Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping

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Abstract | Over the past decade, the identification of cancer-associated factors has been a subject of primary interest not only for understanding the basic mechanisms of tumorigenesis but also for discovering the associated therapeutic targets. However, aminoacyl-tRNA synthetases (ARSs) have been overlooked, mostly because many assumed that they were simply 'housekeepers' that were involved in protein synthesis. Mammalian ARSs have evolved many additional domains that are not necessarily linked to their catalytic activities. With these domains, they interact with diverse regulatory factors. In addition, the expression of some ARSs is dynamically changed depending on various cellular types and stresses. This Analysis article addresses the potential pathophysiological implications of ARSs in tumorigenesis.

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doi:10.1038/nrc3124

Mammalian cells contain a battery of cytoplasmic and mitochondrial aminoacyl-tRNA synthetases (ARSs) that are in charge of cellular protein synthesis. They catalyse the ligation of amino acids to their cognate tRNAs with a high fidelity. As the molecular adaptors that translate mRNA to protein, ARSs exist in the most primitive prokaryotes and are thus both ancient in evolutionary terms and of interest to biologists who are creating synthetic organisms. The ligation of substrate amino acids to their cognate tRNAs proceeds in two steps (FIG. 1). The first reaction involves the activation of amino acids to aminoacyladenylate, consuming one molecule of ATP, and the second delivers activated amino acids to the acceptor end of tRNAs. The fidelity of this reaction depends on the accurate recognition of substrate amino acids and congate tRNAs by ARSs and is further enhanced by a proofreading activity that prevents the ligation of the wrong amino acids to the wrong tRNA. Proofreading can take place before the transfer of aminoacyl-AMP to tRNAs (FIG. 1a) or after the ligation of the amino acid to the acceptor end of tRNAs (FIG. 1b).

Mammalian ARSs carry out the same catalytic function as their prokaryotic counterparts but they contain other domains in addition to their catalytic domains, such as glutathione *S*-transferase (GST), WHEP domains, leucine zipper domains and α-helical appendices[1], which confer the ability to form diverse complexes with each other or with other cellular regulatory factors. This structural complexity seems to be linked to a functional versatility that has only recently been uncovered, and which implicates ARSs in a variety of human diseases[2,3] (BOX 1). It seems that the aberrant expression, mutation and variant formation of ARSs can cause pathologies through multiple routes. For example, a reduction in fidelity can result in the incorporation of erroneous amino acids into nascent polypeptides, which can have substantial consequences depending on the function of the mutated proteins. Even with their catalytic function intact, aberrant cellular localization and molecular interactions of ARSs can disturb normal cell regulatory networks as a result of their non-canonical functions in angiogenesis, immune responses, transcriptional and translational control, and signal transduction. Such disruption of the cellular proteome and signalling pathways can directly cause tumorigenesis or can influence cellular susceptibility to pro-tumorigenic stresses. Despite their potential roles in the control of tumorigenesis, the pathophysiological implications of these enzymes for cancer are not well understood and have not been systematically investigated. In this Analysis article we introduce the potential association of ARSs with cancer from the data obtained from *in vitro*, *in vivo* and systematic *in silico* studies.

ARSs and global translation

The catalytic activities of ARSs can influence both the rate of and the accuracy of protein synthesis. Although some ARSs show cancer-associated overexpression[4,5], it is not clear whether this increase results from the increased demand for protein synthesis in cancer cells or whether

At a glance

- Aminoacyl-tRNA synthetases (ARSs) are 'housekeeping' proteins that are involved in protein translation. They catalyse the ligation of amino acids to their cognate tRNAs with a high fidelity. Mammalian members of this family have additional domains that enable them to interact with various proteins, some of which are implicated in tumorigenesis.
- Eight ARSs form a macromolecular protein complex with three auxiliary factors, designated ARS-interacting multifunctional protein 1 (AIMP1), AIMP2 and AIMP3. This complex is known as the multisynthatase complex (MSC).
- On genotoxic damage, AIMP2 and AIMP3 are translocated to the nucleus where AIMP2 activates p53 directly and AIMP3 activates p53 through the activation of the kinases ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR).
- AIMP2 augments the apoptotic signal of tumour necrosis factor (TNF) through the downregulation of TNF receptor associated factor 2 (TRAF2) and mediates the transforming growth factor-β anti-proliferative signal through the downregulation of fuse-binding protein (FBP). A splice variant of AIMP2, AIMP2-DX2, compromises the tumour suppressive activity of AIMP2 and can induce tumorigenesis.
- Among the ARSs that form the MSC, bifunctional glutamyl-prolyl-tRNA synthetase (EPRS) can function as a translational silencer to suppress the generation of vascular endothelial growth factor A. Lysyl-tRNA synthetase (KRS) can translocate to the nucleus to bind microphthalmia-associated transcription factor, which is an oncogenic transcriptional activator that is implicated in the development of melanoma. KRS is also secreted and induces the production of TNF from macrophages. Glutaminyl-tRNA synthetase (QRS) can interact with apoptosis signal-regulating kinase 1 to suppress apoptotic signals in a glutamine-dependent manner and MRS can increase ribosomal RNA biogenesis in the nucleoli.
- Among free-form ARSs, tryptophanyl-tRNA synthetase (WRS) is secreted, and the truncation of the amino-terminal peptide generates an active cytokine that suppresses angiogenesis. Tyrosyl-tRNA synthetase (YRS) is also secreted and cleaved into N- and C-domains that have pro-angiogenic and immune activation functions, respectively. The C-terminal domain of human YRS is homologous to endothelial-monocyte-activating polypeptide II (EMAPII), which is the C-terminal domain of AIMP1. This functions as an immune-stimulating cytokine that is crucial for the chemotaxis of mononuclear phagocytes and polymorphonuclear leukocytes, and the production of TNF, tissue factor and myeloperoxidase.
- A systematic analysis of the expression of ARSs and AIMPs (ARSN) indicates that these proteins are associated with cancer, and a network model identifies some of the links between ARSN and 123 first neighbour cancer-associated genes.

