Functional and pathologic association of aminoacyl-tRNA synthetases with cancer

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Although key tumorigenic and tumor-suppressive factors have been unveiled over the last several decades, cancer remains the most life-threatening disease. Multimomic analyses of patient samples and an in-depth understanding of tumorigenic processes have rapidly revealed unexpected pathologic associations of new cellular factors previously overlooked in cancer biology. In this regard, the newly discovered activities of human aminoacyl-tRNA synthases (ARSs) deserve attention not only for their pathologic significance in tumorigenesis but also regarding diagnostic and therapeutic implications. ARSs are not only essential enzymes covalently linking substrate amino acids to cognate tRNAs for protein synthesis but also function as regulators of cellular processes by sensing different cellular conditions. With their catalytic role in protein synthesis and their regulatory role in homeostasis, functional alterations or dysregulation of ARSs might be pathologically associated with tumorigenesis. This review focuses on the potential implications of ARS genes and proteins in different aspects of cancer based on various bioinformatic analyses and experimental data. We also review their diverse activities involving extracellular secretion, protein–protein interactions, and amino acid sensing, which are related to cancers. The newly discovered cancer-related activities of ARSs are expected to provide new opportunities for detecting, preventing and curing cancers.

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INTRODUCTION

The well-known function of aminoacyl-tRNA synthetases (ARSs) is to match specific amino acids to their cognate tRNAs and covalently link them for protein synthesis (Fig. 1, left axis)\(^1\). For this reason, most human diseases associated with mutation or aberrant expression of ARSs have been investigated from the point of their roles in translation. However, rapidly accumulating evidence shows that ARSs have evolved to play diverse and crucial roles in system development and homeostasis (Fig. 1, right axis). These new findings suggest that etiological investigation of ARS-associated diseases needs to be considered not only with respect to the catalytic activities of these enzymes for translation but also their noncatalytic roles beyond this process.

Recent genomic, proteomic and bioinformatic analyses have unveiled a pathologic link between human ARSs and various cancers as well as other human diseases. Through unique additional domains, such as WHEP, leucine zipper, and alpha-helices\(^2–6\), ARSs can pathologically participate in sustaining proliferative signals, deregulating cellular energetics, financing tumor-promoting inflammation, and promoting metastasis and angiogenesis. Furthermore, cancer-associated gene expression, mutation, and structural modification of ARSs might be related to their multifunctional properties\(^7\). An irregular event in ARS transcription and translation can result in unrestrained cellular signals that contribute to tumorigenesis. In addition, ARS gene expression patterns can be used as a possible biomarker for cancer\(^8\). Overall, genetic and postgenetic abnormalities of human ARSs appear to be deeply linked to cancer development and survival through unconventional and catalytic activities.

CANCER-ASSOCIATED EXPRESSION OF ARSS

In this section, different ARS expression patterns shown in different cancer types are discussed. Transcriptional and post-transcriptional regulation are also reviewed as factors influencing ARS expression patterns.

Transcription

Alteration of gene expression patterns is a key property of cancer, which might be either a cause or effect of cancer, providing both a tumor-friendly environment and inducing stress responses. Changes in the expression patterns of the genes encoding 37 ARSs and 3 AIMPs (20 cytosolic and 17 mitochondrial ARSs and AIMP (ARS-interacting multifunctional protein) 1, 2, 3) were examined in 19 different cancer types from the open database TCGA, as depicted as a heatmap in Fig. 2a. If ARSs are dedicated only to catalytic activities for translation, their expression is expected to be generally upregulated in cancer cells to meet the increased demand for protein synthesis. However, the heatmap shows no general cancer-associated gene expression pattern...
transcriptionally induced by ATF4. Mechanistically, transcription of 16 ARS-encoding genes is initiated by C/ebp-Atf response element (CARE) enhancers. ATF4 and C/EBP-homologous protein (CHOP) bind to CARE, and TATA-binding protein (TBP) is recruited to the TATA box (Fig. 2b). Following complete formation of the transcription machinery, RNA polymerase II initiates transcription of ARS genes. Considering that ATF4 expression is increased under oxidative stress, ER stress and hypoxia, as well as amino acid deprivation, ARS gene transcription can be changed under tumorigenic conditions.

Another well-known ARS transcription-inducing promoter is the androgen response element (ARE). Hormonal receptor alteration is one of the main characteristics of endocrine cancers. GARS1 and KARS1 display androgen-dependent transcriptional initiation in several hormone-responsive cells, such as prostate cancer cells (Fig. 2c); thus, transcription of GARS1 and KARS1 is initiated in cancers with increases in AREs. The importance of EPRS1 expression in estrogen receptor-positive breast cancer has also been monitored in clinical samples.

**Post-transcriptional control of ARSs**

Posttranscriptional regulation of ARSs, including alternative splicing and alternative polyadenylation, is also associated with tumorigenesis. Alternative splicing occurs in the 5’ untranslated region (UTR) of WARS1, producing exon II-lacking WARS1 (mini-WARS1) mRNA. In contrast to full-length WARS1, mini-WARS1 inhibits vascular endothelial growth factor (VEGF)-induced angiogenesis, which is crucial for a steady supply of nutrients to tumors.

