [67].

In vivo, tumors also display variability in their glutamine dependencies. Vander Heiden and colleagues reported that KRas-driven lung tumors are less dependent on glutamine in mouse lung tumors than they are in cell culture [68]. Metabolism in the lung became more dependent on glucose than in culture. By contrast, glutamine dependence was retained in mouse pancreatic tumors [5]. A possible difference could be the high oxygen content in the lungs such that the cells take advantage of oxidative phosphorylation in the electron transport chain. Alternatively, glutamine may be a better nutrient under hypoxic conditions, especially during early stages of tumorigenesis prior to vascularization. Under hypoxic conditions, most of the pyruvate needs to be reduced to lactate to regenerate NAD^+ to sustain glycolysis and ATP production [69].

Another study using an orthotopic mouse model of human glioblastoma revealed that while tumors in mice brain took up exogenous glutamine and displayed large glutamine pools, they did not use this glutamine for anaplerosis and instead used glucose for pyruvate carboxylation [70]. However, when these cells were cultured *ex vivo*, they were completely glutamine

independent, surviving in culture for over 1 week at a low growth rate [70]. Work by Bishop and colleagues suggested that the *in vivo* requirement for glutamine metabolism depends on genetic background as well as tissue type: Myc-induced liver tumors increased glutamine catabolism by decreasing glutamine synthetase and increasing the GLS1 enzyme, whereas Myc-induced lung tumors display increased expression of both enzymes to increase glutamine pools [71]. Thus, it is likely that the complex microenvironment of tumorigenesis will complicate this fertile area of therapeutic intervention.

Concluding Remarks and Future Perspectives

While at least 25% of all cancers have mutant KRas [72], there has been limited success in treating these cancers due to the difficulty of directly inhibiting the KRas protein, leading many to consider KRas as 'undruggable' [73]. However, the metabolic reprogramming of glutaminolysis in KRas-driven cancer cells to use a transaminase reaction rather than the dehydrogenase reaction [5] creates new therapeutic opportunities. Inhibition of the transaminase reaction creates a synthetic lethality by leading KRas-driven cancer cells to bypass a late-G_1 checkpoint and arrest in S phase where they are susceptible to targeting with S-phase-specific cytotoxic compounds. Moreover, the utilization of aspartate for redox balance in KRas-driven cancer cells [5] also provides a fertile area for therapeutic intervention. This unique property of metabolic reprogramming of glutaminolysis that occurs in KRas-driven cancer cells represents an Achilles' heel for a large percentage of human cancers. While there is excitement regarding the potential for exploiting metabolic reprogramming of glutaminolysis, there remain intriguing questions that will need to be addressed in this exciting area of cancer metabolism (see Outstanding Questions).

Outstanding Questions

To what extent does the tumor microenvironment impact metabolic reprogramming in cancer cells?

Can transaminase enzymes be targeted in humans?

Many of the effects of glutamine are mediated by αKG, including the activation of mTORC1 and PLD activity. How does αKG stimulate these effects?

Do oncogenic mutations other than KRas mutations similarly promote cell cycle progression through the late-G_1 glutamine-dependent cell cycle checkpoint leading to S-phase arrest?

Does the phospholipase D and Arf GTPase requirement for glutamine-induced mTORC1 implicate vesicle trafficking for mTORC1 signals?

To what extent is the aspartate generated by the mitochondrial GOT transamination reaction utilized in the cytosol for redox balance versus nucleotide biosynthesis?

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