ARSs are drivers of cell transformation. In the case of methionyl-tRNA synthetase (MRS), an increase in catalytic activity was reported in human colon cancer[6], and stable overexpression of the MRS substrate $tRNA_i^{Met}$ can cause oncogenic transformation[7]. Overexpression of MRS was also reported in several different types of cancers, such as malignant fibrous histiocytomas, sarcomas, malignant gliomas and glioblastomas[8–11]. Interestingly, these tumours have amplification of the chromosome 12q13 locus, where the gene for MRS resides and overlaps with the gene for C/EBP homologous protein transcription factor (CHOP; also known as GADD153 and DDIT3), which functions as an inhibitor of C/EBP[12]. This amplification probably results in the overexpression of MRS and CHOP, which may promote a favourable milieu for tumour progression. *MRS* and *CHOP* share a 56 bp tail-to-tail complementary sequence at their 3′ untranslated region (UTR), which contains an AU-rich regulatory element (ARE) that is responsible for the destabilization of mRNA[13]. The association between *CHOP* and *MRS* mRNAs could increase the stability of the CHOP transcript[14]. This idea is supported by data showing that a luciferase reporter expression construct containing the full-length 3′ UTR of *CHOP* shows increased expression after deletion of a 55 bp overlap sequence with the MRS transcript. Recently, a somatic frameshift mutation in exon 3 of MRS was also reported in colorectal and gastric cancers[15]. This mutation in the T9 repeat sequence results in a premature stop codon with the loss of the entire catalytic domains of MRS. It is yet to be determined whether (or how) this variant of MRS is implicated in tumorigenesis.

The quality control of ARS-mediated catalysis might also be linked to tumorigenesis, as misfolded or unfolded proteins can trigger various pathologies[16–18]. It is worth noting that mice expressing an editing-defective mutant of alanyl-tRNA synthetase (AlaRS) showed loss of Purkinje cells and ataxia resulting from the accumulation of misfolded proteins[19]. Although the potential importance of canonical ARS function in tumorigenesis needs further investigation, the additional functions that have been identified in mammalian ARSs over recent years are more indicative of a role in tumorigenesis.

AIMPs

In mammalians, eight different ARSs (bifunctional glutamyl-prolyl-tRNA synthetase (EPRS), isoleucyl-tRNA synthetase (IRS), leucyl-tRNA synthetase (LRS), glutaminyl-tRNA synthetase (QRS), lysyl-tRNA synthetase (KRS), arginyl-tRNA synthetase (RRS), aspartyl-tRNA synthetase (DRS) and MRS) form a complex with three non-enzymatic factors, which is known as ARS-interacting multifunctional protein 1 (AIMP1; also known as p43), AIMP2 (also known as p38 and JTV1)[20] and AIMP3 (also known as p18)[21,22] (FIG. 2). This multisynthetase complex (MSC) serves as a molecular reservoir that harbours component enzymes and cofactors until they are dispatched to the target sites whenever necessary[23]. AIMP1–3 provide scaffolding roles in the assembly and integrity of the MSC[21,24–26]. However, these three factors are also translocated to other cellular compartments and are involved in various regulatory pathways[22].

Human AIMP1 is comprised of 312 amino acids and specifically binds to RRS within the MSC[27]. The function of AIMP1 in tumorigenesis is mainly tied to its function as a cytokine (discussed below). The gene encoding AIMP2 is located in chromosome 7p22 and overlaps with the 5′ region of the gene encoding post-meiotic segregation increased 2 (PMS2), which is a mismatch repair endonuclease that is associated with hereditary nonpolyposis colorectal cancer[28]. Within the MSC, AIMP2 is tightly bound to KRS[24,29] and AIMP1 through its leucine zipper, which is located in its amino-terminal region[30]. Initial insights into the function of AIMP2 came from the phenotypic investigation of mice lacking AIMP2. These mice died neonatally, mainly owing to the severe overproliferation of epithelial cells in the lung alveoli[31]. AIMP2 promotes the growth-arresting function of transforming growth factor-β (TGFβ) through ubiquitin-mediated degradation of far upstream sequence element (FUSE)-binding protein (FBP), which is a transcriptional activator of the proto-oncogene *MYC*[32]. FUSE is located around 1.5 kb upstream of the *MYC* promoter[32,33] and attracts FBP, which is required for the activation of *MYC*

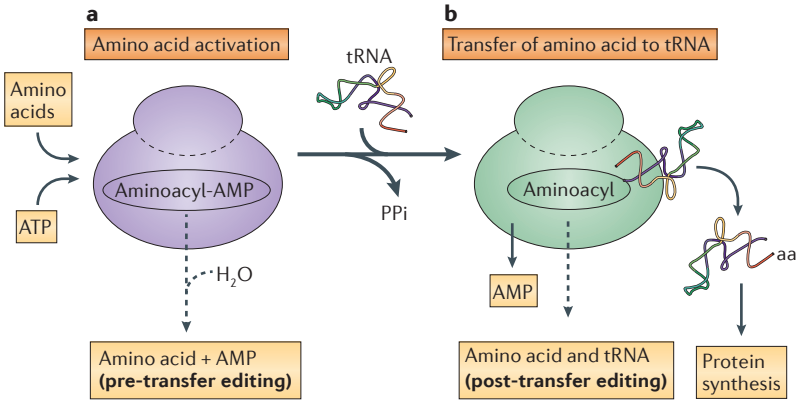

Figure 1 | **Catalytic activity of ARSs.** The reaction proceeds in two steps. In the first step, the substrate amino acids are activated consuming ATP (part **a**), and in the second step, the reaction intermediate, aminoacyladenylate (aminoacyl-AMP), is transferred to the acceptor end of tRNAs (part **b**). The enzymes are highly specific in substrate recognition because misrecognition of substrate amino acids or congate tRNAs will incorporate the wrong amino acids into polypeptides. The fidelity of aminoacylation is further enhanced by proofreading that can take place before and/or after the transfer of activated amino acids to tRNAs (pre-transfer and post-transfer editing)[111]. Some aminoacyl-tRNA synthetases (ARSs) can generate diadenosine polyphosphate[112] as the secondary catalytic reaction[113]. aa, amino acids.

expression. MYC is required for lung cell proliferation during early development but must be downregulated for lung epithelial cell differentiation. TGFβ provides a signal for AIMP2 to dissociate from MSC and to bind FBP. This results in the ubiquitylation-mediated degradation of FBP. AIMP2 was also found to function as a pro-apoptotic mediator in response to tumour necrosis factor (TNF) through TNF receptor-associated factor 2 (TRAF2)[34]. Similar to its interaction with FBP, AIMP2 binds to TRAF2 after a TNF signal and induces the ubiquitylation of TRAF2 through the E3 ligase IAP1 (also known as a BIRC2)[35]. In response to DNA damage, AIMP2 is translocated to the nucleus and binds the tumour suppressor p53 (REF. 36). In this case, AIMP2 binding prevents p53 from ubiquitylation by MDM2 (REF. 37). Together, these data indicate that AIMP2 seems to work as both a pro-apoptotic and an anti-proliferative factor through the regulation of ubiquitylation. In contrast to *Aimp2*-homozygous mice, *Aimp2*-heterozygous mice are born alive but show increased susceptibility to tumour development when they are exposed to lung, colon and skin carcinogens[38], suggesting that the tumour suppressive activities of AIMP2 at molecular and cellular levels are also evident *in vivo*.

