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Aminoacyl-tRNA synthetase-interacting multi-functional protein 1/p43: an emerging therapeutic protein working at systems level

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Background: Drug discovery programs are based on the presumption of one drug–one action–one disease, which is frustrated by the complexity of biological systems. Because the aberration of a single gene often leads to multiple pathological symptoms, we should understand the functional network of the disease-related proteins to develop effective therapy. *Objectives*: To describe how activities of proteins are reflected in phenotypes and their pathological implications using aminoacyl-tRNA synthetase-interacting multi-functional protein 1 (AIMP1). *Methods*: The physiological activities of AIMP1 are unveiled through *in vitro* approaches and *in vivo* phenotyptic investigation. Bioinformatics tool was used to combine all AIMP1-target proteins. *Conclusion*: Although a cytosolic protein, AIMP1 can be secreted as a cytokine to control immune response, angiogenesis and wound healing, and as a glucagon-like hormone for glucose homeostasis. It is involved in the regulation of autoimmune control and TGF-β signaling within the cells. AIMP1-deficient mice developed multiple phenotypes in immune systems, metabolism and body growth. The therapeutic potential of this multi-functional protein with associated biological activities are discussed.

Keywords: aminoacyl-tRNA synthetases, ARS-interacting multi-functional proteins, multiple phenotypes, multi-tRNA synthetase complex, therapeutic proteins, systems biology

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1. Introduction

The discovery of effective therapeutic targets has become a critical determinant for the success of new drug discoveries ever since target-based therapy constituted a main trend. Among various biological components, proteins provide the main source of therapeutic targets because they are in charge of most metabolism, regulation, and frameworks of biological systems. To our surprise, the number of protein-encoding genes in humans turned out to be only 20,000 – 25,000 [1], which is much less than we have predicted based on the complexity of necessary functions to run our body. Ironically, fewer number of structural gene raised more complicated puzzles to deal with because the limited number of genes should be somehow differentiated at protein level to meet the requirement of body complexity. For instance, a single gene can encode multiple forms of protein through alternative splicing, gene fusion, proteolysis, which are further refined by various chemical modifications. However, even without these chemical changes, the same polypeptide can execute multiple functions through its diverse combination with molecular partners.

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Considering that most human diseases express more than a single symptom, it is important to understand the functional diversification of the disease-associated proteins, as well as their functional and regulatory networks, to design an effective method of curing the disease. Owing to advances in functional genomics and proteomic approaches, many unexpected linkages among seemingly unrelated processes are being unveiled and the number of the multi-functional or moonlighting protein is being rapidly increased [2-9]. Anticipating more functions to be found from existing proteins, the norm may be changed from 'one protein–one function' to the more progressive 'one protein–multi-function' concept in the near future.

The activities of functionally promiscuous proteins can be regulated by many different ways including transcription, subcellular localization, homo- or hetero-oligomerization and interactions with substrates or ligands. Thus, far, well known moonlighting proteins include glyceraldehyde-3-phosphate dehydrogenase, lens crystallins, ribosomal proteins, elongation factors, and phosphoglucose isomerase among others [2,8]. Recently, accumulating evidences add aminoacyl-tRNA synthetases (ARSs) and ARS-interacting multi-functional proteins (AIMPs) to the group of multi-functional proteins [5,10,11]. For instance, these enzymes and associated factors were shown to be involved in the control of apoptosis [12,13], proliferation [14], angiogenesis [15-17], inflammation [18,19], DNA repair [20], RNA splicing [21] and silencing [22], immune system [23] and virus packing [24]. For this reason, aberrant expression or activities of ARSs and AIMPs are heavily linked to various human diseases [25]. These enzymes execute such roles mainly through the formation of diverse protein complexes. Among them, the most intriguing is the macromolecular complex consisting of nine different ARSs (glutamyl-prolyl, isoleucyl-, leucyl-, methionyl-, glutaminyl-, lysyl-, aspartyl- and arginyl-tRNA synthetase) and three ARS-interacting multi-functional proteins (AIMP1, AIMP2 and AIMP3 earlier named as p43, p38 and p18, respectively) present in the mammalian system. At present, about half of the component enzymes and non-enzymatic factors were demonstrated to play functions other than protein synthesis. Considering this fact, at least one plausible function for the complex formation seems to provide a platform in controlling the diverse regulatory activities of the component ARSs and AIMPs [26]. Among these complex-forming ARSs and AIMPs, AIMP1/p43 is most prominent in its functional versatility at present. In this paper, we describe diverse activities of AIMP1, its potential association with human diseases as well as its perspective therapeutic functions.