Second, CARS1 is also subjected to a unique splicing mechanism, generating the variant CARS1-N6, in which a specific sequence is inserted at the N-terminal GST domain. This variant inhibits eukaryotic translation elongation factor 1 gamma (EEF1G), which is known to be overexpressed in various cancers, such as esophageal carcinoma, pancreatic cancer, and adenocarcinoma of the colon, suggesting a potential role for CARS1 variants in cancer through association with EEF1G.

An alternative polyadenylation-mediated variant of EPRS1, EPRS1-N1, lacking the PAR1 part in the EPRS1 transcript is found in various human cell lines. Alternative polyadenylation of the EPRS1 transcript starts at the 684th codon and changes UAU to UAA, leading to an incomplete EPRS1 protein. In response to IFN-γ, full-length EPRS1 forms an IFN-γ-activated inhibitor of translation (GAIT) complex along with N1-associated protein 1 (NSAP1), ribosomal protein L13a (L13a), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to inhibit translation of specific target mRNAs (GAIT element RNAs) in monocytic cells. Although EPRS1-N1 also interacts with GAIT-element RNAs, such as VEGFA transcripts, it blocks translational repression of the GAIT complex. Considering the significance of immune cell-mediated microenvironment changes in cancer development, modulating the function of the GAIT complex through this variant may also affect cancer.

**ARS GENE ALTERATION IN CANCER**

Below, ARS gene alterations, including single-nucleotide polymorphisms (SNPs), genetic mutations, and chromosomal rearrangements, are reviewed (Table 1).

**Cancer-associated SNPs**

SNPs in some ARSs are associated with cancer risk or with sensitivity to anticancer therapy. SNPs in the DARSI, NARS1, DARSI and NARS2 genes are reportedly related to cancer. These SNPs were detected in either B-lymphoblastoid cell lines or primary cells of acute lymphoblastic leukemia (ALL) (Table 1). Cells with these
SNPs exhibit altered sensitivity to asparaginase treatment, possibly due to changes in aspartate/asparagine metabolism. Considering the importance of aspartate/asparagine metabolism for the development of cancer, including ALL, these SNPs might play a role in tumorigenesis as well as in sensitivity to anticancer therapy.

Through a case–control study of breast cancer in the Chinese population, SNPs in AARS1, HARS1, RARS1 and WARS1 were found to be associated with an increased risk of breast cancer (Table 1). Considering that these SNPs are observed in 5′ UTRs or introns, they might affect cancer risk by changing gene expression levels. Another study suggested the association of SNPs in CARS1 with an increased risk of gastric cancer in the Chinese population. Through a two-stage case–control study, four SNPs in CARS1 were found to be potentially functional (Table 1). Using the Santa Cruz Genome Browser website and some other web-based analysis tools, the authors predicted that these SNPs disrupt transcription factor response elements or DNA methylation levels, affecting CARS1 expression levels.

**Genetic mutations**

Several point mutations and frameshift mutations in ARS genes are found at considerable frequencies in some types of cancer, with implications for tumorigenesis (Table 1). For example, A or TA deletion in the promoter region of IARS2 is reported in 59% of nonpolyposis colorectal cancer and Turcot syndrome. The (A)10(TA)9 repeat is normally observed in the 5′ upstream position of the IARS2 gene; in the −318~−291 position, alterations of (A)9(TA)8 or absolute deletion of the wild-type allele are frequently observed in tumors. Although the effects of these mutations on IARS2 expression are not yet understood, TA repeat deletion may result in its underexpression. A frameshift mutation in MARS1...
has been reported in colorectal or gastric cancer, and a frameshift caused by deletion of a T in the T9 repeat sequence in exon 3 results in a premature stop codon (p.Leu71CysfsX33), resulting in a lack of the major catalytic domain, nuclear-localizing sequence, and C-terminal protein–protein interaction domain. This mutation frequency is reported in 2.5~6.7% of colorectal cancer and gastric cancer cases.

Chromosomal alterations

Various chromosomal alterations have also been discovered in different types of cancer (Table 1). **CARS1** fused to anaplastic lymphoma kinase (**ALK**) was identified in inflammatory myofibroblastic tumors (**IMTs**). Although **ALK** fusions with different partners are found in various cancers, the **CARS1-ALK** fusion has been reported only in IMT. The **CARS1-ALK** fusion protein is predicted to produce an in-frame chimeric protein containing nearly 80% **CARS1** at the N-terminus and a functional catalytic domain of **ALK** in the C-terminus. For this chromosomal rearrangement, **CARS1** might contribute to neoplastic transformation by increasing the level of functional **ALK** because the fusion would provide the active **CARS1** promoter. Hence, it should be determined whether the **CARS1-ALK** fusion protein itself also contributes to tumorigenesis.