The gene encoding AIMP2 consists of four exons and is subject to alternative splicing. The variant lacking exon 2 (AIMP2-DX2) is highly expressed in lung cancer and has been shown to compromise the tumour suppressive activity of AIMP2 (REF. 39). Although this variant has lost the ability to associate in the MSC, it can still bind p53 and it inhibits the tumour suppressive interaction of full-length AIMP2 with p53. When AIMP2-DX2 expression is increased by exogenous expression, it can induce anchorage-independent cell growth, which is a signature of transformed cells. In addition, treatment with the lung carcinogen benzopyrene induces larger tumours with an increased incidence in AIMP2-DX2-transgenic mice compared with similarly treated wild-type littermates. The *in vivo* delivery of small interfering RNAs (siRNAs) against AIMP2-DX2 in lung cancer xenograft and benzopyrene-induced orthotopic lung cancer models slowed tumour growth. The ratio of AIMP2-DX2/AIMP2 increases according to the stage of lung cancer and shows a positive correlation with reduced survival in patients with lung cancer. All of these data indicate the functional importance of AIMP2 as a tumour suppressor and its variant AIMP2-DX2 as an effective therapeutic target in lung cancer.

AIMP3 is the smallest component in the MSC, and it specifically interacts with MRS[21,25]. *Aimp3*-homozygous mice are early embryonic lethal, indicating the functional importance of AIMP3 for development[40]. *Aimp3*-heterozygous mice are born without developmental defects, but they spontaneously develop various cancers, such as lymphoma, breast cancer, bronchiole epithelium adenocarcinoma and hepatocarcinoma, about 15 months after birth. Mechanistic studies revealed that, after DNA damage, AIMP3 is translocated to the nucleus to bind and to activate the tumour-suppressing kinases, ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR). The cells with a lower level of AIMP3 had reduced activity of p53 and resistance to cell death. Reduced expression of AIMP3 was found in acute myeloid leukaemia, chronic myeloid leukaemia and liver cancers using PCR or western blot analyses. AIMP3 is also activated by oncogenes, such as RAS[41], in which the exogenous introduction of activated RAS increases cellular levels of AIMP3, leading to p53 activation. Moreover, *Aimp3*-heterozygous cells transformed by RAS or MYC showed chromosome instability and uncontrolled cell division. The three-dimensional structure of AIMP3 shows structural similarity to glutamyl-RS and Arc1p, which form a primitive tRNA synthetase complex in yeast, as well as to EF1Bγ and glutathione *S*-transferase[42]. Among several rare mutations of AIMP3 found in patients with chronic myeloid leukaemia, a few mutations in the carboxy-terminal domain abolished the interaction of AIMP3 with ATM[42]. Although reduced activity of AIMP3 leads to spontaneous tumorigenesis, increased expression of AIMP3 enhances cellular senescence through the specific downregulation of lamin A, one of the filament-type proteins that form the nuclear lamina[43]. In this case, AIMP3 binds and specifically induces ubiquitin-mediated degradation of lamin A, but not other lamin isoforms, resulting in a stoichiometric imbalance between the lamins. Mice that constitutively overexpressed AIMP3 showed premature ageing phenotypes, such as reduced body growth, shortened lifespan, alopecia, wrinkled skin with reduced adipocytes, lordokyphosis and reduced bone thickness. In agreement with these findings, the expression level of AIMP3 increases in aged cells and tissues. These results suggest AIMP3 as a crucial factor that has a pivotal role in regulating senescence and tumorigenesis.

As the MSC is part of the essential machinery for protein synthesis, it is counterintuitive that the depletion of the MSC components can induce tumours. Surprisingly,

cells with a depletion or a reduction of AIMP2 or AIMP3 show increased cell proliferation and resistance to death[31,40]. On the basis of these findings, these factors seem to have dual functions depending on their cellular localization. While they are bound in the MSC, they can facilitate protein synthesis. However, when cells are subject to oncogenic stresses, they are rapidly recruited to target sites to prevent these stresses from promoting tumorigenesis.

Non-canonical intracellular activities of ARSs

Many ARSs function in signalling pathways that are linked to the control of tumorigenesis (FIG. 2). In IgE-activated mast cells, for example, KRS is translocated to the nucleus and binds transcription factors such as microphthalmia-associated transcription factor (MITF). MITF is involved in melanocyte development and is a known melanoma oncogene[44]. MITF is sequestered by its interaction with a tumour suppressor, histidine triad nucleotide-binding protein 1 (HINT1), which is a member of the histidine triad (HIT) superfamily[45]. On binding to MITF, KRS can generate a signalling molecule, diadenosine tetraphosphate, that binds HINT1, leading to the release of the bound MITF. On the basis of this connection, nuclear KRS could have a role as a positive regulator of MITF.

Ribosome biogenesis is related to cell proliferation and cancer[46]. During tumorigenesis, the tightly regulated interaction between extracellular signalling and ribosome biogenesis is disrupted. As ribosomal RNA is the major component of the ribosome, the rate of rRNA synthesis may correlate with cancer prognosis[47]. Deregulation of DNA polymerase I, which transcribes rRNA in the nucleolus, can contribute to tumorigenesis, and transcription factors such as p53 and RB are known to control the activity of DNA polymerase I[46]. Interestingly, MRS was shown to be located in nucleoli after a proliferative signal to help with rRNA synthesis[48]. Although the exact function of MRS in rRNA synthesis is still unclear, the deregulation of MRS may be involved in the upregulation of rRNA synthesis, thus accelerating tumorigenesis. MRS can also indirectly control tumour formation through its interaction with AIMP3 (REF. 40). Any mutations in MRS that could influence its interaction with AIMP3 might modulate the tumour suppressive activity of AIMP3 in the nucleus. Apoptosis signal-regulating kinase 1 (ASK1) is activated by various oncogenic stresses[49] to mediate cell death. QRS can bind and suppress the pro-apoptotic activity of ASK1. The interaction between the two proteins was shown to be increased by glutamine, but dissociated by a death signal, such as activation of the pro-apoptotic CD95L–CD95 pathway[50], providing the first evidence that the regulatory interaction of ARSs can be controlled by their substrate amino acids.

