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Aminoacyl-tRNA synthetases of the multi-tRNA synthetase complex and their role in tumorigenesis

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ABSTRACT

In mammalian cells, 20 aminoacyl-tRNA synthetases (AARS) catalyze the ligation of amino acids to their cognate tRNAs to form aminoacylated-tRNAs – essential for decoding genetic information through protein translation [1–3]. There are 20 cytoplasmic AARS – one for each amino acid. The sequence and structure of the catalytic and anticodon binding domains of AARSs are highly conserved from prokaryotes to eukaryotes, supporting their early appearance and critical function in all organisms. Eukaryotic AARSs uniquely harbor novel domains appended during evolution, and which exert non-canonical functions unrelated to translation, but important in multiple complex pathophysiological processes including inflammation, amino acid metabolism, tumorigenesis, and neurodegeneration, among others [4–9]. In humans and other mammals, nine of the cytoplasmic AARSs, in addition to three scaffolding proteins termed AARS-interacting multifunctional proteins 1–3 (AIMP1–3), form a large ~1.25 MDa MSC (Fig. 1a,b) [10]. Altered expression or mutation of multiple MSC-resident AARS correlates with poor cancer prognosis; in contrast, the auxiliary AIMP proteins can exhibit anti-tumorigenic activities [11–13]. The precise physiological function of the MSC remains elusive, however, a critical role in sequestration of constituents with deleterious activities in free form has been proposed [14–16]. Thus, besides expression level, cellular localization of AARSs following release from the MSC can contribute importantly to physiological and pathological function. In many cases, pathogenic mutations do not influence AARS catalytic function, but rather alter their cell localization, and possibly their non-canonical function involved in tumor promotion, thus improving their therapeutic suitability as loss-of-function targets [13, 17]. Specific mechanisms of stimulus-dependent release of constituents are not well understood; however, the recent elucidation of the 3D architecture of the MSC by cross-linking mass-spectrometry (XL-MS) reveals specific interaction domains between constituents, and thus can provide insights into specific sites and domains critical for release [18]. Here, we review our current understanding of the function of MSC AARSs, and their alternative forms in tumor progression and prevention, as well as current therapeutic modalities that target MSC AARSs for cancer prevention and treatment.

Introduction

AARSs are essential enzymes that synthesize building blocks of protein synthesis by ligating amino acids to their cognate tRNAs to form aminoacylated-tRNAs – essential for decoding genetic information through protein translation [1–3]. There are 20 cytoplasmic AARSs – one for each amino acid. The sequence and structure of the catalytic and anticodon binding domains of AARSs are highly conserved from prokaryotes to eukaryotes, supporting their early appearance and critical function in all organisms. Eukaryotic AARSs uniquely harbor novel domains appended during evolution, and which exert non-canonical functions unrelated to translation, but important in multiple complex pathophysiological processes including inflammation, amino acid metabolism, tumorigenesis, and neurodegeneration, among others [4–9]. In humans and other mammals, nine of the cytoplasmic AARSs, in addition to three scaffolding proteins termed AARS-interacting multifunctional proteins 1–3 (AIMP1–3), form a large ~1.25 MDa MSC (Fig. 1a,b) [10]. Altered expression or mutation of multiple MSC-resident AARS correlates with poor cancer prognosis; in contrast, the auxiliary AIMP proteins can exhibit anti-tumorigenic activities [11–13]. The precise physiological function of the MSC remains elusive, however, a critical role in sequestration of constituents with deleterious activities in free form has been proposed [14–16]. Thus, besides expression level, cellular localization of AARSs following release from the MSC can contribute importantly to physiological and pathological function. In many cases, pathogenic mutations do not influence AARS catalytic function, but rather alter their cell localization, and possibly their non-canonical function involved in tumor promotion, thus improving their therapeutic suitability as loss-of-function targets [13, 17]. Specific mechanisms of stimulus-dependent release of constituents are not well understood; however, the recent elucidation of the 3D architecture of the MSC by cross-linking mass-spectrometry (XL-MS) reveals specific interaction domains between constituents, and thus can provide insights into specific sites and domains critical for release [18]. Here, we review our current understanding of the function of MSC AARSs, and their alternative forms in tumor progression and prevention, as well as current therapeutic modalities that target them. There is limited or no data on the relationship of QARS1, IARS1, and RARS1 to cancer, and these are not discussed in detail.

