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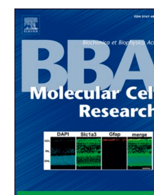

Aminoacyl-tRNA synthetases and amino acid signaling

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ABSTRACT

Aminoacyl-tRNA synthetases (ARSs) are a family of evolutionarily conserved housekeeping enzymes used for protein synthesis that have pivotal roles in the ligation of tRNA with their cognate amino acids. Recent advances in the structural and functional studies of ARSs have revealed many previously unknown biological functions beyond the classical catalytic roles. Sensing the sufficiency of intracellular nutrients such as amino acids, ATP, and fatty acids is a crucial aspect for every living organism, and it is closely connected to the regulation of diverse cellular physiologies. Notably, among ARSs, leucyl-tRNA synthetase 1 (LARS1) has been identified to perform specifically as a leucine sensor upstream of the amino acid-sensing pathway and thus participates in the coordinated control of protein synthesis and autophagy for cell growth. In addition to LARS1, other types of ARSs are also likely involved in the sensing and signaling of their cognate amino acids inside cells. Collectively, this review focuses on the mechanisms of ARSs interacting within amino acid signaling and proposes the possible role of ARSs as general intracellular amino acid sensors.

1. Introduction

Aminoacyl-tRNA synthetases (ARSs) are a family of essential enzymes that are evolutionarily conserved and catalyze the ligation of tRNAs with their cognate amino acids for translation. The aminoacylation of tRNAs by ARSs is a high-fidelity process usually composed of two steps. ARS activates their cognate amino acid using ATP, producing an aminoacyl adenylate (aa-AMP) and a pyrophosphate. Subsequently, the activated amino acid is transferred to the corresponding tRNA. Amino acids are ligated at the 2'-OH or 3'-OH of the tRNA that contains an anticodon complementary to the mRNA codon; class I ARSs bind preferentially to the 2'-OH and class II ARSs bind preferentially to the 3'-OH of the tRNA. Aminoacylated tRNA (also called charged tRNA) is then transferred to ribosomes for translation [1,2].

Aminoacylation of tRNA by ARSs is indeed a pivotal biological process; however, efforts to understand the alternative functions of ARSs have unveiled the fact that ARSs may also exhibit various functions beyond catalytic role in protein synthesis. Through the evolutionary process from simple unicellular organisms to higher-order eukaryotes, while conserving catalytic domains, ARSs have gradually adopted extra domains for interacting with other important cellular factors [1–4]. ARSs may participate in a wide spectrum of cellular processes and contribute to pathophysiological processes, such as angiogenesis, inflammation, obesity and tumorigenesis [1,2].

The nutrient signaling pathways are employed to regulate various signaling cascades that govern cellular energy metabolism and regulate cell growth, proliferation and survival [5]. These pathways usually exert intracellular sensors that are capable of detecting the fluctuations in the abundancy of nutrients [6,7]. Likewise, sensing and maintaining intracellular amino acid levels is certainly crucial biological phenomenon for survival, which ultimately affects the dynamic balance between catabolic and anabolic cellular pathways for efficient utilization of limited resources. Amino acids are the essential building blocks of many important components of life including proteins, hormones, neurotransmitters and other metabolites such as polyamines and creatine [8]. However, amino acid utilizing processes such as protein synthesis are energetically expensive and consume massive amounts of amino acids and ATP. Therefore, cells must develop complex sensing mechanisms that couples the extracellular and intracellular availability of amino acids to diverse cellular processes. Notably, among ARSs, glutaminyl-tRNA synthetase 1 (QARS1) and leucyl-tRNA synthetase 1 (LARS1) have been demonstrated to participate in sensing and transmitting signals related to intracellular levels of their cognate amino acids [9–12].

Collectively, ARSs have the ability to bind their cognate amino acids,

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and are capable of participating in many important protein interactions. Moreover, since major substrates of ARSs, such as amino acids, ATP and tRNAs, are all metabolically important intracellular molecules, this further elucidates the importance of ARSs. Therefore, the further exploration of ARSs' involvement in cellular amino acids signaling pathways would be of great interest. This review covers major discoveries and evidences that suggest the possibility of ARSs acting as the *bona fide* intracellular sensors for their cognate amino acids (Fig. 1). In fact, the idea that ARSs function as amino acid sensors was first recognized and proposed in 2001 after the discovery of the glutamine-dependent antiapoptotic regulation of QARS1 [9]; then this idea was supported by later discoveries that showing evidence of other ARSs' roles in amino acid signaling pathways. Therefore, we discuss how ARSs may interact with other proteins in distinctive signaling cascades to control diverse cellular functions in amino acid-dependent manners.

2. The glutamine sensor QARS1 interacts with ASK1 for antiapoptotic regulation

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase family, which activates c-Jun N-terminal kinase (JNK) and p38 in response to various types of stress signals [13]. ASK1 remains inactive under normal conditions and is activated by the introduction of stress-associated signals, such as oxidative stress, endoplasmic reticulum stress, tumor necrosis factor (TNF) and infection [14–18]. In response to reactive oxygen species (ROS), the redox-responsive protein thioredoxin (Trx) within the ASK1 signalosome is oxidized and dissociates from the complex, which allows autophosphorylation and activation of ASK1 [16,19]. Furthermore, TNF-α receptor-associated factor (TRAF) family proteins can be recruited and reinforce ASK1 signalosome activity once activated by ROS [20]. Importantly, glutamine deprivation is capable of inducing ASK1 expression, and the molecular interaction between QARS1 and ASK1 could be the underlying mechanism for the suppressive nature of glutamine on ASK1 signaling [9].

Glutamine is not only highly utilized as a metabolic fuel in many cells but also recognized as an apoptotic suppressor. Especially in intestinal epithelial cells, glutamine deprivation induces apoptosis [21], whereas supplementation with glutamine can protect intestinal epithelial cells from oxidative stress and apoptosis [22]. Furthermore, many cancer cells have been shown to be highly dependent on the dysregulated glutamine metabolism for their proliferation, and the restriction in glutamine metabolism has been shown to be effective against tumor growth both *in vitro* and *in vivo* by inducing apoptosis [23–25]. Therefore, glutamine might have important roles in protecting cells against apoptosis induced by diverse stimuli. Fundamental mechanisms underlying the protective roles of glutamine specifically under apoptotic conditions have yet to be fully elucidated. However, it was revealed that the relationship between QARS1 and ASK1 responds to intracellular glutamine levels [9], thereby suggesting an interesting model of QARS1 in the regulation of apoptotic signals (Fig. 2).

Ko et al. have shown that QARS1 binds and represses ASK1 in a glutamine-dependent manner, in which increasing glutamine levels enhance their association. Additionally, the catalytic domains of QARS1 and ASK1 are both involved in this association. Thus, QARS1 could be a key modulator of ASK1, correlating cellular glutamine deficiency to apoptotic signals. In early structural studies, bacterial QARS1 was shown to be composed of the anticodon-binding domain and catalytic domain responsible for attachment of glutamine and $tRNA^{Gln}$ [26]. In contrast, human and eukaryotic QARS1 contain an extra domain appended with an N-terminal domain (NTD). This distinctive extension of the domain is crucial for the formation of the multi-tRNA synthetase complex (MSC) and is also important for the propagation of anti-apoptotic signals through the interaction with ASK1 [9,27–29].

Considering that the NTD also plays an important role in the QARS1-ASK1 interaction, the arrangement and interaction between the QARS1 domains could be important in this interaction as well. In a structural study of QARS1, the mutations implicated in neurologic disorders were precisely mapped [29]. Specifically, pathological mutants (*i.e.*, G45V and Y57H) located at the NTD significantly diminished the catalytic activity of human QARS1 while the tRNA binding ability remained intact [29]. Thus, the NTD might have the potential to interact with the distal catalytic domain, which suggests that the QARS1 domains are functionally connected. Therefore, perhaps we can speculate that glutamine binding to the catalytic domain might induce conformational changes in the distal NTD and thus contribute to the QARS1-ASK1 interaction. Additional studies are necessary to determine the detailed mechanism underlying how glutamine binding to QARS1 affects the interaction between QARS1 and ASK1.

Although an in-depth understanding of the exact mechanism in the QARS1 and ASK1 association remains to be elucidated, based on previous studies, we can assume that glutamine binding and/or glutamine

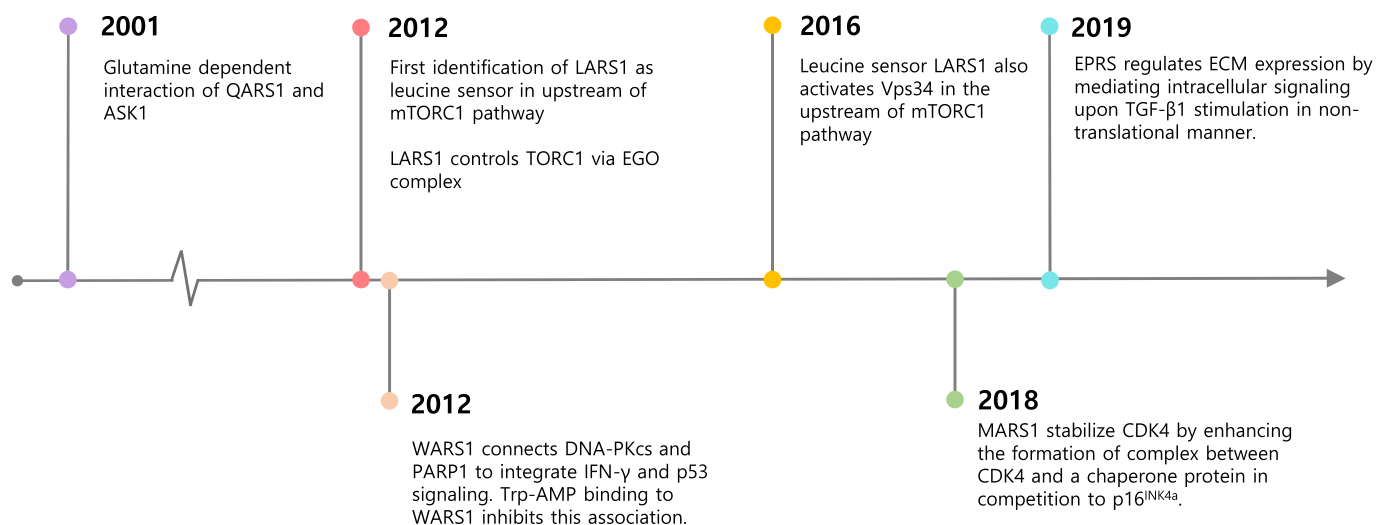
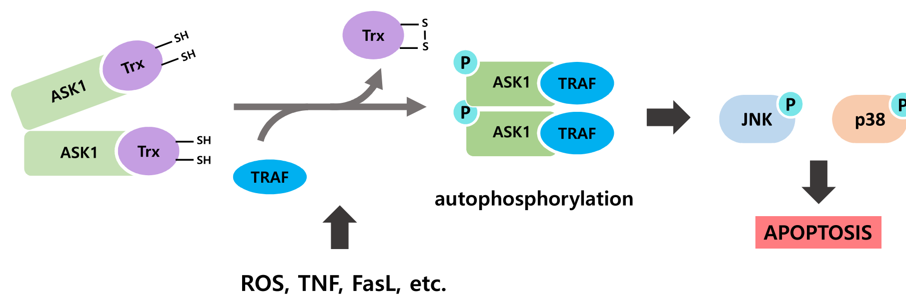


Fig. 1. The key milestones suggest ARSs as potential amino acid sensors. QARS1, glutaminyl-tRNA synthetase 1; ASK1, apoptosis signal-regulating kinase 1; LARS1, leucyl-tRNA synthetase 1; mTORC1, mammalian target of rapamycin complex 1; TORC1, target of rapamycin complex 1; WARS1, tryptophanyl-tRNA synthetase 1; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; PARP1, poly(ADP-ribose) polymerase 1; EPRS 1, glutamyl-prolyl-tRNA synthetase 1; ECM, extracellular matrix; TGF-β1, transforming growth factor beta 1; MARS1, methionyl-tRNA synthetase 1; CDK4, cyclin dependent kinase 4.