Human EPRS is a bifunctional enzyme in which the two catalytic domains are linked by three tandem repeats[51]. In response to interferon-γ (IFNγ), EPRS forms a multi-component complex with other regulatory proteins at a 3′ UTR region that is involved in the translational silencing of target transcripts[52], many of which function during an inflammatory response. The translation of vascular endothelial growth factor A (*VEGFA*), which is a crucial factor for angiogenesis, is also controlled by EPRS through a similar mechanism[53,54]. Thus, EPRS seems to serve as a key gatekeeper of inflammatory gene translation[55].

Secreted ARSs in the tumour microenvironment

Several ARSs have been identified as secreted cytokines that control angiogenesis and immune responses and that may have roles in the tumour microenvironment (FIG. 2). Tryptophanyl-tRNA synthetase (WRS) is 653 amino acids in length and it can lose the N-terminal 47 amino acids through alternative splicing, generating mini-WRS[56,57]. Although both forms of WRS are catalytically active, only mini-WRS functions as an angiostatic cytokine[58]. The production of mini-WRS is stimulated by IFNγ. The N-terminal extension of WRS can also be truncated by proteolytic cleavage with leukocyte elastase[58], generating T1-WRS and T2-WRS, which lack the N-terminal 70 and 93 amino acids, respectively. These two proteolytic products are also active cytokines. The secretion of WRS is mediated by the dissociation from a ternary complex that is formed with annexin II and S100A10 in the cytosol[59]. VE-cadherin, a calcium-dependent adhesion molecule, was identified as a receptor for mini-WRS[60] and is selectively expressed and concentrated at the intercellular junctions of endothelial cells. The angiostatic activity of mini-WRS was mapped to eight amino acids, DTIEEHRQ, which are located within the tRNA anticodon-binding domain[61], implying that WRS uses the same domain for binding to its receptor and to its substrate. The binding of mini-WRS to human umbilical vein endothelial cells inhibits VEGFA-induced activation of ERK and endothelial cell migration. Interestingly, a recent report indicated that low expression levels of WRS are related to an increased risk of disease recurrence and reduced survival of patients with colon cancer[62], although it is not known whether this finding is related to the angiostatic activity of WRS.

Box 1 | **Genetic disorders of ARS and AIMPs linked to human diseases**

Mutations of the genes encoding glycyl-tRNA synthetase (GRS)[102] and tyrosyl-tRNA synthetase (YRS)[103] have been found in patients with Charcot–Marie–Tooth disease, which is one of the most common inherited disorders in motor and sensory neurons. How mutations of GRS or YRS cause this disease is not fully understood. Interestingly, all the identified mutants are not associated with their catalytic activities, implying that the non-catalytic activities of these enzymes could be involved in the pathogenesis of this disease. Among aminoacyl-tRNA synthetase (ARS)-interacting multi-functional proteins (AIMPs), AIMP2 was shown to be a substrate for parkin, an E3 ubiquitin ligase, that is known to cause a familial form of Parkinson's disease[104,105]. Mutant parkin has lost its ability to bind AIMP2, leading to neuronal cell death owing to the increase in the expression level of AIMP2. A homozygous frameshift mutation that inactivates AIMP1 is linked to Pelizaeus–Merzbacher-like disease (PMLD) that is pathologically similar to Pelizaeus–Merzbacher disease, which is a hypomyelinating leukodystrophy[106]. *Aimp1*-knockout mice also display axonal degeneration of motor neurons, defective neuromuscular junctions, muscular atrophy and motor dysfunction[107]. Mutations in human mitochondrial leucyl-tRNA synthetase (LRS)[108] as well as $tRNA^{Leu(UUR)}$ (REF. 109) are associated with type 2 diabetes. In addition, the inactivation of mitochondrial LRS was also found in nasopharyngeal carcinoma[110]. Now that unbiased whole-genome sequencing is rapidly advancing, more alterations in ARS-encoding genes are expected to be found in human cancer samples and cell lines, which in turn could provide further information about the novel functions of these molecules.

Human tyrosyl-tRNA synthetase (YRS) is a 528 amino acid protein that is secreted and cleaved into two separate domains by leukocyte elastase[63,64]. Human YRS contains an ELR motif that is known to be important for cytokine activity[65]. This motif is sequestered by the anticodon-recognition domain in the full-length protein and exposed only when the protein is cleaved[66]. The 364 amino acid N-terminal peptide, also termed mini-YRS (FIG. 2), but not full-length YRS, functions as an angiogenic factor. It stimulates the chemotaxis of endothelial cells and stimulates angiogenesis through the transactivation of vascular endothelial growth factor receptor 2 (VEGFR2)[67]. Mini-YRS is a dose-dependent biphasic cytokine: a high dose of mini-YRS can promote angiogenesis and a low dose can suppress it. The 164 amino acid C-terminal domain of human YRS contains a domain that is homologous to endothelial-monocyte-activating polypeptide II (EMAPII), which is the C-terminal domain of AIMP1 (see below), and which functions as an immune-stimulating cytokine that is distinct from its N-terminal counterpart[64]. It contains a heptapeptide (RVGKIIT) that is crucial for the chemotaxis of mononuclear phagocytes and polymorphonuclear leukocytes, and the production of TNF, tissue factor and myeloperoxidase[63,68]. Human KRS is also secreted from cancer cells in response to TNF, and attracts and stimulates macrophages and peripheral blood mononuclear cells[69].