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MSC-associated AARSs and AIMP\'s and their role in cancer progression

Glutamyl-prolyl-tRNA synthetase (EPRS1)

EPRS1 is an \(\sim 170\) kDa, bifunctional tRNA synthetase with glutamyl-tRNA synthetase activity near the N-terminus and proline-tRNA synthetase activity at the C-terminus (Fig. 1a). The catalytic domains are fused with a linker harboring three helix-turn-helix WHEP domains [19]. The GST-like domain at the N-terminus regulates protein-protein interactions critical for its association with the MSC [20, 21]. A methylation microarray analysis showed that elevated EPRS1 mRNA expression in breast cancer patients is associated with an unfavorable clinical outcome [22]. Likewise, EPRS1 mRNA copy number is associated with increased tamoxifen-resistance and poor prognosis in ER\(^+\) breast cancer cells where it induces estrogen receptor expression and activity [23]. In an alternative mechanism, EPRS1 interaction with several extra-MSC proteins can contribute to cancer progression. For example, EPRS1 interacts with Engrailed1, a homeodomain transcription factor, and disruption of the interaction with synthetic peptides enhances tumor cell apoptosis [24]. In non-small-cell lung cancer (NSCLC) cells, DUS2 interaction with EPRS drives cell proliferation, presumably by enhancing tRNA charging activity [25]. In gastric cancer, over-expression of EPRS1 induces interaction with SYCL2, in turn activating the WNT/\(\beta\)-catenin signaling pathway, driving cell proliferation and tumor growth [26]. IFN-\(\gamma\)-treatment of macrophages, induces EPRS1 phosphorylation and release from the MSC to form the quaternary interferon-\(\gamma\)-activated inhibitor of translation (GAIT) complex with NSAP1, ribosomal protein L13a and GAPDH [27, 28]. The complex silences translation of multiple inflammation-related transcripts, including vascular endothelial growth factor-A, an angiogenic factor critical for growth of breast tumors, among others [29–31]. EPRS1 exhibits a non-canonical function as a terminal effector of the mTORC1-S6K1 axis determining adiposity, and mice bearing an EPRS1 Ser999-to-Ala-loss-of-function mutation are lean. In view of the role of obesity as a determinant of progression of certain cancer types, e.g., breast and ovarian cancer, it will be interesting to test the lean, mutant EPRS1 mice are resistant to these cancers.

Methionyl-tRNA synthetase (MARS1)

MARS1 is an \(\sim 100\) kDa protein harboring two extra-catalytic domains, namely, GST-like and WHEP domains (Fig. 1a) [21]. High level of MARS1 in breast cancer and NSCLC patients is associated with poor clinical outcomes; in bile duct adenocarcinoma, enhanced expression of MARS1 correlates with cell proliferation, migration, and epithelial-mesenchymal transition [32–34]. At the molecular level, MARS1-dependent stabilization of CDK4 contributes to breast cancer progression [35]. Furthermore, by binding and inhibiting ATR, a DNA repair protein, it reduces DNA damage-induced apoptosis resulting in colorectal cancer cell growth [35]. A somatic frameshift mutation in exon 3 of MARS1 was identified in colorectal and gastric cancer, however a potential pathological role has not yet been shown [36]. Finally, unexpected nuclear localization of MARS1 regulates ribosomal RNA production that can contribute to tumorigenesis [37]. MARS1 expression has potential as a diagnostic marker as dual staining of MARS1 and AIMP2-DX2 (see below) has shown utility for detection of lung cancer [39]. The evolutionary addition of the WHEP domain in both MARS1 and EPRS1, combined with the correlation of both proteins as diagnostic markers in cancer, suggests the domain has potential as a therapeutic target.

Fig. 1. Mammalian MSC Constituents and Structure. (a) Domain organization of the MSC-resident AARSs and auxiliary proteins (AIMPs). Each domain is highlighted in different color. Catalytic domains (CD, dark gray), anticodon binding domains (ABD, light gray), WHEP domains (cyan), lysine-rich N-helical domain (N-helix, orange), GST-like domains (GST, purple), leucine zipper (LZ, cyan), N- and C-terminal domains (N/CTD, light green), vertebrate C-terminal (VC, green), and EMAP-II (red) are indicated., (b) 3-dimensional models of human holo-MSC as obtained from cross-linking mass spectrometry studies. Ribbon (left) and space filled (right) models of MSC are illustrated.
Glutaminyl-tRNA synthetase (QARS1)

Mutations in QARS1, an ~88 kDa protein, are mostly associated with neurological disorders [40], and reports of cancer-assocation are limited. Mass-spectrometric analysis of inactive aggregates of PS3 tumor suppressor, that provides chemoresistance in serous ovarian carcinoma cells, revealed preferential interaction with QARS1 (among other proteins) compared to soluble, active PS3, and thus is predicted to contribute to chemoresistance [41]. Moreover, glutamine-dependent binding of QARS1 inactivates the pro-apoptotic protein ASK1, and thus the synthetase might provide the link between glutamine and its known apoptosis suppressor activity [42]. Information on the role of QARS1 in cancer progression is limited, and more studies will be necessary to elucidate its potential role in cancer pathology.

Leucyl-tRNA synthetase (LARS1)

The ~135 kDa LARS1 has been implicated in migration and proliferation of lung cancer cells [43]. Recent studies reveal a critical role of LARS1 in activation of mTORC1, which is upregulated in several cancers and might present an as-yet-unexplored link between the synthetase and tumorigenesis [44,45]. Recent crystallographic elucidation of the structure of mammalian LARS1 is likely to accelerate the search for low molecular weight inhibitors for potential anti-cancer therapy [51]. Although, LARS1 has been studied extensively, a direct association with cancer progression and studies in primary or patient tissues samples is lacking. Future studies will determine if LARS1, indeed, is involved in cancer pathogenesis.