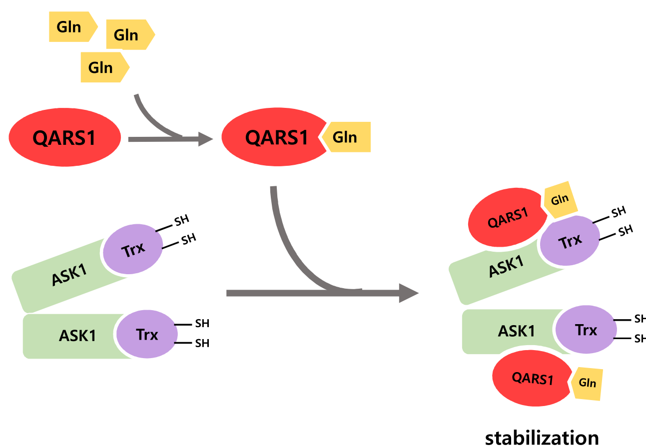


Fig. 2. Schematic representation of the working model for the role of QARS1 and glutamine in the regulation of ASK1 activity. a) Activation of apoptosis signal-regulating kinase 1 (ASK1) upon stimulation by stress signals such as reactive oxygen species (ROS), tumor necrosis factor (TNF) and Fas ligand (FasL). Specifically, ROS facilitate the dissociation of thioredoxin (Trx) from ASK1. After Trx dissociation, ASK1 can be activated by autophosphorylation of the Thr845 site in the kinase domain. Additionally, ASK1 activity can be reinforced by the interaction with TNF-α receptor-associated factor (TRAF). Activated ASK1 causes the activation of JNK and p38 and finally enhances apoptotic responses. Glutaminyl-tRNA synthetase 1 (QARS1) interaction with QARS1, which suppresses ASK1 autophosphorylation, is disrupted under low glutamine conditions. b) QARS1 is associated with ASK1 in a glutamine (Gln)-dependent manner and suppress the activation of ASK1 and apoptotic signaling.

activation by QARS1 could be the essential step in the interaction between QARS1 and ASK1. Recently, interesting research has demonstrated that the reversible glutaminylation of the K688 residue in ASK1 by QARS1 is crucial for their association and the subsequent inhibition of apoptosis induced by the ASK1 signaling pathway [30]. In this study, the authors suggest that aminoacylation on lysine residues could be another type of posttranslational modification and that aa-AMPs are utilized by ARSs for the aminoacylation of substrates in various signaling cascades for sensing and transmitting intracellular amino acid signals. Intriguingly, the authors demonstrated that the only subphysiological levels of amino acids were enough to saturate the tRNA charging activity of ARSs for cells to maintain protein synthesis, whereas aminoacylation of lysine residues on the proteins only occurred only when amino acids were close to physiological levels. This suggests the possibility that ARS functions are segregated based on amino acid levels; while acting as sensors to detect fluctuations in amino acids, their fundamental roles in protein synthesis are saturated most of the time and are altered only during severe depletion of amino acids.

3. The leucine sensor LARS1 couples leucine availability to the mTORC1 signaling pathway

mTOR is a serine/threonine kinase that is well known for its ability to control over a variety of cellular processes, including cellular metabolism, protein synthesis, and autophagy, and is implicated to have roles in pathophysiology associated with cancer, neurodegeneration, obesity and diabetes in response to diverse environmental cues [31–33]. Mammals, unlike yeasts with two target of rapamycin (TOR) genes (*TOR1* and *TOR2*), have only one *MTOR* gene that composes into two distinctive complexes named mechanistic target of rapamycin complex 1 (mTORC1) and mechanistic target of rapamycin complex 2 (mTORC2). Both complexes show distinctive components and upstream regulators and hence differences in downstream signaling. mTORC1 is composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian LST8 (mLST8), proline-rich Akt substrate of 40 kDa (PRAS40) and Dishevelled, EGL-10 and pleckstrin (DEP) domain-containing mTOR interacting protein (DEPTOR). Raptor is responsible for the enhanced recruitment of substrates to mTORC1, and it is also required for the localization of mTORC1 to the lysosomal membrane [34,35]. On the other hand, mTORC2 contains components that are distinct from mTORC1 components, namely, Raptor-independent companion of mTOR (Rictor) and mammalian stress-activated protein kinase-interacting protein (mSin1) and common components such as mLST8 and DEPTOR. While PRAS40 enhances both mTORC1 and mTORC2 functions, DEPTOR acts to suppress both of the complexes. A more detailed review of the structure and composition of mTOR complexes is provided in elsewhere [32].

The mTOR inhibitor rapamycin inhibits S6 kinase (S6K)-mediated phosphorylation of the ribosomal protein S6 and the initiation of mRNA translation [36,37]. 4E-BP1 is another important downstream effector for the activation of mRNA translation, and other well-known targets of mTORC1, such as sterol element-binding protein (SREBP) and Unc-51 like autophagy activating kinase 1 (ULK1) are also metabolically important downstream effectors responsible for the regulation of lipid biosynthesis and autophagy, respectively [38]. Tuberculous sclerosis complex (TSC) and Rheb GTPase are the two important upstream regulators of mTORC1. TSC is a heterotrimeric complex comprising TSC1, TSC2 and Tre-2/BUB2/cdc 1 domain family (TBC1D) [39]; importantly,

TSC2 is able to inhibit Rheb GTPase, which directly interacts and activates mTORC1, by acting as a GTPase-activating protein (GAP) [40].

mTORC1 functions as a master growth regulator that integrates amino acid signaling with cell growth [41]. Leucine is an essential amino acid and is considered an important amino acid due to its effect on the regulation of protein metabolism [42], specifically because of its involvement in the mTORC1 signaling pathway [43]. Rag GTPases have been considered as key modulators that connect amino acid signal to mTORC1 pathway [35,44]. Mammals express four types of Rag GTPases – RagA, RagB, RagC and RagD – that assemble into heterodimers of RagA-RagC or RagB-RagD [35,44–46]. Lysosomal translocation and activation of mTORC1 require the Rag GTPase dimer consisting of RagA or RagB in the GTP-bound form and RagC or RagD in the GDP-bound form [35]. Importantly, amino acids promote lysosomal translocation of mTORC1, where the GTP-bound form RagB containing Rag GTPases heterodimers serves as the docking site for mTORC1 [47].

The signaling sensors placed upstream of mTORC1 have been studied extensively, and several players have been reported to date. Among them, LARS1 was the first identified upstream intracellular leucine sensor that couples leucine levels with mTORC1 activity in human and yeast [10,48]. Specifically, LARS1 with a mutated leucine binding site impaired mTORC1 activation to leucine signals [10]. In fact, there are at least two possible mechanisms associated with the LARS1-mediated activation of mTORC1 when LARS1 senses intracellular leucine signals (Fig. 3). First, LARS1 controls lysosomal translocation and activation of mTORC1 in a Rag GTPase-dependent manner [10]. Recently, the dynamics of the GTP-GDP cycle of Rag GTPases have been identified, and importantly, LARS1 serves as an initiator of the Rag GTPase cycle by accelerating GTP hydrolysis of RagD [49]. Particularly, when LARS1 senses a rise in leucine levels, LARS1 functions as the specific GAP of RagD and renders RagD in the GDP-bound form [10]. Subsequently, GTP hydrolysis of RagD may control the recruitment of Regulator complex, which enhances GTP exchanging of RagB and thus promotes direct interaction of GTP-bound RagB and GDP-bound RagD heterodimer with Raptor, thereby activating mTORC1 pathway [49,50]. Moreover, the stress-induced metabolic protein Sestrin2 also binds to leucine and promotes GTP hydrolysis of RagB by enhancing RagB GAP activity of GATOR1 [51,52], which in turn facilitates the inactivation of RagB-RagD heterodimer and thus terminates the Rag GTPase cycle [49]. Additionally, RagB-RagD heterodimer was dominant over RagA-RagC heterodimer as the key mediator of amino acid signaling to mTORC1 [49]. Collectively, the Rag GTPase cycle is coordinated by LARS1 and Sestrin2 upon leucine stimulation, which turns on and turns off the cycle, respectively [49].

The LARS1-RagD-mTORC1 axis can be selectively inhibited [53]. The pyrazolone compound BC-LI-0186 and its derivatives bind to RagD interacting site of LARS1, inhibiting LARS1-RagD interaction, while they have only little effects on the catalytic activity of LARS1 [53]. BC-LI-0186 suppresses GTP hydrolysis of RagD and hence disrupting lysosomal translocation and activation of mTORC1. The functional importance of LARS1-RagD-mTORC1 axis is supported by the physiological effect of BC-LI-0186. BC-LI-0186 treatment caused a significant reduction in the tumor volume of rapamycin-resistant HCT116 cell xenograft model [53] and in Kras G12D lung cancer mouse model [54]. Moreover, BC-LI-0186 has been suggested to promote robust muscle regeneration that is accompanied by functional recovery, which was abolished by combinatory treatment with an Akt inhibitor, thus indicating the possible involvement of LARS1 in the mTORC1-dependent control of myogenesis [55].

In addition to acting as a specific GAP of RagD, there is the second mechanism associated with the LARS1-mediated activation of mTORC1 that works in parallel with the Rag GTPases. Specifically, LARS1 has also been reported to be an activator of the vacuolar protein sorting 34 (Vps34)-phospholipase D1 (PLD1) signaling pathway upstream of mTORC1 in response to leucine levels [12]. Vps34 is a class III phosphoinositide 3-kinase (PI3K) that participates in many cellular functions, such as autophagy and endocytic sorting, by forming distinctive complexes [56,57]. Amino acid stimulation enhances Vps34-catalyzed production of phosphatidylinositol 3-phosphate (PI3P) from phosphatidylinositol [58,59]. Elevated PI3P levels enhances activation and subsequent recruitment of PLD1 to the lysosomal membrane [60]. Activated PLD1 hydrolyzes phosphatidylcholine (PC) into phosphatidic acid (PA), which binds and activates Rheb GTPase [61,62]. The involvement of LARS1 in the activation of Vsp34-PLD1-mTORC1 axis might suggest the integration of mitogenic signals with nutrient-dependent signals.