2. Multiple pathological phenotypes of AIMP1-deficient mice

Because the significance of protein functions would be reflected by *in vivo* phenotypes resulting from genetic modification of the encoding gene, AIMP1-deficient mice were generated by insertional inactivation of the encoding gene using gene trap method [27]. Although the homozygous mice were delivered following the rule of Mendelian segregation, they manifested high lethality throughout pre and postnatal stages. AIMP1-deficient mice were anatomically normal but the overall body size was dramatically reduced (**Figure 1**). Because body size is determined by many different causes, it is difficult to link any specific function of AIMP1 to this phenotype. AIMP1-deficient mice also showed retarded rate in wound healing and reduced collagen density (**Figure 1**). Systematic analysis of anatomical and histological characteristics revealed that these mice suffer from severe lupus-like auto-immune phenotypes such as infiltration of immune cells to various organs, accumulation of autoantibodies, antinuclear antibodies, glomerular nephritis (**Figure 1**) and these symptoms are more prominent in females [23]. The homozygous mice also showed hypoglycemia implying its function in glucose metabolism (**Figure 1**) [28]. Although they also displayed severe disorders in neural and reproduction systems, these phenotypes require further investigation to understand the functional connections to AIMP1. It is surprising to see all of these distinct phenotypes result from the lack of single gene product, AIMP1. In the next section, we describe distinct activity of AIMP1 at different cellular locations and their pathological linkage to the disclosed disorders.

3. Role of AIMP1 in the molecular assembly of multi-tRNA synthetase complex and protein synthesis

AIMP1 was first discovered as a factor, p43, associated with the multi-ARS complex mentioned above [29-31]. For protein synthesis, this complex is believed to provide an efficient trafficking channel of aminoacyl-tRNAs for protein synthesis [32]. Although the 3D structure of the multi-ARS complex remains to be determined, comprehensive approaches have been applied to look into the physical relationship among the component proteins such as partial dissociation [33], hydrophobic chromatography [34], electron microscopy [34-37], chemical crosslinking [38], genetic approaches [39-41] and systematic depletion analysis [42]. This complex is thought to consist of a few subdomains linked together through three AIMPs [42]. In this complex, AIMP1 is believed to be located at the center [43], interacting with arginyl-tRNA synthetase (RRS), glutaminyl-tRNA synthetase (QRS), methionyl-tRNA synthetase (MRS) and AIMP2/p38. A systematic mapping of the interactions among the components demonstrated that they are interconnected. Moreover, the depletion analysis of the components demonstrated that they are mutually dependent for their cellular stability, although the degree of dependency varies among them. AIMP1 is essential

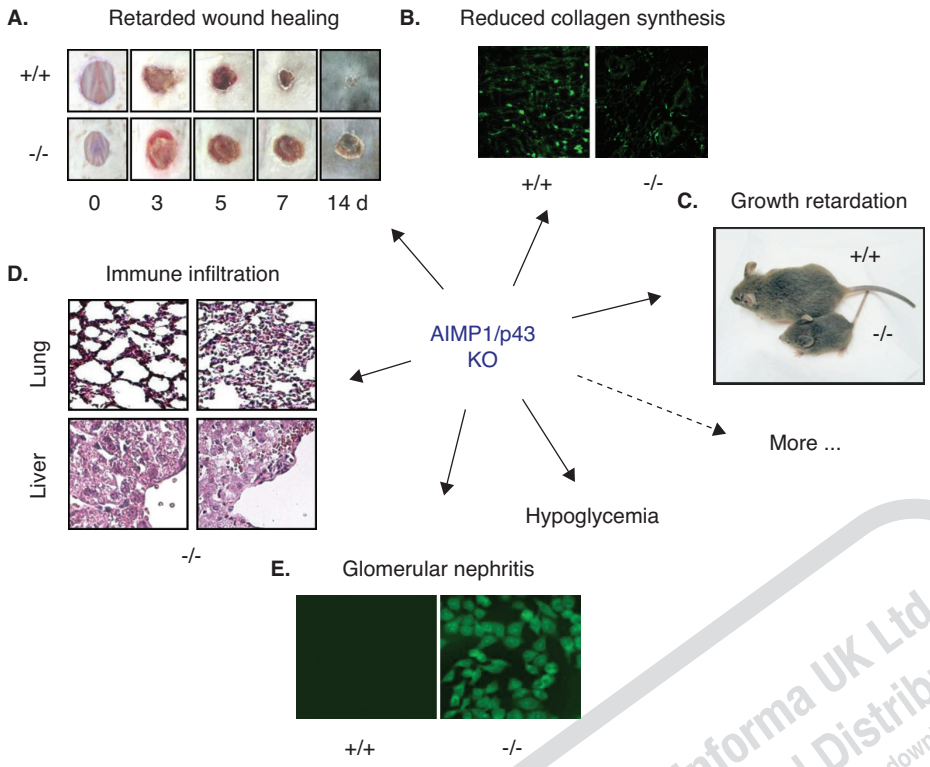

Figure 1. Multiple phenotypes resulting from the depletion of AIMP1. The AIMP1-deficient mice show reduced wound healing and collagen synthesis [47]. **A.** The 0.5 cm diameter full-thickness wound was introduced on the back skin of AIMP1$^{+/+}$ and AIMP1$^{-/-}$ mice and wound closure rate was compared at various time intervals. **B.** The amount of collagen was compared by immunofluorescence staining in the re-epithelialization region of the back skin wound between AIMP1$^{+/+}$ and AIMP1$^{-/-}$ mice. **C.** AIMP1 deficiency also causes severe growth retardation [23]. **D.** The AIMP1-deficient mice also suffer from lupus-like autoimmune disorders [23]. Among many autoimmune phenotypes displayed by this mutant, inflammation in lung and liver is shown by hematoxilyn and eosin staining. **E.** Glomerular immunoglobulin deposition is also observed by immunofluorescence staining of kidney tissues with anti-IgG antibody. In addition, the homozygous mice show disorder in neural and reproduction systems that need further investigation for functional linkage.
Adapted from Am J Pathol 2005;166:387-98 and Am J Pathol 2007;170:2042-54, with permission from the American Society for Investigative Pathology.

for the stability of several components [42], together with two other AIMPs.