**Table 1.** **ARS gene alteration in cancer.**

| Alteration Type       | ARS     | Detail                         | Affected Region                          | Related Cancer Type                      |
|-----------------------|---------|--------------------------------|------------------------------------------|------------------------------------------|
| SNP                   | DARS1   | G/T (rs3768998)                | Aspartate/asparagine metabolism          | Either B-Lymphoblastoid Cell Lines or Acute Lymphoblastic Leukemia |
|                       |         | T/C (rs7587285)                |                                          |                                          |
|                       |         | T/C (rs11893318)               |                                          |                                          |
|                       |         | T/C (rs2322725)                |                                          |                                          |
|                       |         | A/C (rs2278683)                |                                          |                                          |
|                       | NARS1   | C/T (rs2318301)                |                                          |                                          |
|                       |         | C/G (rs540680)                 |                                          |                                          |
|                       | DARS2   | A/G (rs2068871)                |                                          |                                          |
|                       |         | C/T (rs2759328)                |                                          |                                          |
|                       |         | G/T (rs941988)                 |                                          |                                          |
|                       |         | G/T (rs2227589)                |                                          |                                          |
|                       |         | A/G (rs16846526)               |                                          |                                          |
|                       | NARS2   | G/A (rs11237537)               |                                          |                                          |
|                       | AARS1   | G/A (rs34087264)               | S’ cis-eQTL*                             | Breast Cancer                            |
|                       | HARS1   | A/G (rs801186)                 | Intron 11                                |                                          |
|                       | RARS1   | A/G (rs193466)                 | Intron 1                                 |                                          |
|                       | WARS1   | A/G (rs2273802)                | 5’ UTR                                   |                                          |
|                       | CAR5    | A/C (rs384490)                 | Transcription factor binding site        | Gastric Cancer                           |
|                       |         | A/G (rs729662)                 | Exon splicing enhancer                   |                                          |
|                       |         | G/A (rs2071101)                | DNase I Binding Site                     |                                          |
|                       |         | A/G (rs7394702)                | DNase I Binding Site                     |                                          |
| Point Mutation        | IARS2   | A – Deletion                   | Promoter                                 | Nonpolyposis Colorectal Cancer, Turcot Syndrome |
|                       |         | TA – Deletion                  |                                          |                                          |
|                       | RARS1   | miR-15                         | Locus 13q14                              | Chronic Lymphocytic Leukemia, Pituitary Adenoma |
|                       |         | miR-16                         | Locus 13q15                              |                                          |
| Frameshift Mutation   | MARS1   | Premature Stop Codon (Loss of Catalytic Domain) | Exon 3 T9 repeat | Colorectal Cancer, Gastric Cancer |
| Chromosome Rearrangement | CARS1   | CARS1-ALK fusion               | t(2;11)(p23;p15;q31)                      | Inflammatory Myofibroblastic Tumor       |
|                       | MARS1   | TLS-CHOP stabilization         | t(12;16)(q13;p11)                        | Myxoid and Round Cell Liposarcomas       |
| Alternative polyadenylation | EPRS1  | Y864 into Stop Codon           | Y864                                     | Monocyte Cancer                          |
| Deletion              | LARS2   | Entire LARS1 including chromosome loss | 3p21.3                                  | Nasopharyngeal Carcinoma                 |
|                       | CARS1   | Entire CARS1 including chromosome loss | 11p15.5–p15.4 | Wilms Tumor and Embryonal Rhabdomyosarcoma, Adrenocortical Carcinoma, and Lung, Ovarian and Breast Cancer |

ARS gene alterations include SNPs, mutations, and chromosomal rearrangements, and they are uniquely associated with different types of cancer.

*eQTL: expression quantitative trait locus.
controls mRNA stability. Thus, transcripts of TLS-CHOP and MARS1 are hybridized together, and both mRNAs are stabilized. A reporter assay using the 3'UTR of CHOP WT and the AU-rich region deleted form showed reduced protein expression due to AU-rich region inhibition, indicating functional significance of the MARS1 transcript for stabilizing the TLS-CHOP transcript. Because the MARS1 mRNA would also be stabilized by the TLS-CHOP mRNA through the same mechanism, it would be interesting to
mRNA and protein levels of RARS1 in the above cancers suggests that RARS1 expression is regulated by miR-15 and miR-observed, leading to the absence of miRNAs42,43. Interestingly, adenoma, and frequent deletion of the loci 13q14 and 13q15 is downregulated in chronic lymphocytic leukemia and pituitary tissues also showed elevated protein levels of GARS1 and KARS1 in localized and metastatic prostate cancer and normal prostate revealed androgen-dependent increases in AARS1, FARSA, GARS1, Cancers associated protein levels and posttranslational modifications (PTMs) of ARSs are reviewed in the following section.

ARS TRANSLATION IN CANCER

Cancer-associated protein levels and posttranslational modifications (PTMs) of ARSs are reviewed in the following section.

Control of ARS protein levels

Based on overall data from The Human Protein Atlas, different ARS protein levels in 11 different cancer types are displayed in the heatmap in Fig. 3a. IARS1 and IARS2 protein levels are reduced but those of YARS2 and FARS2 increased in most cancer types. In general, cytosolic ARSs show higher protein levels than mitochondrial ARSs in cancer44. Higher levels of KARS1 protein are detected in breast cancer patient tissues45. A proteomic study of ARSs in LNCaP cells revealed androgen-dependent increases in AARS1, FARSA, GARS1, NARS1, TAR1, HARS1, and WAR1 levels. Further investigation of localized and metastatic prostate cancer and normal prostate tissues also showed elevated protein levels of GARS1 and KARS1 in cancer2. The catalytic activity of MAR51 is reported to be increased in colon cancer patient tissues, though protein levels were not directly compared46. Recently, the diagnostic value of MAR51 protein levels in bile duct cancer has been reported47. MAR51 exhibits stronger immunohistochemistry (IHC) staining signals in malignant biliary structures than in nonmalignant specimens49, and elevated LARS1 levels have been observed in 11 different types of non-small-cell lung cancer (NSCLC) and 12 colon cancer cell lines10,48–50. Moreover, clinical validation using NSCLC and colon cancer patient tissue showed higher LARS1 levels than normal tissue51. In contrast to LARS1, IHC using tissue microarrays of colorectal cancer revealed a negative correlation between WARS1 protein level and recurrence risk, lymph node metastasis and a more advanced stage, suggesting the prognostic value of the WARS1 protein level23.