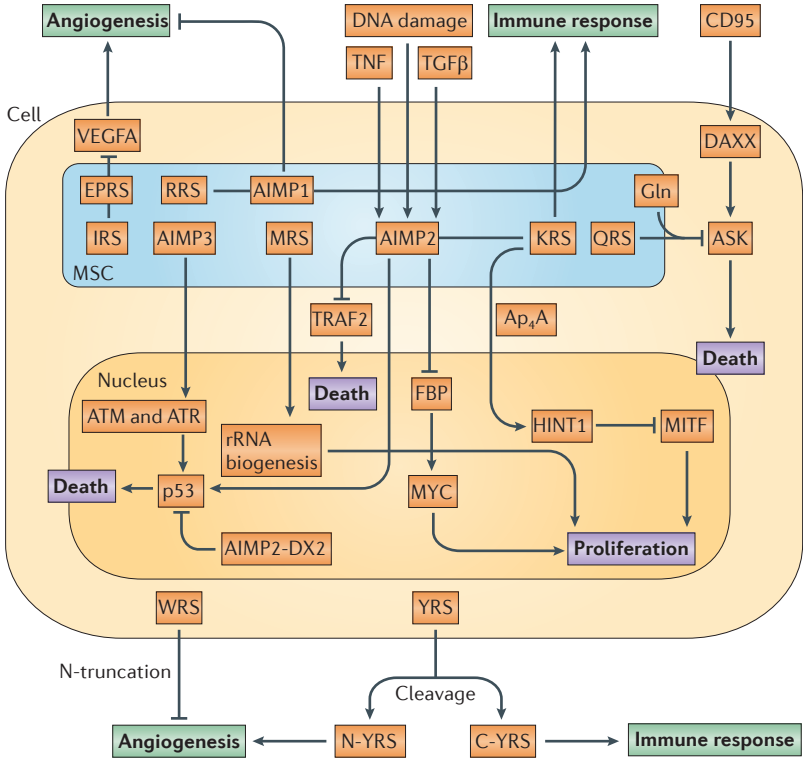

Figure 2 | **Non-canonical activities of ARSs and AIMPs implicated in the control of tumorigenesis.** Eight different aminoacyl-tRNA synthetases (ARSs), bifunctional glutamyl-prolyl-tRNA synthetase (EPRS), isoleucyl-tRNA synthetase (IRS), leucyl-tRNA synthetase (LRS), methionyl-tRNA synthetase (MRS), glutaminyl-tRNA synthetase (QRS), lysyl-tRNA synthetase (KRS), aspartyl-tRNA synthetase (DRS) and arginyl-tRNA synthetase (RRS), form a macromolecular protein complex with three auxiliary factors, designated ARS-interacting multifunctional protein 1 (AIMP1), AIMP2 and AIMP3. This complex is known as the multisynthetase complex (MSC) and is shown in blue. Within the complex, RRS, KRS and MRS specifically interact with AIMP1, AIMP2 and AIMP3, respectively. On genotoxic damage, AIMP2 and AIMP3 are translocated to the nucleus where AIMP2 activates p53 directly[36] and AIMP3 activates p53 through the activation of the kinases ataxia telangiectasia mutated (ATM) and ATM- and Rad3-related (ATR)[40]. AIMP2 also augments the apoptotic signal of tumour necrosis factor (TNF) through downregulation of TNF receptor associated factor 2 (TRAF2)[34] and mediates the transforming growth factor-β (TGFβ) anti-proliferative signal through the downregulation of fuse-binding protein (FBP)[31]. A splice variant of AIMP2, AIMP2-DX2, compromises the tumour suppressive activity of AIMP2 and can induce tumorigenesis[39]. Among the ARSs that form the MSC, EPRS can function as a translational silencer to suppress the generation of vascular endothelial growth factor A (VEGFA), which is an angiogenic factor[53]. KRS can translocate to the nucleus to bind microphthalmia-associated transcription factor (MITF), which is an oncogenic transcriptional activator, and can generate the second catalytic product, diadenosine tetraphosphate (Ap_4A). Ap_4A binds a tumour suppressor histidine triad nucleotide binding protein 1 (HINT1), leading to the release of MITF, which is bound to HINT1 (REF. 114). KRS is secreted and induces the production of TNF from macrophages[69]. QRS can interact with apoptosis signal-regulating kinase 1 (ASK1) to suppress apoptotic signals in a glutamine-dependent manner[50], and MRS can increase ribosomal RNA biogenesis in the nucleoli[48]. Among free-form ARSs, tryptophanyl-tRNA synthetase (WRS) is secreted, and the truncation of the amino-terminal peptide generates an active cytokine that suppresses angiogenesis[58]. Tyrosyl-tRNA synthetase (YRS) is also secreted and cleaved into N- and C-domains that have pro-angiogenic and immune activation functions, respectively[63].

Autoantibodies have also been detected against several other ARSs[70], implying that more ARSs might be secreted or released from diverse cellular sources under physiological and pathological conditions. Although further investigation is needed to understand whether and how these secreted ARSs are involved in tumorigenesis, they seem to be used not only for a host defence mechanism against cancer but also by tumour cells for growth, angiogenesis, metastasis or immune escape.

The C-terminal 165 amino acid domain of AIMP1 was first identified in the culture medium of methylcholanthrene A-induced mouse fibrosarcoma cells as EMAPII[71]. Although EMAPII is somehow derived from AIMP1, the proteolytic cleavage of AIMP1 does not seem to be required for its cytokine activity because AIMP1 is also secreted and functions as an active cytokine[72]. Systemic administration of AIMP1 into human stomach cancer and methylcholanthrene A-induced fibrosarcoma xenograft mouse models showed potent tumour suppressive effects[73,74]. AIMP1 induces the apoptosis of endothelial cells and it also stimulates cell migration at a lower concentration[75]. AIMP1 also activates various immune cells, including monocytes[72], macrophages[76] and bone marrow-derived dendritic cells[77], and induces antigen-specific cytotoxic T lymphocyte responses[78]. The angiostatic and immune-activating activities of AIMP1 seem to work together for its potent tumour suppressive activity. In parallel to the studies on AIMP1, the anticancer potential of EMAPII has been confirmed by independent experiments[79]. ATP synthase subunit-α was suggested to mediate the EMAPII-induced apoptosis of endothelial cells[80] and the opening of the blood–tumour barrier[81]. CXCR3 also seems to be involved in EMAPII-dependent recruitment of endothelial progenitor cells[82]. Although it needs to be determined whether AIMP1 and its C-domain, EMAPII, have distinct cytokine

activities and physiological implications, both have a great therapeutic potential in cancer as they may function as 'double-edged swords' that are able to inhibit tumour vascularization and induce immune activation.