Notably, a recent study revealed an interesting aspect of LARS1 in glucose-dependent regulation of leucine utilization [63]. Activated mTORC1 suppresses autophagy by directly phosphorylating and inhibiting the autophagy-initiating kinase ULK1 [64] and suppressing transcription factor EB (TFEB), which is the transcription factor that controls a wide range of lysosome- and autophagy-related genes [65]. However, during glucose deprivation, mTORC1 is inactivated and thus ULK1 facilitates the activation of autophagy as an adaptative response to the starvation [64]. Yoon et al. identified that ULK1 mediates the phosphorylation at residues in the leucine binding sites of LARS1 and thus suppresses leucine binding and the subsequent leucine-mediated activation of the mTORC1 signaling pathway [63]. Therefore, during low energy status derived by glucose starvation, leucine sensing and mTORC1 activating roles of LARS1 is prohibited, which suppresses the

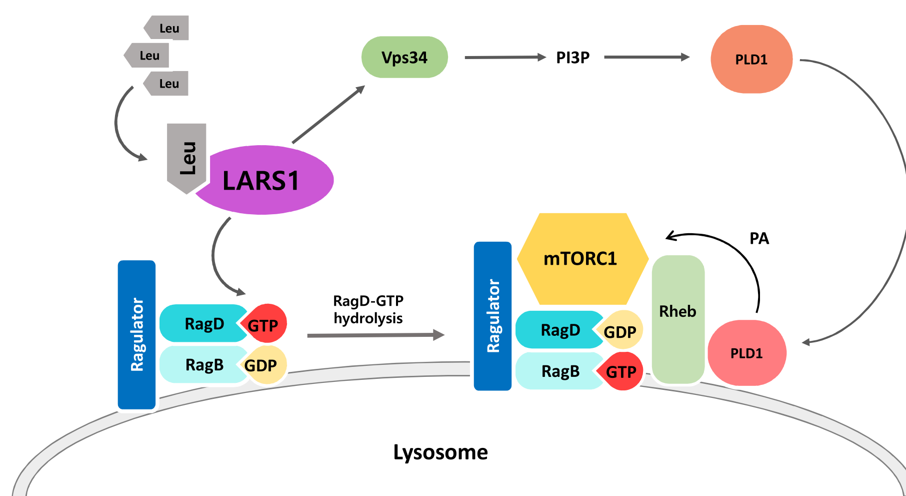


Fig. 3. Schematic representation of LARS1 functioning as an intracellular leucine sensor in upstream of mTORC1 activation. Leucyl-tRNA synthetase 1 (LARS1) may transmit the signal of leucine (Leu) sufficiency inside the cells by two mechanisms. First, LARS1 directly enhances RagD-GTP hydrolysis *via* its endogenous GTPase-activating protein (GAP) activity in a leucine-dependent manner. RagD in the GDP-bound form enhances mechanistic target of rapamycin complex 1 (mTORC1) activation. Second, LARS1 activates the vacuolar protein sorting 34 (Vps34)-phospholipase D1 (PLD1)-mTORC1 signaling pathway in a leucine-dependent manner. Leucine binding to LARS1 is crucial for activation of the Vps34 kinase function. Activated Vsp34 elevates phosphatidylinositol 3-phosphate (PI3P) production, which provokes PLD1 activation and PLD1 translocation to the lysosomal membrane. PLD1 catalyzes phosphatidic acid (PA) from phosphatidylcholine, which binds to and activates the mTORC1 activator Rheb.

high energy consuming protein synthesis process. Furthermore, because leucine utilization in the anabolic processes is reduced, this could contribute to enhanced leucine availability in the catabolic energy generation [63]. This example might indicate the existence of inhibitory mechanisms for the amino acid-sensing role of ARSs in response to stress conditions like low energy status.

Altogether, LARS1 serves as a leucine sensor at the cellular level that regulates protein synthesis by controlling mTORC1 activity in accordance with intracellular leucine availability. Additionally, LARS1 could be capable of controlling the fate of leucine to be used in energy generation or protein synthesis depending on the availability of glucose.

4. WARS1 in tryptophan sensing for the activation of p53

Tryptophanyl-tRNA synthetase 1 (WARS1) is one of the five human ARSs that contains the WHEP domain and catalyzes the aminoacylation of $tRNA^{Trp}$ during translation. WHEP domains are helix-turn-helix domains, and its name is derived from the first letters of WARS1, histidyl-tRNA synthetase 1 (HARS1), and glutamyl-prolyl-tRNA synthetase 1 (EPRS1). In addition to its classical role in aminoacylation, WARS1 has several noncanonical functions. The acquisition of noncanonical functions by WARS1 in part results from the alternative splicing and proteolytic cleavages. The N-terminal proteolytic cleavages that delete 70 and 93 amino acid in length produce the truncated T1 and T2 forms of WARS1, respectively. Both T1 and T2 forms of WARS1 are capable of interacting with vascular endothelial (VE)-cadherin and perform angiostatic activities [66,67]. In addition, WARS1 can be immediately secreted from monocytes upon infectious insults to participate in the priming of innate immunity. Specifically, 154-amino-acid-long N-terminal peptide containing WHEP domain is responsible for triggering of immune stimulation [68].

Importantly, an interesting correlation between WARS1 and p53 phosphorylation in antiproliferative responses was identified [69]. Considering the fact that WARS1 is highly induced by IFN-γ stimulation [70,71], WARS1 might participate in the integration of IFN-γ signaling and the activation of p53, which leads to the generation of strong antiproliferative response. This study demonstrated that in addition to the upregulation of WARS1 expression upon IFN-γ stimulation, enhanced nuclear translocation of WARS1 was detected [69]. Though the nuclear presence of WARS1 has been reported previously [72], the function of nuclear WARS1 was unknown. According to Sajish et al., following translocation to the nucleus, WARS1 arise in a complex where it works as a bridge between poly(ADP-ribose) polymerase 1 (PARP1) and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) [69]. This allows PARP1-mediated polyADP-ribosylation (PARylation) of DNA-PKcs, which activates the kinase function of DNA-PKcs that catalyzes the phosphorylation of p53 and subsequently leads to the activation of antiproliferative responses (Fig. 4) [69]. When WARS1 is absent from the nucleus, DNA-PKcs and PARP-1 are connected by Ku70/80, which work as a bridge instead of WARS1. Ku70/80 orients the C-terminal domain of PARP1 toward DNA-PKcs, promoting phosphorylation of PARP1 by DNA-PKcs [73,74]. In this conformation, because phosphorylated PARP1 is incompetent to mediate PARylation of DNA-PKcs, activation of p53 pathway is suppressed [73]. Importantly, nuclear WARS1 substitutes Ku70/80 and binds both the NTD of PARP1 and the C-terminal kinase domain (KD) of DNA-PKcs with its WHEP domain, and this specific orientation favors PARylation of DNA-PKcs [69].

Surprisingly, in the presence of 5’-O-[N-(L-tryptophanyl)sulfamoyl] adenosine (Trp-SA), a nonhydrolyzable analog of Trp-AMP, conformational changes in WARS1 occur and that prohibit the formation of the ternary complex composed of DNA-PKcs-WARS1-PARP1 and therefore diminish the activation of the p53 pathway [69]. The crystal structure of human WARS1 in the state where the reaction intermediate Trp-AMP occupied the active site showed the closed conformation of WARS1, in which the WHEP domain folds toward the active site [69]. On the other hand, WARS1 without Trp-AMP binding in the active site showed free and disordered WHEP domains, which likely facilitate the formation of the complex [69]. Therefore, this ability of WARS1 to interact with DNA-PKcs and PARP1 was negatively regulated by Trp-AMP or its analog compound Trp-SA occupying the active site of WARS1. This finding implies that this signal transduction cascade involved in the activation of antiproliferative p53 pathway could be dependent on intracellular tryptophan levels, because WARS1 bound with Trp-AMP hinders the activation of the pathway. Tryptophan deficiency and concomitant reduction in Trp-AMP levels might favor nuclear WARS1 to form the active complex between DNA-PKcs and PARP1 to suppress

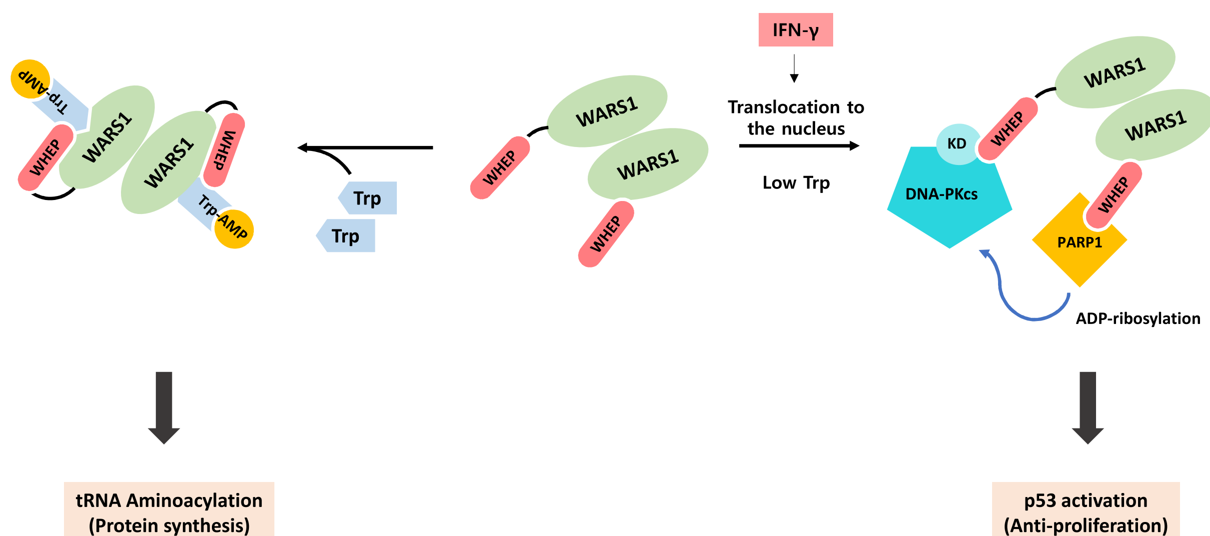


Fig. 4. Schematic model of the tryptophan-dependent function of WARS1 in the nucleus. Interferon- γ (IFN-γ) provokes strong antiproliferative responses partially through activating the p53 signaling pathway. Tryptophanyl-tRNA synthetase 1 (WARS1) translocates to the nucleus and allows the formation of the nuclear complex consisting of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and poly(ADP-ribose) polymerase 1 (PARP1) bound to the WHEP domains of dimeric WARS1. This complex facilitates ADP-ribosylation of DNA-PKcs, which activates its kinase function and concomitantly enhances p53 activation. This interaction held by WARS1 is specifically prevented by Trp-AMP in the active site. When the active site is occupied, WHEP domains shifts to closed conformation and become unavailable for interaction with DNA-KCcs and PARP1. Thus, intracellular tryptophan levels might affect the p53 activity by acting as signals that are detected by WARS1 used to determine the cellular fate in proliferation.

unwanted cell growth during nutrient shortage. Thus, Trp-AMP could work like a secondary messenger that transmits signals regarding the sufficiency of intracellular tryptophan, and WARS1 may function as a tryptophan sensor and decide cell fate by allowing or terminating proliferation by regulating the activation of p53 pathway. We assume that these functions of WARS1 may have been developed to specifically coordinate the nuclear signaling pathway with intracellular nutrient availability.