To determine whether expression of ARS genes is tightly mirrored at the protein level, we compared a heatmap of cancer-associated ARS protein levels with that of ARS gene expression (Fig. 3a). We compared gene and protein levels of ARSs and other housekeeping translational components and ribosome subunits, including RPSA, RPS5, RPS6, RPS13, RPS20, RPLP0, RPL5, RLP8, RPL9, and RPL10A were investigated. Levels of protein and mRNA were calculated using the same method as in Fig. 4a. Cancer types shared by both databases were utilized for the correlation plot. The coefficient of correlation (r-value) and the significance level were only statistically significant for ARSs.

Posttranslational modifications

Because ARS genes are constitutively expressed, their cellular activities, interaction, and cellular localization can be determined by signal- or stress-dependent specific PTMs. iPTMNet is a database that provides functional and structural analyses of posttranslational modifications52, showing 103 different PTMs of ARSs, with significance for KARS1, MARS1, EPRS1 and LARS1 PTMs in cancer cells, mouse models or even in patient samples. KARS1 is phosphorylated at two distinct residues in response to different signals and modulates cancer-associated characteristics (Fig. 4a). First, KARS1 is phosphorylated at T32 by p38MAPK in the presence of laminin. Phosphorylated KARS1 dissociates from MSCs and is translocated to the plasma membrane for interaction with the 67-kDa laminin receptor (67LR). KARS1 stabilizes 67LR, leading to increased cell migration and cancer metastasis53. In contrast, KARS1 is phosphorylated at S207 following activation of the EGFR signaling pathway, which appears to predict disease-free survival of NSCLC54. In addition, N-terminal cleavage of KARS1 occurs in colorectal cancer cell lines. Upon serum starvation, the N-terminal 12 amino acid peptide of KARS1 is cleaved by caspase-8, causing its dissociation from MSCs. Cleaved KARS1 interacts with syntenin for exosome biogenesis and is then secreted via exosomes to trigger macrophage/neutrophil migration and inflammation55. Interestingly, a recent study reported that colorectal cancer patients have higher KARS1 levels in plasma56. In general, it is worth monitoring whether plasma KARS1 is carried by circulating exosomes or as a naked form. KARS1 is also phosphorylated in response to two distinct signals (Fig. 4b). Upon UV irradiation, general control...
nonrepressed-2 (GCN2) phosphorylates MARS1 at S662, decreasing tRNAMet methionylation activity and releasing bound AIMP3, which then translocates to the nucleus for DNA repair and AIMP3 is a tumor suppressor maintaining genomic stability. Dysregulated phosphorylation of MARS1 at S662 may be associated with cancer development. MARS1 is also phosphorylated at S209 and S825 by extracellular signal-related kinase (ERK1/2) in response to oxidative stress. Double-phosphorylated MARS1 exhibits decreased specificity for tRNAMet and charges methionine to nonmethionyl tRNAs, resulting in more frequent methionine incorporation into nascent proteins, which increases ROS levels.

**Fig. 4** Posttranslational modification of ARSs in cancer. 

**a** Full-length KARS1 consists of the N-Helix, ABD and CD Domains. KARS1 is modified to three different forms by different kinds of upstream enzymes. First, KARS1 is phosphorylated at T52 by p38 MAPK in the presence of laminin. KARS1 pT52 is translocated to the plasma membrane for interaction with 67LR and then promotes metastasis. Second, KARS1 is phosphorylated at S207 upon EGFR signaling pathway activation. KARS1 pS207 is translocated to the nucleus and appears to be associated with disease-free survival of NSCLC. Third, the N-terminal 12 amino acid KARS1 is cleaved by caspase-8 to produce ΔKARS1. ΔKARS1 is secreted into the extracellular space in exosomes via interaction with syntenin. 

**b** Full-length MARS1 consists of GST, CD, ABD and WHEP domains. MARS1 is modified to two different forms of MARS1 in response to various input signals. Upon UV irradiation, MARS1 is phosphorylated at S662 by GCN2. MARS1 pS662 has a decreased capability to methionylate tRNAs, resulting in downregulation of global translation. In contrast, MARS1 is doubly phosphorylated at S209 and S825 by ERK1/2 in response to oxidative stress. MARS1 pS209/825 shows increased mismethionyl to noncognate tRNAs due to an increase in Met residues in proteins, which contribute to reducing ROS levels.