Systematic analysis of the ARS network

A cancer-associated genomic profile. Despite the *in vitro* and *in vivo* data discussed above, the link between ARSs and tumorigenesis is not well established. To examine the potential association of this protein family with cancer at a systemic level, we compared the expression profiles and copy number variations of the genes encoding the 20 human cytoplasmic ARSs and AIMP1–3 (REF. 22) (together referred to as ARSN) with those of known cancer-associated genes (CAGs) obtained from the US National Cancer Institute (NCI)'s cancer gene index. Among the genes in the index, we only considered 3,501 genes that showed strong cancer association and clinical indications with no conflicting indications on their cancer association (see Supplementary information S1 (text) and Supplementary information S2 (table)). Using curated protein–protein interactions collected from 11 public databases, we further chose 123 CAGs that can directly interact with the 20 ARSs and three AIMPs (first neighbours), and 1,295 CAGs that may interact with the first neighbour CAGs (second neighbours) (see Supplementary information S3 (text) and Supplementary information S2, S4 (tables)). In addition, we also selected 1,874 genes that are not included in the NCI's cancer gene index and that have no significant cancer-associated expression profiles in more than 22 cancer data sets (*P* values >0.5) (see Supplementary information S5 (table)). These genes were classified as non-cancer-associated genes (non-CAGs).

The gene expression data were obtained from 164 cancer-associated expression data sets from two public databases, the Gene Expression Omnibus database (GEO; see Further information)[83] and the ArrayExpress database (see Further information)[84]. From these, we selected 40 data sets in which the numbers of normal and cancer samples are greater than ten (see Supplementary information S6 (table)). We then categorized the data into ten cancer groups (pancreatic, prostate, lung, breast, colon, kidney, head and neck, haematopoietic and lymphoid, liver and gastric cancer). More than two data sets were available for the same cancer group from independent studies. For each data set in the individual cancer groups, we identified differentially expressed genes between cancer and normal samples using integrative statistical hypothesis testing ($P<0.05$)[85]. For multiple data sets in each cancer group, the *P* values for each gene were summarized using Stouffer's method[86], resulting in the combined *P* values of all the genes in the ten cancer groups. In this analysis, the 23 genes encoding ARS and AIMPs (ARSN) showed expression profiles that are similar to those of the first and second neighbour CAGs in ten different cancer types and that are clearly distinguishable from the pattern of non-CAGs (combined $P<0.0001$) (FIG. 3a; see Supplementary information S7 (text))).

We also investigated the cancer-associated copy number variations of ARS- and AIMP-encoding genes using the data obtained by array comparative genomic hybridization (aCGH) in nine different cancers (skin, gastric, colon, head and neck, haematopoietic and lymphoid, lung, bone, soft tissue and uterine cancer) that are available in the CanGEM database (see Further information)[87]. The pattern of copy number variation of ARSN was also compared with those of first and second neighbour CAGs and non-CAGs. To our surprise, ARS and AIMPs show a high degree of copy number variations in most of the tested cancers, except for skin and uterine cancers, whereas little variation was observed in non-CAGs (FIG. 3b). Another set of copy number variations were obtained from Tumorscape[88] (see Further information)(see Supplementary information S8 (figure)). Several components of ARSN showed high gains (AIMP2, TRS, EPRS and GRS) and losses (QRS, NRS and AIMP1) of the encoding genes, which was largely consistent with those shown in FIG. 3b. We could not assess the cancer-associated sequence alternations and epigenomic characteristics of ARS- and AIMP-encoding genes as there are not enough data available for the ARS and AIMP genes. As some ARSN components are regulated by phosphorylation for their non-canonical activities (as mentioned above), we also examined cancer-associated phosphorylation of ARSN using the data in the PHOSIDA[89] and PhosPhoSitePlus databases[90]. A total of 138 phosphorylations were detected from 20 ARSs and three AIMP3 in cervical, breast, colorectal and leukaemia cells (in the HeLa, MDA-MB-435S, HCT116 and MV4-11 cell lines, respectively) (see Supplementary information S9 (table)), although their pathological implications need further investigation. Combined together, ARSs and AIMPs can be pathologically associated with cancers through their aberrant expression or post-translational modifications. These associations could be a result of the functions or ARSs and AIMPs and/or through their interactions with CAGs.

Cancer-associated interaction network. Using the interactions between 23 ARSs and AIMPs and 123 first neighbours, a network map was established to display how each of the ARSs and AIMPs could be functionally linked to the components of different biological pathways (FIG. 4a). In this map, node colour indicates the extent of cancer-associated deregulation and node size indicates the degree of centrality that reflects the number of direct interactions between ARSN and CAGs[86,91,92]. The edge thickness indicates the degree of cancer-dependent co-association of the interacting pair[93–95] (see Supplementary information S10 (text)). Most ARSs and AIMPs showed cancer-associated deregulation compared with those of the 123 CAGs. Among the components of ARSN, relatively higher cancer-associated deregulation was shown by KRS, LRS, TRS, RRS, DRS, AIMP2 and AIMP3 (thick red nodes). Also, most of the ARSs and AIMPs showed similar node sizes, indicating that they have similar numbers of interactions with CAGs. In the map, some interacting pairs such as