Alterations in tryptophan metabolism have been implicated in immune responses and immunosurveillance in cancer [75,76]. Indoleamine 2,3-dioxidase (IDO) is a pivotal enzyme placed in the center of tryptophan metabolic reprogramming, which is co-induced with WARS1 upon IFN-γ stimulation in cancer cells and myeloid cells such as dendritic cells, macrophages and microglia [70,77]. Activated IDO rapidly consumes tryptophan to generate kynurenine, a natural ligand of aryl hydrocarbon receptor (AhR), and causes local depletion of tryptophan [75]. Kynurenine and tryptophan depletion both contribute to the negative regulation of immune responses by suppressing effector T cell activation and T cell tolerance and enhancing the hyperactivation of regulatory T cells (Tregs) [78–81]. As mentioned above, if tryptophan depletion is sensed by WARS1 and thus leads to the activation of p53 pathway, this mechanism could be the fundamental reason for why reduced activation and growth in bystander T cells in the tumor microenvironment occurs. Moreover, a recent study revealed that the upregulation of WARS1 in IFN-γ-treated cells is correlated with high affinity tryptophan uptake into IDO-expressing cells, thus enabling their survival while degrading tryptophan [82]. Although the detailed mechanism by which WARS1 may enhance the tryptophan transportation has not been elucidated, the IFN-γ-mediated induction of WARS1 and IDO is clearly the key element in reprogramming of tryptophan metabolism, including the uptake and utilization of tryptophan.

Intriguingly, a reduction in WARS1 expression is detected in several types of cancer and it correlates with shorter patient survival and poor prognosis [83–85]. Additionally, reduced expression of WARS1 was reported in brain, breast, colorectal and renal cancers according to the Oncomine cancer profiling database and The Cancer Genome Atlas (TCGA). The downregulation of WARS1 might imply the abolishment of nuclear WARS1 mediated activation of p53 pathway, thus incapacitating WARS1-dependent suppression of cell proliferation. Future investigations on WARS1 might provide a unique therapeutic target in cancer immunotherapy and autoimmunity.

5. MARS1 associates methionine levels with CDK4 stabilization

Methionine is often regarded as one of the modulators of the cell cycle since methionine deprivation is reported to be accompanied by cell cycle arrest [86,87]. Cancer cells often show increasing dependence on methionine, and the stress resulting from methionine restriction may lead to growth restriction [88]. Interestingly, increased expression of methionyl-tRNA synthetase (MARS1) has also been reported in several types of cancer. For instance, neoplastic regions in lung tissue with elevated MARS1 expression are associated with poor prognosis, shorter disease-free survival and more advanced stages of cancer [89]. Elevated expression of MARS1 was also observed in glioblastoma, malignant gliomas, osteosarcomas, lipoma and malignant fibrous histiocytomas [4,90–93]. An abnormal elevation in the activity or expression of cell cycle regulators such as cyclin-dependent kinase 4 (CDK4) are frequently observed in many human cancers [94–96]. However, the correlation between methionine, MARS1 and cyclin-dependent kinases (CDKs) in cell cycle regulation have not been elucidated. Surprisingly, a recent study demonstrated that while the suppression of MARS1 had only a minor effect on global translation, it caused specific inhibition of the cell cycle, particularly on the G1 to S phase transition [97].

The timely activation and inactivation of cell cycle regulator complexes comprised of cyclins and CDKs are required in cell cycle regulation. Among these complexes, the CDK4 and cyclin D1 complex regulates the G1 to S phase transition and is crucially correlated with cellular fate decisions concerning quiescence and growth [98]. To ensure proper regulation of the cell cycle in response to various signals, several partners of CDK4 are involved. The tumor suppressor $p16^{INK4a}$ interferes in a direct manner with CDK4 and cyclin D1 by suppressing the CDK4-cycline D1 interaction and hence causing cell cycle arrest [99,100]. Some human cancers, such as pancreatic cancer, lung cancer and breast cancers, are associated with deletions and/or the hypermethylation in the promoter region of the $p16^{INK4a}$-encoding gene *CDKN2A* [101–103]. The $p16^{INK4a}$-negative cancers are highly susceptible to tumor progression due to the loss of control over CDK4 activity.

In the aforementioned research, Kwon et al. showed that MARS1 specifically interacts and stabilizes CDK4 and augment its complex formation with heat shock protein 90 (HSP90) and cell division cycle 37, HSP90 cochaperone (CDC37) in a competitive manner with the tumor suppressor $p16^{INK4a}$ (Fig. 5) [97]. In this study, the authors have demonstrated that the knockdown of MARS1 or the pharmacologic inhibition of MARS1 using methionine analogs caused a reduction in CDK4 levels and subsequent cell cycle arrest. Among 13 methionine analogs tested, Fmoc-Sec(Mob)-OH (FSMO) showed the most potent inhibition of the CDK4 signaling pathway, and at high concentrations, it inhibited protein synthesis and even the methionine activation step [97]. Since FSMO was expected to bind with the catalytic domain within MARS1 that is important for methionine activation, the authors additionally proved that the Lys-Met-Ser-Lys-Ser (KMSKS) conserved motif, which is crucial for amino acid binding and activation in the catalytic sites, was important for the MARS1-CDK4 interaction. They further demonstrated that the K596Q mutation within this motif, which is crucial for methionine activation, caused the loss of CDK4 binding ability of MARS1 [97]. Therefore, methionine binding and the production of Met-AMP is likely an important step in the mechanistic interaction with CDK4. Additionally, when the CDK4 interaction with MARS1 is weak, it will bind with $p16^{INK4a}$ instead; thus, CDK4-HSP90-CDC37 complex formation becomes unfavorable, and CDK4 is susceptible to ubiquitination and proteasomal degradation [104].

Therefore, since the methionine binding and/or methionine activation ability of MARS1 is likely crucial for the MARS1-CDK4 interaction, this might partially explain the increasing dependency of methionine in cancer cells. Also, high MARS1 expression in cancers could favors MARS1 to interact and stabilize CDK4 and hence facilitates cell proliferation. Thus, we may speculate that MARS1 is another amino acid sensor that couples intracellular methionine levels with the regulation of cell proliferation.

6. EPRS integrates proline signaling for fibrogenesis

Among ARSs, glutamyl-prolyl-tRNA synthetase (EPRS) is a uniquely fused bifunctional protein, that is composed of glutamyl-tRNA synthetase (ERS) and prolyl-tRNA synthetase (PRS) encoded by the *EPRS* gene. In all known metazoans, except for *Caenorhabditis elegans*, EPRS is encoded as a fused gene, and cytosolic EPRS resides in the MSC and catalyzes the aminoacylation of both glutamate and proline to their cognate tRNAs [105]. These two synthetase domains of EPRS are bridged by a flexible linker usually consisting of one or more helix-turn-helix WHEP domains. Despite the common helix-turn-helix structure, WHEP domains have highly diverse sequences, and duplications or occasional losses are common among different animals [106]. Perhaps these divergences are attempts to acquire the capability of interacting with various kinds of protein partners.

Liver fibrosis is characterized by the excessive deposition of extracellular matrix (ECM) [107], and is accompanied by abnormal cell proliferation that may progress into advanced organ dysfunction and even to hepatocellular carcinoma (HCC) [108]. Hepatic stellate cells (HSCs) are considered the major source of liver ECM, and collagen I is the most abundant component of ECM that is produced by HSCs [109,110]. Idiopathic pulmonary fibrosis (IPF) is a chronic fibrotic

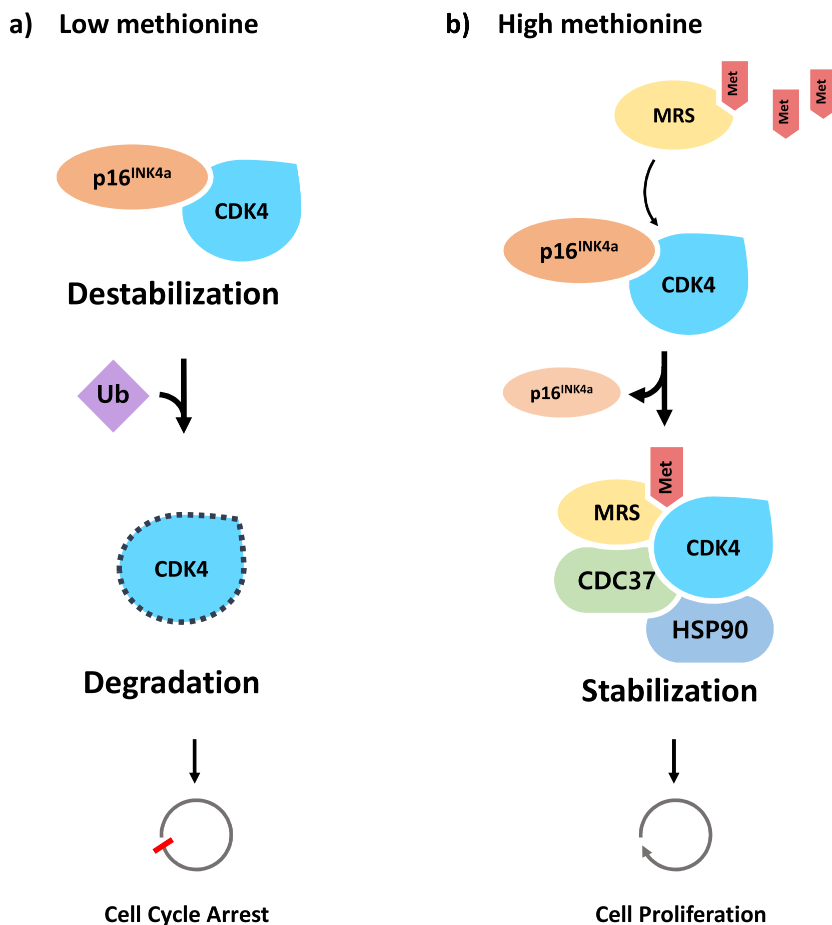


Fig. 5. Simplified model of the regulation of CDK4 stability by MARS1 and p16^{INK4a}. a) The interaction of cyclin-dependent kinase 4 (CDK4) with the tumor suppressor p16^{INK4a} leads to the destabilization of CDK4 and thus enhances its ubiquitin (Ub) attachment and proteasomal degradation, which could be facilitated under low methionine conditions. b) Under sufficient or high methionine levels, Methionyl-tRNA synthetase 1 (MARS1) is associate with CDK4 in a competitive manner with p16^{INK4a}, thereby enhancing the formation of the stabilized complex of CDK4 with heat shock protein 90 (HSP90) and cell division cycle 37, HSP90 cochaperone (CDC37). Catalytic sites responsible for methionine (Met) binding and methionine activation are crucial for the association between MARS1 and CDK4. Additionally, the binding of methionine analogs inhibits the MARS1-CDK4 interaction (not shown in the figure). Intracellular methionine levels might adjust the extent of the interaction between MARS1 and CDK4, thereby correlating methionine sufficiency in the cell cycle.

interstitial pulmonary disease of unknown cause that is also characterized by the abnormal accumulation of ECM [111–113]. Transforming growth factor beta 1 (TGF-β1) is considered the major player in fibrogenesis, and its aberrant activity and its downstream mediators are characterized in progressive organ fibrosis [110,114]. TGF-β1-mediated signal transducers and activators of transcription (STAT) signaling pathways activates lung fibroblasts and promotes epithelial-mesenchymal transformations (EMTs) [113–116]. The formation of the protein complex consisting TGF-β receptor 1 (TGFβR1) and Janus kinase 1 (JAK1) enables the activation of STAT3 *via* the mediation of mothers against decapentaplegic homolog 3 (SMAD3) [117]. Intriguingly, halofuginone (HF) treatment in human patient-derived fibroblasts reduced TGF-β1-triggered collagen synthesis, while the expression levels of the TGF-β1 or TGF-βR1 gene were not affected [118]. Importantly, recent studies suggested that EPRS may be involved in the transcriptional control of ECM components by participating in the intracellular signal transduction upon TGF-β1 stimulation (Fig. 6.) [119,120]. According to Song et al., STAT signaling activation might occur in a prolyl-tRNA synthetase (PRS)-dependent manner, and the formation of a complex consisting of TGF-β1R1, EPRS, Janus kinases (JAKs) and STAT6 is crucial for the expression of ECM components, including collagen [119,120]. This phenomenon was observed in both LX2 HSCs and liver tissue from fibrotic mice [119], and in A549 alveolar epithelial cells and the lung tissue from bleomycin-treated animal models [120].