**c** Upon stimulation with IFN-γ, EPRS1 is phosphorylated at S886 and S999 by CDK5 and S6K1, respectively. EPRS1 pS886/999 forms the GAIT complex to regulate translation of GAIT elements. 

**d** LARS1 is phosphorylated at S720 by ULK1 in response to glucose starvation. LARS1 pS720 shows decreased leucine binding capability, resulting in decreased tRNA leucylation and mTORC1 stimulation. CD, catalytic domain; ABD, anticodon-binding domain; ROS, reactive oxygen species.
Cells expressing phosphorylation-deficient mutants are more sensitive to oxidative stresses, suggesting that these cells defend against oxidative stress by utilizing S209/S825 phosphorylated MARS1 to promiscuously charge methionine to many different tRNAs. This mechanism may also be functionally related to the reactive oxygen species (ROS)-managing mechanism in cancer.

As mentioned above, EPRS1 forms the GAIT complex in the presence of IFN-γ to regulate GAIT element-containing mRNAs, including VEGFA. Two-step phosphorylation at S886 and S999...
mediated by cyclin-dependent kinase 5 (CDK5) and ribosomal protein S6 kinase beta-1 (S6K1), respectively, is necessary for EPR5 to dissociate from MscS and subsequently associate with the GAIT complex (Fig. 4c)61,62. pS886 is required for interaction with NAP1, L13a and GAPDH, and pS999 directs binding to elf4G for formation of the functional GAIT complex.

Posttranslational modification of LARS1 was recently identified. Under glucose deprivation, Unc-51-like autophagy activating kinase 1 (ULK1) phosphorylates LARS1 at S720 (Fig. 4d). This modification is reported to decrease leucine binding affinity, inhibiting tRNA<sub>α</sub> leucylation and mechanistic target of rapamycin complex 1 (mTORC1)-stimulating activities to save energy. In addition, cells expressing phosphomimetic mutants showed increased leucine degradation for energy generation in rhabdomyosarcoma cell lines63. Although the exact role of LARS1 phosphorylation at S720 in cancer is not fully understood, this work suggests that LARS1 modulates its leucine binding capability under metabolic stress in cancer, such as glucose starvation, thereby providing a metabolic adaptation and survival strategy.

CANCER-ASSOCIATED FUNCTIONS

In addition to a catalytic role in translation to meet the increased demand for protein synthesis for cancer cell growth, ARSs can be involved in the processes of tumorigenesis in multiple ways. First, ARSs play unique roles in the extracellular space (Fig. 5). Second, ARSs mediate a broad spectrum of cellular signaling pathways via specific protein–protein interactions with diverse cellular factors (Fig. 6, upper). Third, ARSs control their catalytic and signaling activities in an amino acid-dependent manner (Fig. 6, lower). Fourth, ARSs generate the second messenger molecules diadenosine polyphosphates (ApnAs). Among their diverse regulatory activities, we discuss below the functions of ARSs associated with cancer development and maintenance.

Secretory functions

Although ARSs primarily operate in intracellular locations, they are known to mediate cell-to-cell communication. Indeed, they are secreted from cells such as macrophages and cancer cells as naive or modified forms in response to specific environmental stimuli and exert unique extracellular activities (Fig. 5).

For instance, TNF-α induces secretion of KARS1 from cancer cells to the extracellular space (Fig. 5a). Although the specific mechanism by which TNF-α mediates KARS1 secretion is not fully understood, it was reported that secreted KARS1 stimulates proinflammatory responses when it acts on immune cells such as macrophages40. Cancer cells are also capable of secreting CAR5 upon stimulatory signals such as TNF-α and the ER stress inducer tunicamycin (Fig. 5a)45. Secreted CAR5 activates immune responses by directly interacting with toll-like receptor 2 (TLR2) on antigen-presenting cells, including dendritic cells. UNE-C1 in the catalytic domain of CAR5 was determined to serve as the binding domain for TLR2. The CAR5 UNE-C1 domain shows a synergistic effect with cancer antigens and several immune checkpoint inhibitors in in vivo cancer models, suggesting further potential for the domain to be developed as an immunoadjuvant to activate antitumor immunity.

In contrast, GARS1 is secreted from macrophages when stimulated by Fas ligand (Fig. 5b). Secreted GARS1 interacts with cadherin 6 (CDH6) on neighboring cancer cells, leading to phosphatase 2A (PP2A) release, which deactivates the ERK signaling pathway19. Administration of purified recombinant GARS1 induces tumor regression in vivo in tumor models19, suggesting its potential as a novel anticancer agent.

After the discovery of autoantibodies specific for TARS1 in human sera65, TARS1 was predicted to be secreted from cells, and the role of secreted TARS1 was confirmed in blood vessel formation (Fig. 5c)66. After VEGF or TNF-α stimulation, TARS1 is secreted from human endothelial cells. TARS1 treatment of endothelial cells, fertilized chicken embryos and zebrafish increases blood vessel formation in different in vitro and in vivo models66,67. Further investigation of the exact mechanism by which secreted TARS1 increases angiogenesis would provide functional insight into the roles of other ARSs, including YARS1 and TARS1, in tumor-associated vasculization.