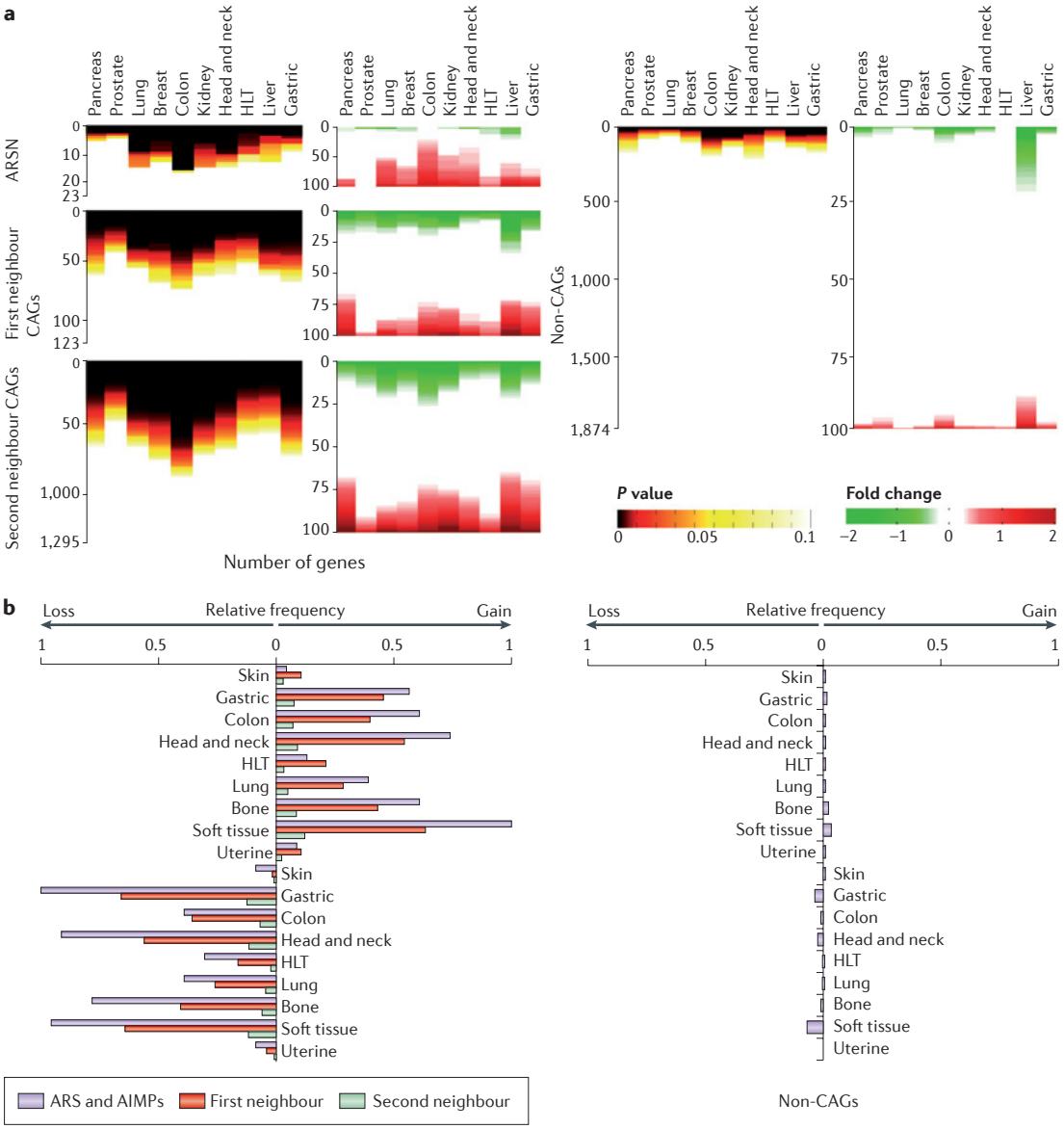

Figure 3 | **Expression profiles and copy number variations of ARS-encoding genes in comparison to CAGs and non-CAGs. a** | The comparison of gene expression profiles between the genes encoding 20 human cytoplasmic aminoacyl-tRNA synthetases (ARSs) and three ARS-interacting multi-functional proteins (AIMPs) (collectively termed ARSN), 123 cancer-associated genes (CAGs) that are directly linked to ARSs and AIMPs (first neighbours), 1,295 CAGs that are linked to the first ARS-neighbours (second neighbours) using the US National Cancer Institute (NCI) cancer gene index and 1,874 non-cancer associated genes (non-CAGs) is shown. Cancer-associated differential expression of each gene group was indicated by *P* values (left column) and fold changes (right column). The *P* values and the fold changes were sorted in ascending order. The *P* values are displayed in a red colour gradient: dark red ($P<0.01$), red ($0.01< P<0.05$) and white ($P>0.05$). The fold changes are represented by a red–green gradient showing cancer-specific increased expression and decreased expression, respectively. **b** | The comparison of copy number variations of ARS and AIMP-encoding genes, first, second neighbour CAGs and non-CAGs is shown. Copy number variations in the nine cancer types were obtained from the CanGEM database. For each gene group, the relative frequency of copy number variations in each cancer type is presented. HLT, haematopoietic and lymphoid tissue.

GRS–CD44, VRS–EEF1A1 and KRS–MITF are connected with thick edges, implying their potentially tight co-association in cancer.

We estimated the association of each ARS or AIMP with biological processes in the network as the mean shortest path between each of the ARSs and AIMPs and the proteins in the cellular pathways[96]. Most of ARSs and AIMPs showed strong connections to diverse biological pathways (FIG. 4b; see Supplementary information S11 (text)). Compared with others, CRS showed the lowest connection to most of the pathways. We further evaluated the association of ARSs with ten different cancers as the cancer-associated deregulation in the individual cancer groups. About 50% of the components of ARSN