Arginyl-tRNA synthetase (RARS1)

Marked tumorigenic activity of RARS1 has been reported. About a 2-fold increase in activity of the ~75 kDa RARS1 was shown in malignant rat hepatoma cells characterized by increased growth rate compared to healthy rat liver cells [52]. Reduced expression of microRNAs miR-15a and miR-16-1 in pituitary adenomas, due to regional deletion, increases expression of RARS1, which in turn diminishes extracellular secretion of the tumor suppressor, AIMP1, and accelerates tumor growth [53,54]. The possibility that RARS1 acts as a “sponge” trapping the tumor suppressor AIMP1 is a mechanism that needs further investigation. We and others have shown that RARS1 and AIMP1 interact within the MSC (Fig. 1b) [18,55] however, the possible role of RARS1 in releasing AIMP1 from the MSC has not been investigated. Following chromosomal translocation in nasopharyngeal carcinoma (NPC) fusion of RARS1 with MAD1L1 induces cancer stem cell-like characteristics driving head and neck tumor growth [56,57].

Asparyl-tRNA synthetase (DARS1)

~58 kDa DARS1 was shown to be an oncogene driving progression of the most prevalent type of renal cell carcinoma, namely, clear cell renal cell carcinoma (ccRCC). DARS-AS1 is a long noncoding RNA (lncRNA) positioned near the DARS1 gene [58]. DARS-AS1 upregulates DARS1 by sequestering its inhibitor, miR-194–5p, thereby contributing to malignancy in ccRCC. Likewise, DARS-AS1 is oncogenic in acute myeloid leukemia (AML) where it exhibits a negative correlation with the prognostic factor, miR-425, regulating TGF-β expression [59]. DARS-AS1 also accelerates proliferation of cervical cancer cells via miR-638–5p/JAG1 axis to regulate Notch pathway [60]. Interestingly, single nucleotide polymorphisms (SNPs) in DARS1 are associated with altered asparaginase activity, and might contribute to asparaginase sensitivity in childhood acute lymphoblastic leukemia [61].

Lysyl-tRNA synthetase (KARS1)

~68 kDa KARS1 is highly over-expressed in multiple cancers [62–64]. MSC disassociation and membrane re-localization of KARS1 is associated with dissemination of small groups of cells from colon cancer spheroids; the KARS1/ERKs/paxillin signaling axis is required for this dissemination [65,66]. KARS1 promotes cell-cell and cell-extracellular matrix adhesion required for colon cancer cell migration [67]. Likewise, KARS1 interaction with laminin receptor 67LR prevents ubiquitin-dependent degradation of the receptor and promotes cancer progression [68]. Caspase-8 mediated N-terminus cleavage of KARS1 induces disassociation from the MSC and binding to syntenin which facilitates exosomic secretion, and ultimately macrophage migration and inflammation [69]. In spheroids, KARS1 remodels the tumor microenvironment by inducing M2 macrophage polarization contributing to tumor metastasis [70]. Ser207 phosphorylation of KARS1 by epidermal growth factor receptor (EGFR), primarily in NSCLC patients with EGFR mutations, induces nuclear translocation of KARS1, and is associated with improved mean disease-free survival [71]. In melanoma cells free KARS1 can generate diadenosine tetraphosphate, i.e., Ap4A, which binds HINT1, thereby disrupting its interaction with microphthalmia transcription factor (MITF), resulting in MITF-dependent transcription and oncogenic activity [72,73]. Furthermore, KARS1 SNPs are associated with DNA-damage response and increased cancer occurrence [74,75]. The abundant evidence showing pleiotropic activities of post-translationally modified KARS1 that influence multiple cancer progression pathways, suggests it is an important MSC AARS involved in carcinogenesis, and a likely target for therapeutic intervention.

Aminoacyl-tRNA synthase complex-interacting protein 1 (AIMP1)