Proteolytic cleavages also appear to be involved in the secretion or proper extracellular function of some ARSs (Fig. 5d). As discussed above, serum starvation triggers N-terminal truncation of KARS1 via activated caspase-8, leading to exosome-mediated secretion from cancer cells for proinflammatory activities56,68. In response to apoptotic signals, YARS1 is secreted and then cleaved by elastase to generate N-terminal mini-YARS1 and C-terminal EMAPII-like domains. The C-terminal EMAPII-like domain increases the migration of mononuclear phagocytes and TNF-α production, and N-terminal mini-YARS1 induces the migration of polymorphonuclear leukocytes and HUVECs and angiogenesis69–71. Mini-YARS1, but not full-length YARS1, has such activities because cleavage of the C-terminal EMAPII-like domain enables exposure of the ELR motif on mini-YARS1, through which it binds to CX-C motif chemokine receptor 1/2 (CXCR1/2)72. By binding to CXCR1/2, mini-YARS1 appears to transactivate VEGF receptor 2 (VEGFR2) as well as downstream angiogenesis-associated signaling molecules for blood vessel formation41. In contrast to YARS1, truncated forms of WAR51 exhibit angiostatic function. Upon stimulation with IFN-γ, either full-length or mini-WAR51, the alternative splicing form of WAR51, as described above, is secreted from cells. In the extracellular space, the N-terminal end of WAR51 is digested by elastase to produce the T1 and T2 forms of the protein54,56. Similar to YARS1, only truncated forms, but not full-length WAR51, show angiostatic activity. T2-WAR51 binds to VE-cadherin on endothelial cells through interaction between the tryptophan residue of VE-cadherin and the catalytic pocket of WARS173. Binding of T2-WAR51 to VE-cadherin decreases VEGF-mediated activation of the ERK pathway, suggesting that its antiangiogenic function is mediated by VE-cadherin41.

Protein–protein interactions

ARSs also have diverse regulatory activities via interactions with different cellular factors77. The functional ARS interactome broadly covers major signaling pathways to control cellular growth,
proliferation and death, crucially influencing the process of tumorigenesis (Fig. 6)78. SARS1 and TARS1 control VEGFA levels at different stages via interactions with their specific partners79,80. SARS1 interacts with the transcription factor YY1 and then represses transcriptional activation of VEGFA. Interaction between SARS1 and YY1 seems to be crucial for repression of VEGFA transcription, as zebrafish with knockdown of either SARS1 or YY1 develop more intersegmental vessels79. TARS1 regulates translation of some mRNAs, including VEGFA, through the formation of a novel translation initiation complex composed of TARS1 and eukaryotic translation initiation factor 4E family member 2 (eIF4E2). The TARS1 and eIF4E2 complex successfully initiates translation of some mRNAs that are selected via the specific RNA-binding ability of TARS1 and the cap-binding ability of eIF4E280. Although the functional significance of VEGFA regulation in cancer was not directly examined in either study, dysregulated interaction of the two factors may affect cancer growth via VEGF expression.

AIMP2-DX2 is a splicing variant of AIMP2 lacking exon 2 and is known to disturb the tumor-suppressive activities of AIMP2 through competitive interaction with target proteins81,82. SARS1 and TARS1 control VEGFA levels at different stages via interactions with their specific partners79,80. SARS1 interacts with the transcription factor YY1 and then represses transcriptional activation of VEGFA. Interaction between SARS1 and YY1 seems to be crucial for repression of VEGFA transcription, as zebrafish with knockdown of either SARS1 or YY1 develop more intersegmental vessels79. TARS1 regulates translation of some mRNAs, including VEGFA, through the formation of a novel translation initiation complex composed of TARS1 and eukaryotic translation initiation factor 4E family member 2 (eIF4E2). The TARS1 and eIF4E2 complex successfully initiates translation of some mRNAs that are selected via the specific RNA-binding ability of TARS1 and the cap-binding ability of eIF4E280. Although the functional significance of VEGFA regulation in cancer was not directly examined in either study, dysregulated interaction of the two factors may affect cancer growth via VEGF expression.

AIMP2-DX2 is a splicing variant of AIMP2 lacking exon 2 and is known to disturb the tumor-suppressive activities of AIMP2 through competitive interaction with target proteins81,82. Overexpression of AIMP2-DX2 correlates positively with cancer progression, and a recent study reported that AIMP2-DX2 is stabilized through interaction with heat shock protein 70 (HSP70)83. HSP70 binding to AIMP2-DX2 appears to prevent the association of Siah E3 ubiquitin protein ligase 1 (Siah1) with AIMP2-DX2, reducing AIMP2-DX2 degradation. Chemical inhibition of AIMP2-DX2 and HSP70 interaction successfully decreases AIMP2-DX2 levels in cells and induces tumor regression in an in vivo mouse model, suggesting the interface of AIMP2-DX2 and HSP70 as a novel target to control cancer.