Notably, HF is one of the febrifugine derivatives that is known to inhibit collagen expression, and its mechanism of action involves the catalytic inhibition of EPRS, which can be recovered by proline supplementation [121]. However, the exact mechanism of how HF-mediated inhibition of EPRS is involved in the pathogenesis of TGF-β1/STAT signaling-induced fibrotic diseases remained unclear. One possible explanation for the mechanism of action of HF is the indirect activation of the amino acid response (AAR) pathway. Amino acid deprivation or inhibition of ARSs may lead to the accumulation of free uncharged tRNAs that bind and activate general control non-derepressible 2 (GCN2), which facilitates the phosphorylation of eukaryotic translational inhibition factor 2α (eIF2α) and leads to the induction of activating transcription factor 4 (ATF4) and the suppression of global protein synthesis [121]. While GCN2 is usually considered important for receiving the signals related to intracellular amino acid levels, the idea that uncharged tRNAs function as activators of GCN2 has been recently challenged. Unlike mammalian cells, yeast GCN2 activation by uncharged tRNAs could not be established *in vitro* [122]. In addition, alternative direct activators of yeast GCN2 have been identified, which include ribosomal P-stalk-derived proteins [123]. Moreover, a recent investigation revealed an intriguing relationship between GCN2 and ribosomes by analyzing the brains of mice lacking the ribosome rescue factor guanosine triphosphate-binding protein 2 (GTPBP2) and a specific neuronal tRNA (tRNA$^{Arg}_{UUU}$) [124]. These mice showed a high incidence of stalled translational elongation complexes in their ribosomal profiling as well as elevated GCN2-induced eIF2α phosphorylation. However, there were no signs of the accumulation of uncharged tRNA [124]. Thus, these results suggest that other than tRNA, stalled ribosomes could be alternative activators of GCN2. If stalled ribosomes can be sensed by GCN2, then there could be a direct functional relationship between GCN2 and the translation elongation machinery. The existence of such a relationship was proven in the most recent study in which human GCN2 was directly activated by ribosomes, particularly by domain II of the ribosomal P-stalk protein uL10 [125]. Although GCN2 is certainly an essential effector that regulates protein synthesis to control amino acid catabolism, the above observations and reports clearly

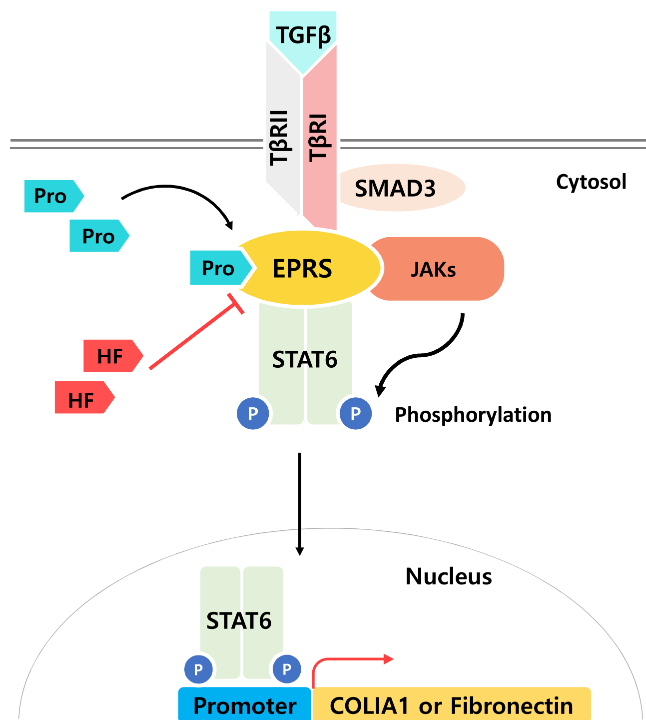


Fig. 6. Working model for EPRS1 mediated signaling during TGF-β stimulated expression of ECM materials. Transforming growth factor beta 1 (TGF-β) binds to TGF-β receptor 1 (TGFβR1), facilitating the formation of a signaling complex consisting of TGFβR1, mothers against decapentaplegic homolog 3 (SMAD3), Janus kinases (JAKs) and signal transducers and activators of transcription 6 (STAT6), which is crucial for the JAK-mediated STAT6 phosphorylation. Subsequently, activated STAT6 enters the nucleus and induces the transcription of extracellular matrix (ECM) genes, including collagen α1 (COLA1) and fibronectin. Halofuginone (HF) binding to the catalytic site of glutamyl-prolyl-tRNA synthetase 1 (EPRS1) abolishes the EPRS1-mediated interaction of JAKs and STAT6. However, proline (Pro) supplementation could reverse this effect. ERPS1 may participate in complex formation depending on the intracellular proline availability to control the overall production of proline-rich ECM components such as collagens.

demonstrate the controversies in the activation mechanism of GCN2 in nutrient sensing pathways.

Interestingly, Song et al. revealed that HF treatment abrogated both TGF-β1 mediated expression of collagen I and fibronectin by preventing the interaction between EPRS and STAT6, thus resulting in reduced STAT6 activation [119]. Therefore, blocking the EPRS catalytic site by HF might also lead to the inhibition of the protein-protein interaction between EPRS and STAT6. Importantly, proline supplementation after HF treatment could partially rescue the suppressive effects of HF in this interaction, which further emphasizes the importance of the catalytic subunits of EPRS in the production of the ECM [119]. Moreover, the suppression of other ARSs, including LARS1, lysyl-tRNA synthetase (KRS) or glycyl-tRNA synthetase (GRS), did not show significant effects on the mRNA levels of diverse ECM chains; however, TGF-β1 treatment elevated ECM production despite the suppression of these ARSs [119]. Therefore, the above findings suggest that EPRS regulates ECM production partly by a unique mechanism that is independent of the GCN2 signaling pathway, which is intertwined with the TGF-β1/STAT6 signaling pathway. The necessity of monitoring intracellular proline utilization could be the underlying rationale for EPRS-mediated ECM regulation, because the most abundant protein in the ECM, type I collagen, is a proline-rich protein that consists of approximately 20% of prolines and 3- or 4- hydroxyprolines [126]. Additionally, the recent evidence has shown that the proline metabolism is important in the collagen protein synthesis upon TGF-β-dependent onset of IPF [127]. Because EPRS translation can be selectively upregulated in response to the integrated stress response (ISR), this implies that EPRS has adaptative roles during stress conditions [128]. Excessive production of proline-rich ECM components could be unfavorable for cells due to proline depletion; therefore, EPRS might be employed as a proline sensor or a checkpoint to sustain adequate intracellular proline levels and the rate of ECM production in cells.

Additionally, the linker domain WHEP is usually involved in the noncanonical functions of EPRS. First, three WHEP domains in the human EPRS are essential in forming the IFN-γ-activated inhibitor of translation (GAIT) complex that silences the translation of multiple mRNAs, including vascular endothelial growth factor A (VEGFA) [129,130]. Second, the WHEP domain binds with fatty acid transport protein 1 (FATP1) to facilitate uptake of long-chain fatty acids upon insulin stimulation to adipocytes [131]. Whether or not the WHEP domain is also responsible for the EPRS interaction with STAT6 and JAK in the context of EPRS working as a proline sensor needs to be addressed. Moreover, further studies are required to evaluate the dependency of EPRS on proline during the formation of the GAIT complex and the interaction with FATP1. Therefore, a deeper understanding of the mechanisms of EPRS involved in fibrosis might guide the ongoing efforts to identify novel therapeutic targets of fibrotic diseases.

7. Concluding remarks

ARSs are enzymes responsible for the manufacturing of building blocks for proteins in all kinds of living organisms. ARSs have been considered to exert exclusively in translation, but their functions have been expanded continuously throughout evolution, giving rise to the acquisition of unique roles for each ARS in controlling complex multicellular systems. Therefore, ARSs resembling the characteristics of nutrient sensors might indicate the existence of demands during evolution for an integrated system to control various physiological functions in addition to protein synthesis. To actively adjust to the changing environment for survival, a primary requirement would be to develop highly sensitive mechanisms that are capable of sensing amino acids from the surrounding environment. The intrinsic capability of ARSs in the binding of amino acids during protein synthesis could be a very appealing feature in this manner. Therefore, the expansion of extra domains in the architecture of ARSs throughout the evolution might have been motivated by the development of multifunctional sensors that are capable of interacting with diverse effectors according to the environmental demands. Considering the emerging evidence for novel roles of ARSs in amino acid signaling pathways that was discussed in this review, ARSs could be the genera *bona fide* sensors designed specifically for sensing amino acids.