AIMP1 is an ~34 kDa protein with tumor suppressor activity that distinguishes it from other MSC constituents. Purified AIMP1 has potent anti-tumor activity as shown in mouse xenograft models, and inhibition of cell cycle progression in gastric cancer-bearing mice [76,77]. AIMP1 administration increases expression of the potent tumor-suppressing cytokines TNF-α and IL-1β [77]. With a sub-clinical dose of paclitaxel, AIMP1 combination therapy reduces growth of stomach cancer cells and lung cancer xenografts [76]. The tumor suppressor activity of AIMP1 is supported by its reduced expression in gastric and colorectal cancer tissues compared to adjacent normal tissue, as well as under hypoxic conditions where its high expression is associated with diminished risk of glioblastoma [78–80]. In pituitary adenomas AIMP1 regulates AIMP1 secretion where it exerts potent anti-tumor activity [54]. Proteolytic cleavage of AIMP1 generates the multi-functional inflammatory cyto- kine endothelial monocyte-activating polypeptide II (EMAP II) [54] (discussed in detail below). AIMP1 has been associated with poor survival in patients with head and neck squamous cell carcinoma [81]. In laryngeal squamous cell carcinoma AIMP1, in cooperation with leukotriene A4 hydrolase (LTA4H), promotes cell proliferation, migration, and invasion by binding fascin actin-bundling protein 1 [82]. As both full-length AIMP1 and its cleaved product EMAP II exhibit anti-cancer activities, strategies have been developed to improve the stability of the recombinant proteins, primarily by reducing aggregation [83]. AIMP1 influences diverse immune response. It negatively regulates the expansion of myeloid-derived suppressor cells (MDSCs) involved in immune suppression in breast tumor-bearing mice, and reduces their differentiation and induction of Treg cell differentiation [84]. Alternatively, AIMP1 stimulates anti-tumorigenic NK cells in a TNF-α-dependent manner, and TH1 polarization by regulating cytokine and costimulatory molecule expression as well as p38 MAPK signaling [85,86]. It stimulates antigen-presenting cells to secrete IL-12 by increasing NF-κB occupancy on the IL-12 promoter leading to enhanced TH1 responses [87]. In fact, anti-CD3 single-chain Fv fusion with AIMP1 serves as a DNA adjuvant enhancing TH1 cell-mediated immune response [88].
Variants of MSC constituents and their role in cancer progression

Endothelial-monocyte-activating polypeptide-II (EMAP-II)

EMAP-II is a 22 kDa proteolytically-cleaved fragment of AIMP1, first purified from conditioned medium of methycholeanthrene A-induced murine fibrosarcoma cells, and exhibiting endothelial cell (EC)-activating and monocyte pro-migratory properties [101,102]. EMAP-II overexpression renders TNF (tumor necrosis factor)-α-resistant soft tissue sarcoma sensitive to TNFa therapy, and is dependent on TNF-receptor-1 relocalization from Golgi storage pools to the cell plasma membrane [103-105]. In prostate adenocarcinoma cells, chemotherapeutic agents induce EMAP-II expression, thereby potentiating localized activation of host cell effector mechanisms, as well as anti-angiogenic and pro-apoptotic activities in melanoma lines [106,107]. EMAP-II binds the EC α5β1 receptor directing its internalization and interaction with PSM7, a component of the proteasome degradation pathway that induces HIF-1α degradation [108]. Tumor-bearing mice treated with a combination of EMAP-II and photodynamic therapy (PDT), in which photochemical generation of cytotoxic singlet oxygen leads to localized tumor destruction, significantly reduces tumorigenesis compared to PDT alone [109]. Elevated EMAP-II expression correlates with improved survival of glioblastoma patients, and following trans-retinoic acid treatment of squamous carcinoma cells, is thought to be a mechanism of retinoid chemoprevention [110,111]. Recombinant EMAP-II therapy in mice retards tumor growth by targeting vascular endothelium, and possibly inducing cytoskeletal remodeling [112,113]. EMAP-II increases the blood-tumor barrier permeability in glioblastoma, and the effectiveness of chemotherapy, by interacting with the ATP synthase a subunit and altering tight junction complexes between brain EC [114,115]. In glioblastoma stem cells, EMAP-II regulates G2/M cell cycle arrest by inhibiting simultaneous activation of the PI3K/Akt pathway activation and FoxO1 [116,117]. Also in glioblastoma stem cells, combination therapy of EMAP-II with temozolomide upregulates miR-590–3p and represses the PI3K/AKT/mTOR signaling pathway [118]. In primary uveal melanoma, expression of EMAP-II correlates with enhanced expression of EC ICAM-1 which causes macrophage activation and localized vascular damage [119]. In certain situations, for example in colorectal cancer, EMAP-II exhibits tumor-protective activity, facilitating tumor evasion of immune surveillance by inducing apoptosis in activated lymphocytes and ECs [120-122]. In addition, hypoxia facilitates EMAP-II-dependent lymphocyte killing and immune evasion [123].

AIMP1 and its proteolytic cleavage product EMAP-II both exhibit anti-tumorigenic properties; however, there are substantial differences between their functions. AIMP1, unlike EMAP-II, upregulates pro-inflammatory chemokines, e.g., IL-8, MIP-1, MIP-2α, MCP-1, and IL-8, among others [124]. EMAP-II is secreted from apoptotic or necrotic cells following caspase-mediated cleavage [125], possibly suggesting a desperate effort by host cells to defend against cancerous cells.