EPRS1 forms the GAIT complex to inhibit the proinflammatory response. KARS1 inhibits NEDD4 and stabilizes 67LR through its interaction with 67LR. KARS1-mediated Ap4A production increases the transcriptional activity of MITF by liberating HINT1. SARS1 inhibits VEGFA transcription through its interaction with YY1; the TARS1-eIF4E2 complex increases translation initiation of VEGFA. Oncogenic AIMP2-DX2 is stabilized by its association with HSP70, leading to cell transformation. LARS1 mediates mTORC1 activation through leucine-dependent interactions with either RagD or Vps34. QARS1 decreases apoptosis through glutamine-dependent interactions with ASK1. MAR5 competes with p16INK4a for interaction with CDK4. Interaction between MAR5 and CDK4 is possibly dependent on methionine, and the MAR5-CDK4-HSP90-CDC37 complex increases the stability of CDK4. WARS1 mediates PARylation of DNA-PKcs, leading to p53 activation. Association of WARS1, PARP-1, and DNA-PKcs might also be dependent on tryptophan.

Intracellular amino acid sensory activities

After the functional significance of amino acids as signaling molecules was suggested, cellular amino acid sensors have attracted much attention84–88. As ARSs specifically recognize corresponding amino acids for their catalytic activities, they have intrinsic potential to sense the intracellular levels of amino acids. For instance, LARS1 senses intracellular leucine levels to activate the mTORC1 pathway (Fig. 6)84. Mechanistically, leucine-bound LARS1 is translocated to lysosomes and interacts with RagD GTPase. Through conversion of RagD-GTP to RagD-GDP, mTORC1 is recruited to lysosomes and activated, promoting cell

**Fig. 6 Intracellular signaling functions of ARSs via diverse protein-protein interactions.** ARSs mediate diverse intracellular and extracellular signaling pathways by protein-protein interactions, some of which are further controlled by amino acid sensing and by generating second messengers such as diadenosine polyphosphates. KARS1 inhibits NEDD4 and stabilizes 67LR through its interaction with 67LR. KARS1-mediated Ap4A production increases the transcriptional activity of MITF by liberating HINT1. SARS1 inhibits VEGFA transcription through its interaction with YY1; the TARS1-eIF4E2 complex increases translation initiation of VEGFA. Oncogenic AIMP2-DX2 is stabilized by its association with HSP70, leading to cell transformation. LARS1 mediates mTORC1 activation through leucine-dependent interactions with either RagD or Vps34. QARS1 decreases apoptosis through glutamine-dependent interactions with ASK1. MAR5 competes with p16INK4a for interaction with CDK4. Interaction between MAR5 and CDK4 is possibly dependent on methionine, and the MAR5-CDK4-HSP90-CDC37 complex increases the stability of CDK4. WARS1 mediates PARylation of DNA-PKcs, leading to p53 activation. Association of WARS1, PARP-1, and DNA-PKcs might also be dependent on tryptophan.
proliferation and growth. In this context, LARS1 functions as a GTPase-activating protein (GAP) in the Rag GTPase cycle in coordination with other leucine sensors, such as Sestrin2. The role of LARS1 in the mTORC1 pathway was also shown in the axis of vacuolar protein sorting 34 (Vps34)-phospholipase D1 (PLD1). Leucine-bound LARS1 activates Vps34, accumulating phosphatidylinositol 3-phosphate (PI-3-P) for PLD1 activation. Activated PLD1 is recruited to lysosomes and generates phosphatidic acid (PA) for activation of mTORC1. Overall, LARS1 appears to control the activity of mTORC1 through multiple pathways in a leucine-dependent manner. The functional significance of LARS1 as a leucine sensor for mTORC1 activation in cancer has been further investigated in different cancer cell lines and in vivo models. Chemical inhibition of the interaction between LARS1 and RagD decreases the proliferation and increases the death of colon and lung cancer cell lines but not normal cell lines. In addition, a chemical inhibitor induces tumor regression in a mouse xenograft model using colon and lung cancer cell lines, even though the cells show rapamycin resistance. Considering that LARS1 is overexpressed in some cancers, including myeloid leukemia, pancreatic cancer, renal cancer, cervical cancer and skin cancer (Fig. 3), targeting the leucine-sensing-mediated function of LARS1 has therapeutic potential against cancer.

Glutamine is one of the most crucial amino acids in tumor progression. Glutamine depletion induces apoptosis, whereas glutamine supplementation protects cells through various molecular pathways. Interestingly, QARS1 was shown to mediate the antiapoptotic property of glutamine. Mechanistically, QARS1 forms a protein complex with apoptosis signal-regulating kinase 1 (ASK1) in a glutamine-dependent manner. In the presence of glutamine, QARS1 and ASK1 interact through their C-terminal domains, decreasing the kinase activity of ASK1 for apoptosis. MARS1 was previously shown to stabilize cyclin-dependent kinase 4 (CDK4), which forms a complex with cyclin D1 and regulates the cell cycle transition from G1 to S phase. MARS1 contributes to proper folding of CDK4, along with heat shock protein 90 (HSP90) and cell division cycle 37 (CDC37), which then interacts with cyclin D1. Although direct evidence for methionine-mediated interaction between MARS1 and CDK4 was not provided, methionine binding-deficient mutants of MARS1 and a methionine analog.
FSMO, reduced interaction with CDK4, implying that interaction between these two proteins may be sensitive to the binding status of methionine to MARS1. p16INK4a is a tumor suppressor that negatively regulates the CDK4 and cyclin D1 complex, which activates oncoenzymes such as Rb and E2F98,100. The effect of MARS1-mediated CDK4 stabilization is more prominent in p16INK4a-negative cancers because MARS1 and p16INK4a appear to compete for interaction with CDK4. Indeed, p16INK4a-negative cancer cell lines show a higher positive correlation for MARS1 and CDK4 protein levels than p16INK4a-positive cancer cell lines. Thus, targeting MARS1 using methionine analogs may be an attractive way to control p16INK4a-negative cancer via CDK4.