Further studies to unveil the unique functions of each ARS in amino acid sensing would definitely expand our scope of interest in ARSs and our understanding of nutrient homeostasis and metabolic regulation inside of cells. For instance, regarding ARSs' amino acid sensing ability, it could be important to examine whether there is preferred form between MSC-bound form and free form in how they exert their regulatory functions. Also, although disease-related mutations and splicing patterns of ARSs have been reported frequently, insights into their pathological mechanisms are insufficient [132–136]. Therefore, improving the mechanistic understanding might provide us with new foundations for investigating the roles of ARSs in diseases such as fibrosis and cancer and might also be used in the development of novel therapeutics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S.W. Lee, B.H. Cho, S.G. Park, S. Kim, Aminoacyl-tRNA synthetase complexes: beyond translation, J. Cell Sci. 117 (2004) 3725–3734.
[2] M. Guo, P. Schimmel, Essential nontranslational functions of tRNA synthetases, Nat. Chem. Biol. 9 (2013) 145–153.
[3] M. Guo, X.L. Yang, P. Schimmel, New functions of aminoacyl-tRNA synthetases beyond translation, Nat. Rev. Mol. Cell Biol. 11 (2010) 668–674.
[4] S. Kim, S. You, D. Hwang, Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping, Nat. Rev. Cancer 11 (2011) 708–718.
[5] S. Marshall, Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer, Science's STKE: signal transduction knowledge environment 2006 (2006) re7.
[6] L. Chantranupong, R.L. Wolfson, D.M. Sabatini, Nutrient-sensing mechanisms across evolution, Cell 161 (2015) 67–83.
[7] A. Efeyan, W.C. Comb, D.M. Sabatini, Nutrient-sensing mechanisms and pathways, Nature 517 (2015) 302–310.
[8] S. Bröer, A. Bröer, Amino acid homeostasis and signalling in mammalian cells and organisms, The Biochemical journal 474 (2017) 1935–1963.
[9] Y.G. Ko, E.Y. Kim, T. Kim, H. Park, H.S. Park, E.J. Choi, S. Kim, Glutamine-dependent antiapoptotic interaction of human glutaminyl-tRNA synthetase with apoptosis signal-regulating kinase 1, J. Biol. Chem. 276 (2001) 6030–6036.
[10] J.M. Han, S.J. Jeong, M.C. Park, G. Kim, N.H. Kwon, H.K. Kim, S.H. Ha, S.H. Ryu, S. Kim, Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway, Cell 149 (2012) 410–424.
[11] G. Bonfils, M. Jaquenoud, S. Bontron, C. Ostrowicz, C. Ungermann, C. De Virgilio, Leucyl-tRNA synthetase controls TORC1 via the EGO complex, Mol. Cell 46 (2012) 105–110.
[12] M.S. Yoon, K. Son, E. Arauz, J.M. Han, S. Kim, J. Chen, Leucyl-tRNA Synthetase activates Vps34 in amino acid-sensing mTORC1 signaling, Cell Rep. 16 (2016) 1510–1517.
[13] K. Hattori, I. Naguro, C. Runchel, H. Ichijo, The roles of ASK family proteins in stress responses and diseases, Cell Commun Signal 7 (2009) 9.
[14] H. Ichijo, E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, Y. Gotoh, Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways, Science 275 (1997) 90–94.
[15] H. Nishitoh, M. Saitoh, Y. Mochida, K. Takeda, H. Nakano, M. Rothe, K. Miyazono, H. Ichijo, ASK1 is essential for JNK/SAPK activation by TRAF2, Mol. Cell 2 (1998) 389–395.
[16] M. Saitoh, H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, H. Ichijo, Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1, EMBO J. 17 (1998) 2596–2606.
[17] H. Nishitoh, A. Matsuzawa, K. Tobiume, K. Saegusa, K. Takeda, K. Inoue, S. Hori, A. Kakizuka, H. Ichijo, ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats, Genes Dev. 16 (2002) 1345–1355.
[18] A. Matsuzawa, K. Saegusa, T. Noguchi, C. Sadamitsu, H. Nishitoh, S. Nagai, S. Koyasu, K. Matsumoto, K. Takeda, H. Ichijo, ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity, Nat. Immunol. 6 (2005) 587–592.
[19] K. Tobiume, M. Saitoh, H. Ichijo, Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer, J. Cell. Physiol. 191 (2002) 95–104.
[20] G. Fujino, T. Noguchi, A. Matsuzawa, S. Yamauchi, M. Saitoh, K. Takeda, H. Ichijo, Thioredoxin and TRAF family proteins regulate reactive oxygen species-dependent activation of ASK1 through reciprocal modulation of the N-terminal homophilic interaction of ASK1, Mol. Cell. Biol. 27 (2007) 8152–8163.
[21] H.T. Papaconstantinou, K.O. Hwang, S. Rajaraman, M.R. Hellmich, C. M. Townsend Jr., T.C. Ko, Glutamine deprivation induces apoptosis in intestinal epithelial cells, Surgery 124 (1998) 152–159 (discussion 159-160).
[22] L. Moine, G. Diaz de Barboza, A. Perez, M. Benedetto, N. Tolosa de Talamoni, Glutamine protects intestinal calcium absorption against oxidative stress and apoptosis, comparative biochemistry and physiology, Part A, Molecular & integrative physiology 212 (2017) 64–71.
[23] L. Chen, H. Cui, Targeting glutamine induces apoptosis: a Cancer therapy approach, Int. J. Mol. Sci. 16 (2015) 22830–22855.
[24] B.J. Altman, Z.E. Stine, C.V. Dang, From Krebs to clinic: glutamine metabolism to cancer therapy, Nat. Rev. Cancer 16 (2016) 619–634.
[25] L. Yang, S. Venneti, D. Nagrath, Glutaminolysis: a Hallmark of Cancer metabolism, Annu. Rev. Biomed. Eng. 19 (2017) 163–194.
[26] R. Breton, D. Watson, M. Yaguchi, J. Lapointe, Glutamyl-tRNA synthetases of Bacillus subtilis 168T and of Bacillus stearothermophilus. Cloning and sequencing of the gltX genes and comparison with other aminoacyl-tRNA synthetases, J. Biol. Chem. 265 (1990) 18248–18255.
[27] T. Kim, S.G. Park, J.E. Kim, W. Seol, Y.G. Ko, S. Kim, Catalytic peptide of human glutaminyl-tRNA synthetase is essential for its assembly to the aminoacyl-tRNA synthetase complex, J. Biol. Chem. 275 (2000) 21768–21772.
[28] S.B. Rho, M.J. Kim, J.S. Lee, W. Seol, H. Motegi, S. Kim, K. Shiba, Genetic dissection of protein-protein interactions in multi-tRNA synthetase complex, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 4488–4493.
[29] J. Ognjenovic, J. Wu, D. Matthies, U. Baxa, S. Subramaniam, J. Ling, M. Simonovic, The crystal structure of human GlnRS provides basis for the development of neurological disorders, Nucleic Acids Res. 44 (2016) 3420–3431.
[30] X.D. He, W. Gong, J.N. Zhang, J. Nie, C.F. Yao, F.S. Guo, Y. Lin, X.H. Wu, F. Li, J. Li, W.C. Sun, E.D. Wang, Y.P. An, H.R. Tang, G.Q. Yan, P.Y. Yang, Y. Wei, Y. Z. Mao, P.C. Lin, J.Y. Zhao, Y. Xu, W. Xu, S.M. Zhao, Sensing and transmitting intracellular amino acid signals through reversible lysine Aminoacylations, Cell Metab. 27 (2018) 151–166 (e156).
[31] D.A. Guertin, D.M. Sabatini, An expanding role for mTOR in cancer, Trends Mol. Med. 11 (2005) 353–361.
[32] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell 149 (2012) 274–293.
[33] Y.M. Kim, J.M. Park, D. Grunwald, D.H. Kim, An expanded role for mTORC1 in autophagy, Molecular & cellular oncology 3 (2016), e1010958.
[34] H. Nojima, C. Tokunaga, S. Eguchi, N. Oshiro, S. Hidayat, K. Yoshino, K. Hara, N. Tanaka, J. Avruch, K. Yonezawa, The mammalian target of rapamycin (mTOR) partner, raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif, J. Biol. Chem. 278 (2003) 15461–15464.
[35] Y. Sancak, T.R. Peterson, Y.D. Shaul, R.A. Lindquist, C.C. Thoreen, L. Bar-Peled, D.M. Sabatini, The rag GTPases bind raptor and mediate amino acid signaling to mTORC1, Science 320 (2008) 1496–1501.
[36] J. Chung, C.J. Kuo, G.R. Crabtree, J. Blenis, Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases, Cell 69 (1992) 1227–1236.
[37] L. Beretta, A.C. Gingras, Y.V. Svitkin, M.N. Hall, N. Sonenberg, Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation, EMBO J. 15 (1996) 658–664.
[38] K. Duvel, J.L. Yecies, S. Menon, P. Raman, A.I. Lipovsky, A.L. Souza, E. Triantafellow, Q. Ma, R. Gorski, S. Cleaver, M.G. Vander Heiden, J. P. MacKeigan, P.M. Finan, C.B. Clish, L.O. Murphy, B.D. Manning, Activation of a metabolic gene regulatory network downstream of mTOR complex 1, Mol. Cell 39 (2010) 171–183.
[39] P.B. Crino, K.L. Nathanson, E.P. Henske, The tuberous sclerosis complex, N. Engl. J. Med. 355 (2006) 1345–1356.
[40] A.R. Tee, B.D. Manning, P.P. Roux, L.C. Cantley, J. Blenis, Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb, Current biology: CB 13 (2003) 1259–1268.
[41] R. Zoncu, L. Bar-Peled, A. Efeyan, S. Wang, Y. Sancak, D.M. Sabatini, mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase, Science 334 (2011) 678–683.
[42] M.H. Stipanuk, Leucine and protein synthesis: mTOR and beyond, Nutr. Rev. 65 (2007) 122–129.
[43] K.G. Foster, D.C. Fingar, Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony, J. Biol. Chem. 285 (2010) 14071–14077.
[44] E. Kim, P. Goraksha-Hicks, L. Li, T.P. Neufeld, K.L. Guan, Regulation of TORC1 by rag GTPases in nutrient response, Nat. Cell Biol. 10 (2008) 935–945.
[45] A. Schürmann, A. Brauers, S. Massmann, W. Becker, H.G. Joost, Cloning of a novel family of mammalian GTP-binding proteins (RagA, RagBs, RagB1) with remote similarity to the Ras-related GTPases, J. Biol. Chem. 270 (1995) 28982–28988.
[46] T. Sekiguchi, E. Hirose, N. Nakashima, M. Ii, T. Nishimoto, Novel G proteins, rag C and rag D, interact with GTP-binding proteins, Rag A and Rag B, J Biol Chem 276 (2001) 7246–7257.
[47] Y. Sancak, L. Bar-Peled, R. Zoncu, A.L. Markhard, S. Nada, D.M. Sabatini, Ragulator-rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids, Cell 141 (2010) 290–303.
[48] N. Segev, N. Hay, Hijacking leucyl-tRNA synthetase for amino acid-dependent regulation of TORC1, Mol. Cell 46 (2012) 4–6.
[49] M. Lee, J.H. Kim, I. Yoon, C. Lee, M. Fallahi Sichani, J.S. Kang, J. Kang, M. Guo, K.Y. Lee, G. Han, S. Kim, J.M. Han, Coordination of the leucine-sensing rag GTPase cycle by leucyl-tRNA synthetase in the mTORC1 signaling pathway, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E5279–E5288.
[50] L. Bar-Peled, L.D. Schweitzer, R. Zoncu, D.M. Sabatini, Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1, Cell 150 (2012) 1196–1208.
[51] R.L. Wolfson, L. Chantranupong, R.A. Saxton, K. Shen, S.M. Scaria, J.R. Cantor, D. M. Sabatini, Sestrin2 is a leucine sensor for the mTORC1 pathway, Science 351 (2016) 43–48.
[52] J.S. Kim, S.H. Ro, M. Kim, H.W. Park, I.A. Semple, H. Park, U.S. Cho, W. Wang, K. L. Guan, M. Karin, J.H. Lee, Sestrin2 inhibits mTORC1 through modulation of GATOR complexes, Sci. Rep. 5 (2015) 9502.
[53] J.H. Kim, C. Lee, M. Lee, H. Wang, K. Kim, S.J. Park, I. Yoon, J. Jang, H. Zhao, H. K. Kim, N.H. Kwon, S.J. Jeong, H.C. Yoo, J.H. Kim, J.S. Yang, M.Y. Lee, C.W. Lee, J. Yun, S.J. Oh, J.S. Kang, S.A. Martinis, K.Y. Hwang, M. Guo, G. Han, J.M. Han, S. Kim, Control of leucine-dependent mTORC1 pathway through chemical intervention of leucyl-tRNA synthetase and RagD interaction, Nat. Commun. 8 (2017) 732.