AIMP2-DX2

AIMP2-DX2, a splicing variant of AIMP2 lacking exon 2, is pathologically associated with cancer and is induced by carcinogen treatment, in contrast to the tumor suppressive activity of the parent AIMP2 [126]. For example, AIMP2-DX2 overexpression is associated with increased proliferation, migration, and invasion of nasopharyngeal carcinoma cells [127]. Autoantibodies against AIMP2-DX2 in sera of lung cancer patients is associated with poor outcomes [128]. In contrast, down-regulation of AIMP2-DX2 inhibits chemo-resistant ovarian and lung cancer cell growth [129,130]. In addition to influencing solid tumor growth, the ratio of AIMP2-DX2/AIMP2 expression correlates with poor prognosis in hematological cancers [131]. With respect to molecular mechanism, AIMP2-DX2 fails to associate with the MSC, and competitively binds p53 to interfere with pro-apoptotic activities of AIMP2 [132]. Its interaction with HSP70 increases cell transformation and chemical intervention suppresses cancer cell growth in vitro and in vivo [132]. Likewise, targeted degradation by ribozymes efficiently reduces cancer cell growth [133]. AIMP2-DX2 knockdown in mice reduces lung cancer progression by inhibiting the
AKT1-mTOR-p70S6K1 signaling [134]. AIMP2-DX2 is an example of how tumor cells take advantage of host cellular machinery to generate novel proteins supporting their own survival.

**Threonyl t-RNA synthetase-like protein 2 (TARS2)**

TARS2 is the result of a gene duplication of TARS1 bearing a novel N-terminal extension that directs inclusion in the MSC [135,136]. Little is known about the variant, but it has been observed in pediatric brain tumor cells [137]. Moreover, pathway analysis revealed an association of TARS2 with poor bladder cancer outcome [138]. As a newly-identified MSC constituent, more studies are required to elucidate its tumor-related function.

**Function of the MSC**

Despite extensive investigation for several decades, the function of the MSC remains uncertain [139,140]. Inclusion in the MSC can contribute to stability of constituents [141]. Interaction of the MSC with ribosomes has been proposed to enhance translation efficiency due to direct channeling of charged tRNA’s to the emerging polypeptide chain [142,143]. However, MSC association does not appear to regulate catalytic activity of constituent tRNA synthetases, and thus alternative hypotheses have been suggested [14]. Appendage of domains during evolution that are absent in prokaryotic AARSs can facilitate protein-protein interactions, particularly with other MSC constituents, but also can dictate non-canonical (i.e., moonlighting) functions unrelated to their primary function in interpretation of the genetic code [19, 144,145]. MSC has been proposed to be a “depot” that sequesters constituents that in free form might exhibit detrimental activities [14,15]. In contrast, MSC constituents released in a stimulus-dependent manner can exhibit condition-specific, beneficial functions; because release does not require mRNA or protein synthesis, the mechanism has the dual efficiencies of speed as well as minimal energy requirement.

**MSC: Pro- or anti-tumorigenic?**

As multiple AARSs are upregulated in cancer cells, it is tempting to speculate it as a pro-tumorigenic consequence of rapid cell-division requiring highly active translational machinery. However, upregulation of only selected AARSs in specific cancers, while maintaining basal expression in others, argues against this mechanism. The correlation between MSC release of AARSs (discussed below) and their pro-tumorigenic properties also suggests cancer-causative activity. The acquisition of appended domains allows the MSC-associated AARSs to perform both cancer-causing functions and enhanced protein synthesis, which together can stimulate tumor growth.

Contrary to the MSC-resident AARSs, the auxiliary proteins AIMP1–3 primarily exhibit anti-tumorigenic functions. These conflicting observations ambiugate the overall ex-translational role of the MSC. The AIMPs lack enzymatic activities and they are generally considered to be scaffolding proteins involved in the assembly and structure of the large MSCs found in higher eukaryotes, but not in prokaryotes bearing much smaller MSCs [10]. Nonetheless, the AIMPs participate in multiple protein-protein interactions, primarily with appended domains of MSC-resident AARSs [18]. The important role of AIMPs in AARS sequestration within the MSC has been shown in studies in which AIMP knockdown leads to AARS release [141]. Thus, AIMPs exhibit dual anti-tumorigenic functions — exerted both as extra-MSC activities and by preventing release of pro-tumorigenic AARSs from the MSC. Thus, the pro- or anti-tumorigenic activity of the MSC is likely to be stimulus- and condition-dependent, with the overall activity determined by the specific constituents released.

Apart from the nine MSC-associated AARSs, the eleven extra-MSC AARSs are associated with several diseases including cancer [4,146]. Similar to MSC-associated AARSs, many extra-MSC AARSs also acquired non-catalytic, appended domains during evolution, provoking a question: why are these AARSs not incorporated in the MSC or MSC-like multi-protein complexes? Many of these extra-MSC AARSs are secretory in nature and have anti-tumorigenic properties contrary to the MSC-associated AARSs [147-153]. Extra-MSC localization might allow for more rapid or efficient transport across the plasma membrane. Also, sequestration of MSC-resident AARSs might reduce pro-tumorigenic activities, unlike the anti-tumorigenic activities of extra-MSC AARSs. It is possible that during evolution, when the AARSs were gaining additional domains, the auxiliary proteins selectively sequestered the pro-tumorigenic/deleterious AARSs into a multi-protein MSC, thereby regulating their non-canonical functions without affecting their canonical function in translation [16].