WARS1-mediated poly(ADP-ribose)ylation (PARylation) of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) also exhibits a potential connection with amino acid binding93,94. After IFN-γ stimulation, the WARS1 protein level increases, and at least some of this increased WARS1 population plays a role in activating DNA-PKcs and p53 through DNA-PKcs PARylation. Although the effect of tryptophan on the formation of WARS1, poly(ADP-ribose) polymerase-1 (PARP-1) and the DNA-PKcs complex has not been clearly elucidated, S-O-[N-(9-L-tryptophanyl) sulfamoyl] adenosine (Trp-SA), a Trp-AMP analog, dissociates this triple complex; thus, catalytic site occupation might be crucial for this function. Overall, the functional significance of Trp or Trp-SA analogs on IFN-γ-induced cancer cell death should be further investigated.

**CONCLUDING REMARKS**

ARSs utilize amino acids as reaction substrates, consuming ATP as an energy source and the cellular tRNA pool as the vehicle to carry charged amino acids to ribosomes. Thus, metabolic balance among amino acids in coordination with ATP and tRNAs is crucial for the protein synthesis rate and fidelity. When intracellular levels of the reaction substrates are high, ARSs can enhance protein synthesis via dual pathways of catalysis and signal transduction (Fig. 7a). In cells with lower levels of amino acids, ATP and tRNAs, ARSs may reduce or stop protein synthesis and function to reprogram metabolism to return amino acid, ATP and tRNA levels to normal (Fig. 7b). Thus, the cellular pool of ARSs contributes to the homeostasis of the cellular metabolome and proteome not only via their catalytic activities but also via their multifarious regulatory capability.

As described above, multiple genomic and transcriptomic analyses show that ARS-encoding genes are specifically under- or overexpressed in different types of cancer cell lines. Cancer-associated genetic alterations of ARSs, including SNPs, splicing variants, single mutations, and deletions, have also been found. In addition, cancer-related PTMs and secretion of ARSs have been reported. Considering the diversity of cancer-associated changes in ARS-encoding genes and proteins, ARSs may be involved in cancer formation in a systemic manner rather than through the participation of only a few of them. Regardless of the cancer-associated changes in ARSs at the gene and protein levels, these changes would ultimately affect homeostatic control of the metabolome (particularly related to amino acids, energy and RNAs). In theory, ARSs can function as cancer drivers or supporters. As a cancer driver, a disrupted ARS pool would cause an imbalance of the metabolome and proteome, resulting epigenetic and genetic changes and eventually provoking cancer (Fig. 7c). Alternatively, enhanced expression or mutations and aberrant forms of ARSs can increase their catalytic activities and signaling pathways to support the increased demand for protein synthesis required for cancer cell growth (Fig. 7d).

From a therapeutic point of view, diverse biological activities of human ARSs indicate their potential as therapeutic targets and agents for cancer treatment. For instance, inhibition of the catalytic site95 and noncatalytic site of LARS1 responsible for interaction with RagD have been shown to be effective in controlling the tumor-promoting mTORC1 pathway96. Furthermore, chemical intervention of interaction between KARS1 and 67LR in the cell membrane is effective against cancer metastasis95. Targeting a splicing variant of AIMP2 at the interface with HSP70 also effectively suppresses tumor growth97. Secreted GAR1 and CAR1 exhibit potent anticancer activities via their specific and unique modes of action98. The potential of ARSs as therapeutic targets has not been seriously explored because they are essential enzymes for protein synthesis, with concern for a general effect on the body. Nevertheless, recent unexpected discoveries regarding their specific roles in diverse regulatory pathways are rapidly opening a new possibility for ARSs as druggable target families. First, global protein synthesis is not much affected, even when cellular expression of ARSs is significantly suppressed or their catalytic activities are inhibited. Perhaps cellular levels of ARSs are higher than those required to meet the demand of global protein synthesis for highly differentiated normal cells. Thus, even if a cellular ARS is crippled by transcriptional suppression or catalytic inhibition, it may not seriously affect global protein synthesis and cell viability, as expected. Second, only a small portion of cellular ARSs is actually used for their epi-translational activities as exerted by the extracellular space, cell membrane and nucleus. Thus, targeting ARSs with regard to these activities would specifically modulate pathologically relevant activities while not affecting global protein synthesis. In general, specific targeting of a portion of ARSs that are involved in epitranslational activities might show highly specific and potent efficacy toward pathological phenotypes of diseases. Third, the diverse-yet-idiiosyncratic activities of ARSs provide multiple options for developing drugs not only for cancer but also for other refractory diseases with no effective drugs available.

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Y.S. collected and assembled the data. Y.S. and I.Y. wrote the manuscript. J.M.H. conceived of and designed the review. I.Y., J.M.H. and S.K. revised and edited the manuscript. J.M.H. and S.K. provided supervision. All authors have read and agreed to the publication of this version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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