[54] E.Y. Kim, J.G. Lee, J.M. Lee, A. Kim, H.C. Yoo, K. Kim, M. Lee, C. Lee, G. Han, J. M. Han, Y.S. Chang, Therapeutic effects of the novel Leucyl-tRNA synthetase inhibitor BC-LI-0186 in non-small cell lung cancer, Therapeutic advances in medical oncology 11 (2019), https://doi.org/10.1177/1758835919846798.
[55] K. Son, J.S. You, M.S. Yoon, C. Dai, J.H. Kim, N. Khanna, A. Banerjee, S. A. Martinis, G. Han, J.M. Han, S. Kim, J. Chen, Nontranslational function of leucyl-tRNA synthetase regulates myogenic differentiation and skeletal muscle regeneration, J. Clin. Invest. 129 (2019) 2088–2093.
[56] J.M. Backer, The regulation and function of class III PI3Ks: novel roles for Vps34, Biochem. J. 410 (2008) 1–17.
[57] R.C. Russell, H.X. Yuan, K.L. Guan, Autophagy regulation by nutrient signaling, Cell Res. 24 (2014) 42–57.
[58] M.P. Byfield, J.T. Murray, J.M. Backer, hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase, J. Biol. Chem. 280 (2005) 33076–33082.
[59] T. Nobukuni, M. Joaquin, M. Roccio, S.G. Dann, S.Y. Kim, P. Gulati, M.P. Byfield, J.M. Backer, F. Natt, J.L. Bos, F.J. Zwartkruis, G. Thomas, Amino acids mediate mTOR/raptor signaling through activation of class 3 phosphatidylinositol 3OH-kinase, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 14238–14243.
[60] M.S. Yoon, G. Du, J.M. Backer, M.A. Frohman, J. Chen, Class III PI-3-kinase activates phospholipase D in an amino acid-sensing mTORC1 pathway, J. Cell Biol. 195 (2011) 435–447.
[61] Y. Fang, M. Vilella-Bach, R. Bachmann, A. Flanigan, J. Chen, Phosphatidic acid-mediated mitogenic activation of mTOR signaling, Science 294 (2001) 1942–1945.
[62] Y. Fang, I.H. Park, A.L. Wu, G. Du, P. Huang, M.A. Frohman, S.J. Walker, H. A. Brown, J. Chen, PLD1 regulates mTOR signaling and mediates Cdc42 activation of S6K1, Current biology: CB 13 (2003) 2037–2044.
[63] I. Yoon, M. Nam, H.K. Kim, H.S. Moon, S. Kim, J. Jang, J.A. Song, S.J. Jeong, S. B. Kim, S. Cho, Y. Kim, J. Lee, W.S. Yang, H.C. Yoo, K. Kim, M.S. Kim, A. Yang, K. Cho, H.S. Park, G.S. Hwang, K.Y. Hwang, J.M. Han, J.H. Kim, S. Kim, Glucose-Dependent Control of Leucine Metabolism by Leucyl-tRNA Synthetase 1, Science, 2019.
[64] J. Kim, M. Kundu, B. Viollet, K.L. Guan, AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1, Nat. Cell Biol. 13 (2011) 132–141.
[65] J.A. Martina, Y. Chen, M. Gucek, R. Puertollano, MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB, Autophagy 8 (2012) 903–914.
[66] E. Tzima, J.S. Reader, M. Irani-Tehrani, K.L. Ewalt, M.A. Schwartz, P. Schimmel, VE-cadherin links tRNA synthetase cytokine to anti-angiogenic function, J. Biol. Chem. 280 (2005) 2405–2408.
[67] Q. Zhou, M. Kapoor, M. Guo, R. Belani, X. Xu, W.B. Kiosses, M. Hanan, C. Park, E. Armour, M.H. Do, L.A. Nangle, P. Schimmel, X.L. Yang, Orthogonal use of a human tRNA synthetase active site to achieve multifunctionality, Nat. Struct. Mol. Biol. 17 (2010) 57–61.
[68] Y.H. Ahn, S. Park, J.J. Choi, B.K. Park, K.H. Rhee, E. Kang, S. Ahn, C.H. Lee, J. S. Lee, K.S. Inn, M.L. Cho, S.H. Park, K. Park, H.J. Park, J.H. Lee, J.W. Park, N. H. Kwon, H. Shim, B.W. Han, P. Kim, J.Y. Lee, Y. Jeon, J.W. Huh, M. Jin, S. Kim, Secreted tryptophanyl-tRNA synthetase as a primary defence system against infection, Nat. Microbiol. 2 (2016) 16191.
[69] M. Sajish, Q. Zhou, S. Kishi, D.M. Valdez Jr., M. Kapoor, M. Guo, S. Lee, S. Kim, X. L. Yang, P. Schimmel, Trp-tRNA synthetase bridges DNA-PKcs to PARP-1 to link IFN-gamma and p53 signaling, Nat. Chem. Biol. 8 (2012) 547–554.
[70] M.C. Yadav, E.M. Burudi, M. Alirezaei, C.C. Flynn, D.D. Watry, C.M. Lanigan, H. S. Fox, IFN-gamma-induced IDO and WRS expression in microglia is differentially regulated by IL-4, Glia 55 (2007) 1385–1396.
[71] M. Jin, Unique roles of tryptophanyl-tRNA synthetase in immune control and its therapeutic implications, Exp. Mol. Med. 51 (2019) 1.
[72] V.I. Popenko, N.E. Cherny, S.F. Beresten, J.L. Ivanova, V.V. Filonenko, L. L. Kisselev, Immunoelectron microscopic location of tryptophanyl-tRNA synthetase in mammalian, prokaryotic and archaebacterial cells, Eur. J. Cell Biol. 62 (1993) 248–258.
[73] Y. Ariumi, M. Masutani, T.D. Copeland, T. Mimori, T. Sugimura, K. Shimotohno, K. Ueda, M. Hatanaka, M. Noda, Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase in vitro, Oncogene 18 (1999) 4616–4625.
[74] S. Jin, S. Kharbanda, B. Mayer, D. Kufe, D.T. Weaver, Binding of Ku and c-Abl at the kinase homology region of DNA-dependent protein kinase catalytic subunit, J. Biol. Chem. 272 (1997) 24763–24766.
[75] M. Platten, E.A.A. Nollen, U.F. Röhrig, F. Fallarino, C.A. Opitz, Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond, Nat. Rev. Drug Discov. 18 (2019) 379–401.
[76] A. Amobi, F. Qian, A.A. Lugade, K. Odunsi, Tryptophan catabolism and Cancer immunotherapy targeting IDO mediated immune suppression, Adv. Exp. Med. Biol. 1036 (2017) 129–144.
[77] D.H. Munn, A.L. Mellor, Indoleamine 2,3 dioxygenase and metabolic control of immune responses, Trends Immunol. 34 (2013) 137–143.
[78] F. Fallarino, U. Grohmann, S. You, B.C. McGrath, D.R. Cavener, C. Vacca, C. Orabona, R. Bianchi, M.L. Belladonna, C. Volpi, P. Santamaria, M.C. Fioretti, P. Puccetti, The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells, Journal of immunology Baltimore, Md. 176 (2006) (1950) 6752–6761.
[79] J.D. Mezrich, J.H. Fechner, X. Zhang, B.P. Johnson, W.J. Burlingham, C. A. Bradfield, An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells, Journal 1 Baltimore, Md. 185 (2010) (1950) 3190–3198.
[80] J.P. Routy, B. Routy, G.M. Graziani, V. Mehraj, The Kynurenine pathway is a double-edged sword in immune-privileged sites and in Cancer: implications for immunotherapy, International journal of tryptophan research: IJTR 9 (2016) 67–77.
[81] C. Bilir, C. Sarisozen, Indoleamine 2,3-dioxygenase (IDO): only an enzyme or a checkpoint controller? Journal of Oncological Sciences 3 (2017) 52–56.
[82] M. Miyanokoshi, T. Yokosawa, K. Wakasugi, Tryptophanyl-tRNA synthetase mediates high-affinity tryptophan uptake into human cells, J. Biol. Chem. 293 (2018) 8428–8438.
[83] A. Ghanipour, K. Jirström, F. Pontén, B. Glimelius, L. Påhlman, H. Birgisson, The prognostic significance of tryptophanyl-tRNA synthetase in colorectal cancer, in: Cancer epidemiology, biomarkers & prevention 18, a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 2009, pp. 2949–2956.
[84] E.L. Paley, D.E. Paley, T. Merkulova-Rainon, P.R. Subbarayan, Hypoxia signature of splice forms of tryptophanyl-tRNA synthetase marks pancreatic cancer cells with distinct metastatic abilities, Pancreas 40 (2011) 1043–1056.
[85] M. Campone, I. Valo, P. Jézéquel, M. Moreau, A. Boissard, L. Campion, D. Loussouarn, V. Verriele, O. Coqueret, C. Guette, Prediction of recurrence and survival for triple-negative breast Cancer (TNBC) by a protein signature in tissue samples, Molecular & cellular proteomics: MCP 14 (2015) 2936–2946.
[86] D.M. Kokkinakis, X. Liu, R.D. Neuner, Modulation of cell cycle and gene expression in pancreatic tumor cell lines by methionine deprivation (methionine stress): implications to the therapy of pancreatic adenocarcinoma, Mol. Cancer Ther. 4 (2005) 1338–1348.
[87] R.M. Hoffman, S. Yano, Tumor-Specific S/G2-Phase Cell Cycle Arrest of Cancer Cells by Methionine Restriction, Methods in molecular biology (Clifton, N.J.) 1866 (2019) 49–60.
[88] D.M. Kokkinakis, X. Liu, S. Chada, M.M. Ahmed, M.M. Shareef, U.K. Singha, S. Yang, J. Luo, Modulation of gene expression in human central nervous system tumors under methionine deprivation-induced stress, Cancer Res. 64 (2004) 7513–7525.
[89] E.Y. Kim, J.Y. Jung, A. Kim, K. Kim, Y.S. Chang, Methionyl-tRNA synthetase overexpression is associated with poor clinical outcomes in non-small cell lung cancer, BMC Cancer 17 (2017) 467.
[90] G. Reifenberger, K. Ichimura, J. Reifenberger, A.G. Elkahloun, P.S. Meltzer, V. P. Collins, Refined mapping of 12q13-q15 amplicons in human malignant gliomas suggests CDK4/SAS and MDM2 as independent amplification targets, Cancer Res. 56 (1996) 5141–5145.
[91] M. Nilbert, A. Rydholm, F. Mitelman, P.S. Meltzer, N. Mandahl, Characterization of the 12q13-15 amplicon in soft tissue tumors, Cancer Genet. Cytogenet. 83 (1995) 32–36.
[92] J.L. Palmer, S. Masui, S. Pritchard, D.K. Kalousek, P.H. Sorensen, Cytogenetic and molecular genetic analysis of a pediatric pleomorphic sarcoma reveals similarities to adult malignant fibrous histiocytoma, Cancer Genet. Cytogenet. 95 (1997) 141–147.
[93] A. Forus, V.A. Florenes, G.M. Maelandsmo, O. Fodstad, O. Myklebost, The protooncogene CHOP/GADD153, involved in growth arrest and DNA damage response, is amplified in a subset of human sarcomas, Cancer Genet. Cytogenet. 78 (1994) 165–171.
[94] H.K. Reddy, R.V. Mettus, S.G. Rane, X. Graña, J. Litvin, E.P. Reddy, Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis, Cancer Res. 65 (2005) 10174–10178.
[95] Q. Yu, E. Sicinska, Y. Geng, M. Ahnström, A. Zagozdzon, Y. Kong, H. Gardner, H. Kiyokawa, L.N. Harris, O. Stål, P. Sicinski, Requirement for CDK4 kinase function in breast cancer, Cancer Cell 9 (2006) 23–32.
[96] M. Malumbres, M. Barbacid, Cell cycle, CDKs and cancer: a changing paradigm, Nat Rev Cancer 9 (2009) 153–166.
[97] N.H. Kwon, J.Y. Lee, Y.-l. Ryu, C. Kim, J. Kong, S. Oh, B.S. Kang, H.W. Ahn, S. G. Ahn, J. Jeong, H.K. Kim, J.H. Kim, D.Y. Han, M.C. Park, D. Kim, R. Takase, I. Masuda, Y.-M. Hou, S.I. Jang, Y.S. Chang, D.K. Lee, Y. Kim, M.-W. Wang, S. Kim Basappa, Stabilization of Cyclin-Dependent Kinase 4 by Methionyl-tRNA Synthetase in p16INK4a-Negative Cancer, ACS Pharmacology & Translational Science 1 (2018) 21–31.
[98] E.A. Musgrove, C.E. Caldon, J. Barraclough, A. Stone, R.L. Sutherland, Cyclin D as a therapeutic target in cancer, Nat. Rev. Cancer 11 (2011) 558–572.
[99] L. Stepanova, M. Finegold, F. DeMayo, E.V. Schmidt, J.W. Harper, The oncoprotein kinase chaperone CDC37 functions as an oncogene in mice and collaborates with both c-myc and cyclin D1 in transformation of multiple tissues, Mol. Cell. Biol. 20 (2000) 4462–4473.
[100] L. Bockstaele, K. Coulonval, H. Kooken, S. Paternot, P.P. Roger, Regulation of CDK4, Cell Div. 1 (2006) 25.
[101] Q. Yu, E. Sicinska, Y. Geng, M. Ahnstrom, A. Zagozdzon, Y. Kong, H. Gardner, H. Kiyokawa, L.N. Harris, O. Stal, P. Sicinski, Requirement for CDK4 kinase function in breast cancer, Cancer Cell 9 (2006) 23–32.
[102] A.K. Witkiewicz, K.E. Knudsen, A.P. Dicker, E.S. Knudsen, The meaning of p16 (ink4a) expression in tumors: functional significance, clinical associations and future developments, Cell cycle (Georgetown, Tex.) 10 (2011) 2497–2503.
[103] M.C. Casimiro, M. Velasco-Velazquez, C. Aguirre-Alvarado, R.G. Pestell, Overview of cyclins D1 function in cancer and the CDK inhibitor landscape: past and present, Expert Opin. Investig. Drugs 23 (2014) 295–304.
[104] H. Wang, T. Goode, P. Iakova, J.H. Albrecht, N.A. Timchenko, C/EBPalpha triggers proteasome-dependent degradation of cdk4 during growth arrest, EMBO J. 21 (2002) 930–941.
[105] E. Berthonneau, M. Mirande, A gene fusion event in the evolution of aminoacyl-tRNA synthetases, FEBS Lett. 470 (2000) 300–304.