**Factors determining constituent release from the MSC**

**Post-translational modifications (PTMs)**

Phosphorylation is the principal PTM that drives release of individual components from the MSC (Fig. 2). In the first known example, IFN-γ activation of macrophages causes Cdks- and S6K1-dependent dual phosphorylation of human EPRS1 at Ser886 and Ser999, thereby stimulating its release from the MSC [27,154]. Cdks and S6K1 are upregulated in multiple cancers, and might contribute to the role of EPRS1 in tumorigenesis [155,156]. Insulin stimulates phosphorylation of EPRS1 in adipocytes, but its connection to cancer has not been explored [157]. Likewise, EPRS1 Ser999 phosphorylation following viral infection induces release from the MSC to inhibit viral replication, but the role of this modification in cancer is unknown [158]. Ser662 phosphorylation of MARS1 by Gcn2 following UV irradiation induces a conformational change that causes AIMP3 release from the MSC; subsequent interaction with p53 in the nucleus induces tumor suppressive activities (Fig. 2) [159]. In contrast, MARS1 phosphorylation by ERK1/2 at Ser209 and Ser825 does not induce MSC release, but causes mis-acylation, a phenomenon associated with cancer progression [160]. Proteomic analysis of DARS1 identified phosphorylation sites at Tyr239 and Ser249 and six acetylation sites at Lys55, 110, 213, 241, 330, and 453, although their impact on localization is unknown [161]. Likewise, structural studies identified DARS1 Ser146 phosphorylation in human cells, but the proposed role in MSC release has not been validated [162]. Ser207 phosphorylation of KARS1 induces release from the MSC and relocation to the nucleus where it binds and activates microphthalmia-associated transcription factor (MITF), a protein implicated in melanoma (Fig. 2) [163,164]. EGFR-mediated Ser207 phosphorylation of KARS1 in lung cancer cells and in NSCLC patients has been described, and interestingly, phospho-KARS1 immunostaining is associated with better prognosis in these patients [71]. Thr52 phosphorylation causes KARS1 re-localization from the MSC to the plasma membrane where it inhibits ubiquitin-dependent degradation of the 67-kDa laminin receptor, thereby enhancing cell migration [68]. DNA damage-mediated phosphorylation of AIMP2 by JNK causing re-localization from the MSC to the nucleus where it binds and stabilizes p53 [165]. Similarly, Ser156 phosphorylation of AIMP2 by p38 MAPK in HeLa cells causes release from the MSC and anti-tumorigenic activity following interaction with nuclear Smurf2 (Fig. 2) [93]. Likewise, AIMP3 phosphorylation by ERK is linked to anti-tumorigenic activities, however, its release from the MSC remains to be explored [96]. Although phosphorylation is the principal known PTM inducing MSC constituent release, modifications yet to be determined are worthy of consideration in future studies.

**Protease-mediated cleavage**

Biochemical studies have shown that controlled proteolysis can generate active mammalian AARS proteoforms (Fig. 2) [142]. Yeast two-hybrid screen showed calpain-2 interacts not only with LARS1, but also cleaves other MSC constituents including EPRS1, MARS1, DARS1,
QARS1, RARS1, and AIMP1, resulting in generation of fragments that escape the MSC (Fig. 2) [166]. Similarly, caspase 7-mediated cleavage releases the EMAP-II domain of AIMP1 that stimulates migration of mononuclear phagocytes [167]. AIMP1 cleavage by elastase in apoptotic U937 cells generates p43-derived apoptosis release factor [168]. N-terminal truncation of QARS1 by calpain prevents MSC incorporation and directs nuclear localization [166]. Caspase-8-mediated cleavage of the N-terminus of KARS1 induces release from the MSC, secretion via exosomes, and inflammation (Fig. 2) [69]. Caspase-mediated cleavage at EPRS1 Asp929 in the third WHEP domain generates neoEPRS1, an N-terminal proteoform of unknown in vivo function [169].