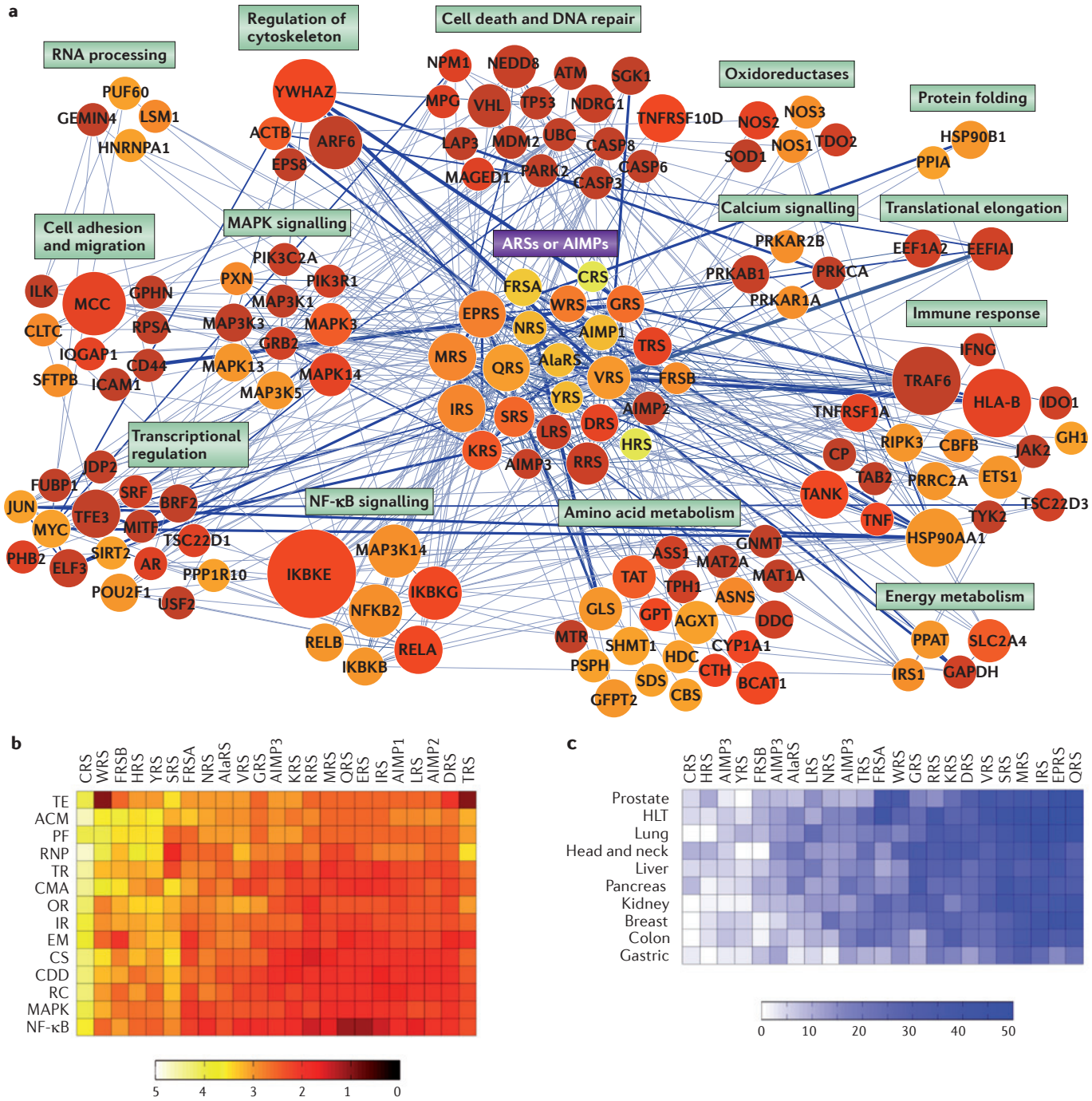

Figure 4 | **Hypothetical network model showing connections of ARSs and AIMPs with their CAG interactors. a** | This network model shows the links between 20 aminoacyl-tRNA synthetases (ARSs) and three ARS-interacting multi-functional proteins (AIMPs) with 123 cancer-associated genes (CAGs) (first neighbours). The network model was built with the pre-defined interactions using Cytoscape. The network nodes were arranged according to their associated GO biological process (GOBP) so that the nodes with similar GOBPs could be closely located. Nodes with multiple GOBPs are located next to the modules of GOBPs where the majority of their interactors are located (see Supplementary information S3 (text)). **b** | The mean shortest paths showing the close association of ARSs and AIMPs with the cellular processes represented in the network in part **a** are shown. Most of the ARSs and AIMPs had a low mean shortest path except for CRS, implying their close association with all the cancer pathways in the network (see Supplementary information S4 (table)). **c** | A heat map shows the association of the individual ARSs and AIMPs with ten types of cancer. The cancer association score for each of the ARSs and AIMPs reflects the degree of deregulation in the corresponding cancer. ACM, amino acid metabolism; AlaRS, alanyl-tRNA synthetase; CDD, cell death and DNA repair; CMA, cell migration and adhesion; CS, calcium signalling; DRS, aspartyl-tRNA synthetase; EM, energy metabolism; GRS, glycyl-tRNA synthetase; IR, immune response; IRS, isoleucyl-tRNA synthetase; KRS, lysyl-tRNA synthetase; LRS, leucyl-tRNA synthetase; MAPK, MAPK signalling; MRS, methionyl-tRNA synthetase; NF-κB, NF-κB signalling; OR, oxidoreductases; PF, protein folding; QRS, glutaminyl-tRNA synthetase; RC, regulation of cytoskeleton; RNP, RNA processing; RRS, arginyl-tRNA synthetase; TE, translational elongation; TR, transcriptional regulation; WRS, tryptophanyl-tRNA synthetase; YRS, tyrosyl-tRNA synthetase.

showed a stronger association with ten cancers (FIG. 4c). Among the other 50%, four proteins (CRS, HRS, AIMP3 and YRS) showed the weakest association with the listed cancers. Although these data do not indicate any specific connection of ARSN to biological processes or cancers, idiosyncratic connection patterns of ARSs and AIMPs to biological processes and cancer types may imply that they have unique roles in tumorigenesis. Although in-depth mechanistic studies are needed to understand the pathological implications for cancer-associated genomic variations and interactions, systematic analyses of ARS and AIMPs in connection with CAGs further support their potential importance in cancer biology.

ARSs and AIMPs as therapeutic targets

ARSs and AIMPs may be explored as therapeutic targets against cancer. Alternatively, the secreted ARS-derived bioactive peptides can work as pharmacological agents themselves. The potential exploitation of AIMP2 (REF. 39) as a therapeutic target has been discussed above. The suppression of the oncogenic variant of AIMP2 reduced the growth of lung cancer *in vivo*. In addition, the diverse protein–protein interactions between ARSs with other cancer-associated proteins could provide novel therapeutic targets. In this regard, it is worth noting that the interaction between AIMP1 and tumour-specific antigen endoplasmin (a homologue of gp96) can be chemically modulated to control autoimmune responses[97]. It remains conceptually possible that ARS-derived cytokines can be explored as therapeutic agents against cancer, and this possibility has been validated by the studies using AIMP1 and EMAPII[73,98–100].

Conclusions

The roles of ARSs and AIMPs as cell regulatory factors have been largely neglected for a few reasons. First, their functions outside of protein synthesis were not expected, as they had been classified as housekeeping enzymes dedicated to translation. Second, for a similar reason, the presence of ARSs in unexpected cellular locations or in cellular complexes has often been regarded as experimental artefact. Third, even if non-conventional functions of ARSs have been validated, they are considered as evolutionary co-incidences that happened in a few special cases. However, there is now increasing evidence indicating that the multiple functions of ARSs are systematic and are controlled through sophisticated mechanisms in response to various cellular stimuli. Perhaps, dynamic cellular localization, specific recognition of amino acids, ATP and tRNAs, and versatile protein–protein interactions render these enzymes a molecular 'emergency response' that can swiftly respond to various cellular stresses to maintain cellular homeostasis. As many translational components are rapidly emerging as key factors to control tumorigenesis[101], the unexpected regulatory network of these ancient enzymes brings new insights into the pathogenic process of cancer and presents a potential therapeutic opportunity.

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Acknowledgements
This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2008-359-C00024), the Global Frontier Project grant (NRF-M1AXA002-2010-0029785) and by [R31-2008-000-10103-0] and [R31-2008-000-10105-0] from the World Class University project of the Ministry of Education, Science and Technology.

Competing interests statement
The authors declare no competing financial interests.

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