[106] P.S. Ray, P.L. Fox, Origin and evolution of glutamyl-prolyl tRNA synthetase WHEP domains reveal evolutionary relationships within Holozoa, PLoS One 9 (2014), e98493.
[107] O.A. Gressner, C. Gao, Monitoring fibrogenic progression in the liver, Clinica chimica acta; international journal of clinical chemistry 433 (2014) 111–122.
[108] D. Schuppan, M. Ashfaq-Khan, A.T. Yang, Y.O. Kim, Liver fibrosis: direct antifibrotic agents and targeted therapies, Matrix biology: journal of the International Society for Matrix Biology 68-69 (2018) 435–451.
[109] J.J. Maher, R.F. McGuire, Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo, J. Clin. Invest. 86 (1990) 1641–1648.
[110] A.M. Gressner, R. Weiskirchen, Modern pathogenetic concepts of liver fibrosis suggest stellate cells and TGF-beta as major players and therapeutic targets, J. Cell. Mol. Med. 10 (2006) 76–99.
[111] D.J. Lederer, F.J. Martinez, Idiopathic pulmonary fibrosis, N. Engl. J. Med. 378 (2018) 1811–1823.
[112] X.M. Zhou, G.L. Wang, X.B. Wang, L. Liu, Q. Zhang, Y. Yin, Q.Y. Wang, J. Kang, G. Hou, GHK peptide inhibits Bleomycin-induced pulmonary fibrosis in mice by suppressing TGFbeta1/Smad-mediated epithelial-to-Mesenchymal transition, Front. Pharmacol. 8 (2017) 904.
[113] J. Milara, G. Hernandez, B. Ballester, A. Morell, I. Roger, P. Montero, J. Escriva, J. M. Lloris, M. Molina-Molina, E. Morcillo, J. Cortijo, The JAK2 pathway is activated in idiopathic pulmonary fibrosis, Respir. Res. 19 (2018) 24.
[114] A.K. Ghosh, S.E. Quaggin, D.E. Vaughan, Molecular basis of organ fibrosis: potential therapeutic approaches, Experimental biology and medicine (Maywood, N.J.) 238 (2013) 461–481.
[115] D.V. Pechkovsky, C.M. Prele, J. Wong, C.M. Hogaboam, R.J. McAnulty, G. J. Laurent, S.S. Zhang, M. Selman, S.E. Mutsaers, D.A. Knight, STAT3-mediated signaling dysregulates lung fibroblast-myofibroblast activation and differentiation in UIP/IPF, Am. J. Pathol. 180 (2012) 1398–1412.
[116] M. Pedroza, T.T. Le, K. Lewis, H. Karmouty-Quintana, S. To, A.T. George, M. R. Blackburn, D.J. Tweardy, S.K. Agarwal, STAT-3 contributes to pulmonary fibrosis through epithelial injury and fibroblast-myofibroblast differentiation, FASEB J. 30 (2016) 129–140.
[117] L.Y. Tang, M. Heller, Z. Meng, L.R. Yu, Y. Tang, M. Zhou, Y.E. Zhang, Transforming growth factor-beta (TGF-beta) directly activates the JAK1-STAT3 Axis to induce hepatic fibrosis in coordination with the SMAD pathway, J. Biol. Chem. 292 (2017) 4302–4312.
[118] T. Turgeman, Y. Hagai, K. Huebner, D.S. Jassal, J.E. Anderson, O. Genin, A. Nagler, O. Halevy, M. Pines, Prevention of muscle fibrosis and improvement in muscle performance in the mdx mouse by halofuginone, Neuromuscular disorders: NMD 18 (2008) 857–868.
[119] D.G. Song, D. Kim, J.W. Jung, S.H. Nam, J.E. Kim, H.J. Kim, J.H. Kim, S.J. Lee, C. H. Pan, S. Kim, J.W. Lee, Glutamyl-prolyl-tRNA synthetase induces fibrotic extracellular matrix via both transcriptional and translational mechanisms, FASEB J. 33 (2019) 4341–4354.
[120] D.G. Song, D. Kim, J.W. Jung, S.H. Nam, J.E. Kim, H.J. Kim, J.H. Kim, C.H. Pan, S. Kim, J.W. Lee, Glutamyl-Prolyl-tRNA Synthetase regulates epithelial expression of Mesenchymal markers and extracellular matrix proteins: implications for idiopathic pulmonary fibrosis, Front. Pharmacol. 9 (2018) 1337.
[121] T.L. Keller, D. Zocco, M.S. Sundrud, M. Hendrick, M. Edenius, J. Yum, Y.J. Kim, H.K. Lee, J.F. Cortese, D.F. Wirth, J.D. Dignam, A. Rao, C.Y. Yeo, R. Mazitschek, M. Whitman, Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase, Nat. Chem. Biol. 8 (2012) 311–317.
[122] J. Dong, H. Qiu, M. Garcia-Barrio, J. Anderson, A.G. Hinnebusch, Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain, Mol. Cell 6 (2000) 269–279.
[123] A. Jimenez-Diaz, M. Remacha, J.P. Ballesta, J.J. Berlanga, Phosphorylation of initiation factor eIF2 in response to stress conditions is mediated by acidic ribosomal P1/P2 proteins in Saccharomyces cerevisiae, PLoS One 8 (2013), e84219.
[124] R. Ishimura, G. Nagy, I. Dotu, J.H. Chuang, S.L. Ackerman, Activation of GCN2 Kinase by Ribosome Stalling Links Translation Elongation with Translation Initiation, eLife, 5, 2016.
[125] A.J. Inglis, G.R. Masson, S. Shao, O. Perisic, S.H. McLaughlin, R.S. Hegde, R. L. Williams, Activation of GCN2 by the ribosomal P-stalk, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 4946–4954.
[126] S.M. Krane, The importance of proline residues in the structure, stability and susceptibility to proteolytic degradation of collagens, Amino Acids 35 (2008) 703–710.
[127] R.B. Hamanaka, E.M. O'Leary, L.J. Witt, Y. Tian, G.A. Gokalp, A.Y. Meliton, N. O. Dulin, G.M. Mutlu, Glutamine metabolism is required for collagen protein synthesis in lung fibroblasts, Am. J. Respir. Cell Mol. Biol. 61 (5) (2019 Nov) 597–606.
[128] S.K. Young, T.D. Baird, R.C. Wek, Translation regulation of the Glutamyl-prolyl-tRNA Synthetase gene EPRS through bypass of upstream open Reading frames with noncanonical initiation codons, J. Biol. Chem. 291 (2016) 10824–10835.
[129] A. Arif, J. Jia, R. Mukhopadhyay, B. Willard, M. Kinter, P.L. Fox, Two-site phosphorylation of EPRS coordinates multimodal regulation of noncanonical translational control activity, Mol. Cell 35 (2009) 164–180.
[130] J. Jia, A. Arif, P.S. Ray, P.L. Fox, WHEP domains direct noncanonical function of glutamyl-Prolyl tRNA synthetase in translational control of gene expression, Mol. Cell 29 (2008) 679–690.
[131] A. Arif, F. Terenzi, A.A. Potdar, J. Jia, J. Sacks, A. China, D. Halawani, K. Vasu, X. Li, J.M. Brown, J. Chen, S.C. Kozma, G. Thomas, P.L. Fox, EPRS is a critical mTORC1-S6K1 effector that influences adiposity in mice, Nature 542 (2017) 357–361.
[132] Z. Xu, Z. Wei, J.J. Zhou, F. Ye, W.S. Lo, F. Wang, C.F. Lau, J. Wu, L.A. Nangle, K. P. Chiang, X.L. Yang, M. Zhang, P. Schimmel, Internally deleted human tRNA synthetase suggests evolutionary pressure for repurposing, structure, London, England 20 (2012) (1993) 1470–1477.
[133] W.S. Lo, E. Gardiner, Z. Xu, C.F. Lau, F. Wang, J.J. Zhou, J.D. Mendlein, L. A. Nangle, K.P. Chiang, X.L. Yang, K.F. Au, W.H. Wong, M. Guo, M. Zhang, P. Schimmel, Human tRNA synthetase catalytic nulls with diverse functions, Science 345 (2014) 328–332.
[134] T. Kanaji, M.N. Vo, S. Kanaji, A. Zarpellon, R. Shapiro, Y. Morodomi, A. Yuzuriha, K. Eto, R. Belani, M.H. Do, X.L. Yang, Z.M. Ruggeri, P. Schimmel, Tyrosyl-tRNA synthetase stimulates thrombopoietin-independent hematopoiesis accelerating recovery from thrombocytopenia, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E8228–e8235.
[135] A.B. Tolstrup, A. Bejder, J. Fleckner, J. Justesen, Transcriptional regulation of the interferon-gamma-inducible tryptophanyl-tRNA synthetase includes alternative splicing, J. Biol. Chem. 270 (1995) 397–403.
[136] J.E. Kim, K.H. Kim, S.W. Lee, W. Seol, K. Shiba, S. Kim, An elongation factor-associating domain is inserted into human cysteinyl-tRNA synthetase by alternative splicing, Nucleic Acids Res. 28 (2000) 2866–2872.