**Single nucleotide polymorphisms/point mutations in MSC constituents**

Multiple single amino acid changes in EPRS1, LARS1, DARS1, IARS1, and MARS1 have been reported but MSC localization and their potential roles in cancer remain to be explored [170–173]. Interestingly, several mutations in DARS1 (Arg460His, Pro464Leu) that cause hypomyelination with brain stem and spinal cord involvement and leg spasticity were found that might induce release from the MSC [172]. In the case of QARS1, R403W mutation causes neurodegenerative disease; however, localization of the mutant remains to be explored [40]. D2G mutation in the RARS1 N-terminus releases it from the MSC and causes Pelizaeus-Merzbacher disease (Fig. 2) [174]. In KARS1, P200L and P263V mutations reduce its affinity for AIMP2 and the MSC, and causes optic neuropathy (Fig. 2) [175]. The KARS1 P200L mutation site is close to Ser207, the residue that when phosphorylated causes release from MSC, consistent with a role of local structure in MSC retention. Other neurological disease-causing KARS1 mutations including Phe127Leu and Pro297Ala, reduce dimerization; as KARS1 is present as a dimer in the MSC, reduced dimerization might influence its localization [176]. Mutations in AIMPs 1 and 2 have been observed in neurodegenerative diseases, whereas multiple point mutations in AIMP3 (Thr35/Ser40, Thr76, Thr80, Ser87, Val106 and Arg144) are seen in chronic myeloid leukemia patients. Few of these mutations reduce the affinity of AIMP3 for ATM but nor for MARS1, but their altered function or localization has not been investigated [177–179]. Most AARS mutations mentioned here have been associated with non-cancer pathologies. The adaptation of whole exome sequencing likely will facilitate identification of additional AARS mutations, and their potential association with cancer incidence and progression. Whether the mutations discussed here predispose patient’s cancer is not known, and cell-based studies where similar mutation are introduced, for example by CRISPR/Cas9 technology, will
clarify MSC localization of these mutant proteins along with additional functions, and further illuminate the role of MSC release of constituents on tumorigenic functions.

Role of MSC structure in constituent release

The assembly and structure of the MSC can provide important clues to mechanisms of constituent release. A collection of sub-complexes has been revealed by biochemical and structural studies, showing key interactions, but the role of these complexes in MSC assembly is unclear [21,55,180]. According to a recent 3-dimensional model, all of the constituents have substantial surface exposure [18]. The structure is organized in a way that many constituents have limited interactions with just a few neighbors, possibly facilitating release. Post-translational modification, most often phosphorylation can alter the structure of a constituent or interfere with a critical binding domain interface. The latter is exemplified by phosphorylation of KARS1 in the domain that interacts with the N-terminus of AIMP2, thereby inducing a steric clash, release from the MSC, and subsequent entry into the nucleus [163]. In nearly all cases only a small fraction of a constituent is released. It is unclear why only a small amount is released as artificial exclusion of the entire pool of a constituent from the MSC does not alter protein synthesis efficiency [16]. Possibly the signaling pathway inducing the post-translational modification is limiting or transitory. EPRS1 release is an exception to this principle since at least half is released from the MSC upon stimulus-dependent phosphorylation[27,157]. In either case, the amount of a protein released post-modification is sufficient to trigger robust cellular activities, including effecting cancer progression.

AARSs and immune regulation: a potential link to cancer development and progression

Immune dysregulation and inflammation are major mechanisms influencing cancer incidence, progression, and dissemination, and chronic inflammation due to overactive immune cells is associated with an increased risk of cancer malignancy [181,182]. Tumor-associated macrophages in particular have essential roles in tumor progression [183]. AARSs are influenced by diverse immunological conditions and stresses within the tumor microenvironment [184]. For example, TNF-α-induced secretion of KARS1 binds macrophages, feeding forward to exacerbate TNF-α secretion thus amplifying the inflammatory state [185]. Similarly, AIMP1 induces macrophase expression of multiple inflammatory cytokines in a MAPK and NF-κB-dependent fashion [124]. CD23, a cell-surface receptor for AIMP1 further induces TNF-α secretion [186]. EMAPII, the secreted proteolytic product of AIMP1, induces an acute inflammatory response and recruit macrophages [187]. The high affinity of T-cell receptor (TCR) for microbial peptide:MHC class II complex upregulates AIMP3 and IL-2R proteins, promoting Th1 cell differentiation and inflammation [188]. EPRS1, upregulated in multiple cancers, exhibits an anti-inflammatory function in which it participates in translational silencing of inflammation-related agents in myeloid cells, for example VEGFA [27,29]. EPRS1 activates TGF-β/STAT signaling, and TGF-β induces Th17 cell differentiation a principal effectors of cancer progression [189–191]. Likewise, halofuginone, a potent inhibitor of the prolyl-tRNA synthetase activity of EPRS1, suppresses inflammatory response by inhibiting Th17 cell differentiation [192]. Inhibition of MARS1 suppresses Drosophila inflammatory and immune response genes and mutations in DARS1 causes leukocyte- phalopathy along with spinal cord abnormalities mimicking neuro-inflammatory disease [172,193]. Homozygous mutations in IARS1 cause very early-onset inflammatory bowel disease associated with T-cell dysfunction [194]. Many AARSs likely have multi-facted roles, alone or in concert with other proteins, driving cancer progression where it can function as a proto-oncogene by driving specific signaling pathways, and can also dysregulate the immune environment that by itself can promote cancer.

Anti-cancer therapeutics targeting AARSs

MSC AARSs can stimulate initiation and progression of multiple cancers, and thus therapeutics targeting these AARSs are under development [5]. These drugs can target either AARSs catalytic activity within the tumor or undesirable protein-protein interactions that drive tumorigenesis. Halofuginone, an inhibitor of the prolyl-tRNA synthetase catalytic activity of EPRS1 [195] inhibits breast and prostate cancer metastasis by reducing TGF-β and BMP-regulated pathways, and sensitizes lung cancer for chemotherapy by suppressing NRF2 accumulation [196,197]. Although, a major mechanism of halofuginone function is inhibition of collagen biosynthesis, it also activates the amino acid starvation response in cancer cells due to its inhibition of prolyl-tRNA synthetase activity [195,198,199,200]. As EPRS1 is over-expressed in many cancers, inhibition of prolyl-tRNA synthetase activity by halofuginone might contribute to its tumor-suppressive activity; however, a direct link between halofuginone-mediated inhibition of EPRS1 and reduction in cancer cell growth has not been shown. Xanthoangelol and 4-hydroxycerin bind EPRS1 and prevent its interaction with SCYL2, thereby abrogating the WNT/GSK-3/β-catenin signaling pathway and reducing patient-derived xenograft tumor growth [26]. Small molecules derived from quinoline, 1,3-benzoxazine, and 1,3-oxazine compete with methionine and adenosine binding sites in MARS1, and are effective in suppressing AF59 and HCT116 cell proliferation [201]. Inhibitors of LARS1, e.g., AN2690 and its derivatives, exhibit anti-tumor activity in U2OS and SKOV3 cells by activating p21-mediated cell apoptosis [202]. Similarly, the LARS1 inhibitor BC-LI-0186 at nanomolar concentration exhibits potent anti-tumor activity against lung cancer cells [203]. Recent studies highlight the role of LARS1 in mTORC1 activation and competitive inhibitors of LARS1, e.g., leucinol and leucyladenylate sulfate derivatives, prevent leucine-mediated activation of mTORC1 [204–209]. Similarly, BC-LI-0186 prevents LARS1 lysosomal localization and mTORC1 activation by blocking RagD-LARS1 interaction [51]. Small molecule inhibitors against KARS1, e.g., SL-1910, reduce migration of MDA-MB-231 cells, whereas BC-K_YH16899 inhibits the interaction of KARS1 and 67 kDa laminin receptor, thereby suppressing membrane localization in cells and metastasis in mouse models [210,211]. Inhibition of caspase-8-mediated cleavage of KARS1 abrogates its secretion via exosomes and reduces inflammation [69]. Thiazolo[5,4-b]pyridines (SL-1910) is a KARS1 inhibitor that reduces cell migration and prevents tumor metastasis in mice [210]. L-canaavanine is a structural analog of L-arginine that as an RARS1 substrate is competitively incorporated into nascent proteins. L-canaavananyl-containing proteins are less basic than wild-type, thereby influencing essential protein structures and ionic interactions, resulting in cell death. Canavanine is specifically sequestered by the pancreas, and is predicted to be useful as an adjuvant therapy for pancreatic cancer [212]. Small molecule inhibitors targeting AIMP2-DX2 mRNA, e.g., BC-DX101, degrade the transcript and in reduce AIMP2-DX2 level and tumor size in mice [213]. In lung carcinogenesis, AIMP2-DX2 induction by oncogenes promotes drug resistance by binding and inhibiting p14/ARF. SLCB050, blocks the interaction between AIMP2-DX2 induction by oncogenes promotes drug resistance by binding and inhibiting p14/ARF. SLCB050, blocks the interaction between AIMP2-DX2 and p14/ARF in vitro, and in vivo reduces the viability of small cell lung cancer cells in a mouse model of K-ras-driven lung tumorigenesis [214]. BC-DX1-843 is a sulfonamide-based drug that selectively inhibits AIMP2-DX2 binding to HSP70 resulting in its selective Siah-1-mediated ubiquitination and degradation in lung cancer cells [126,215]. Pyrimethamine is an anti-parasite drug that also induces selective, ubiquitin-mediated degradation of AIMP2-DX2, and suggests potential repurposing as an anti-tumor therapeutic agent [216]. As the association of AARSs with specific cancer types becomes stronger, investigation of existing inhibitors and generation of novel therapeutics is likely to accelerate. Several currently available inhibitors mentioned here are currently in clinical trials for cancer and other disorders [13]. However, much more basic research and translational development will be necessary to establish the potential of AARSs as therapeutic targets.
Concluding remarks

MSC constituents, particularly the AARSs, are essential for cell viability under normal conditions due to their principal function in interpretation of the genetic code. However, it is becoming evident that the same constituents can induce pathology, including cancer progression. Recent appreciation of MSC structure and dynamics is contributing to our understanding of the mechanisms of activation of MSC constituents, and their potential to exhibit non-canonical moonlighting functions. Early and ongoing studies targeting MSC components show promising anti-tumor outcomes. Continued investigation will be necessary to understand the repertoire of functions that the MSC and its constituents perform, elucidate mechanisms of activation, and develop novel therapeutics that inhibit its constituent release, activity, or protein-protein interactions, and ultimately inhibit tumor initiation and progression.

Author contribution statement

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