Interestingly, many complex-forming ARSs contain unique N- or C-terminal peptide appendices thought to be involved in their versatile molecular interactions [39]. The 146 aa containing N-terminal domain of AIMP1 interacts with the N-terminal appendix of RRS and QRS, as well as with the leucine zipper motif of AIMP2 [44-46]. The interaction of AIMP1 with RRS augments aminoacylation activity of RRS [44]. Yet, despite the fact that AIMP1 improves the cellular stability of the associated enzymes and the catalysis of the associated enzyme, AIMP1 seems to be dispensable in normal physiological conditions or in the cells cultivated in complete medium because cellular protein synthesis and cell growth are not seriously affected by the depletion of AIMP1 [47]. Thus, the multiple phenotypes shown in AIMP1-deficient mice may not necessarily result from its potential role in protein synthesis. Perhaps, the functional importance of AIMP1 in protein synthesis may become obvious at starving or stressful conditions.

4. AIMP1 in immune control

The functional relationship of AIMP1 to immune reaction originated from the discovery of a polypeptide with cytokine activity in the culture medium of murine methylcholanethrene A-induced fibrosarcoma cells [48]. This peptide was named endothelial monocyte activating polypeptide II (EMAPII) [31,49], for its ability to induce activation of tissue factor in human umbilical vein endothelial cells. Interestingly, this polypeptide turned out to be the C-terminal part of AIMP1 associated with multi-ARS complex. To generate the 166 aa C-terminal domain equivalent to EMAPII, 312 aa human AIMP1 is cleaved by caspase-7 on apoptosis [50]. Because of this initial finding, AIMP1 has been thought to be an inactive precursor for EMAPII. However, subsequent investigations demonstrated that AIMP1 itself is also secreted from intact mammalian cells and works as a cytokine to trigger pro-inflammatory response through monocytes and macrophages (**Figure 2**) [18]. Considering the critical role of AIMP1 in the stability of the multi-ARS complex [42], the

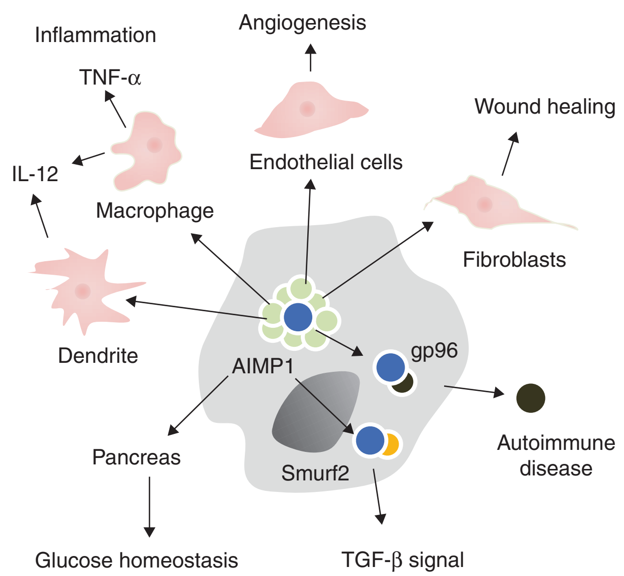

Figure 2. Diverse extra- and intracellular activities of AIMP1. It is harbored in multi-tRNA synthetase complex to facilitate the assembly and stability of the component enzymes and associated factors [42]. It also resides in endoplasmic reticulum, holding heat-shock protein gp96 to prevent its aberrant extracellular exposure that may trigger autoimmune phenotypes [23]. It can also prevent ubiquitylation of Smurf2 [102], which is the negative regulator of TGF-β signaling pathway, thereby controlling TGF-β-dependent cell growth control. It is also secreted to work on immune, endothelial cells and fibroblasts to give the indicated activities depending on the target cells. AIMP1 is also enriched in pancreatic α cells and secreted out to the blood on hypoglycemia for the recovery of blood glucose level [28].

proteolytic cleavage of AIMP1 may lead to disintegrate the multi-ARS complex rather than to release EMAPII domain from the bound complex. The secretion of intact AIMP1 is found in different types of cells including prostatic adenoma cells, immune and transfected cells under various conditions such as apoptosis, drug treatment, cytokine stimulation, heat shock and hypoxia [18,51-54]. The C-terminal domain of AIMP1 shows partial homology with those of a few different inflammatory cytokines such as RANTES (regulated on activation, normal T-cell expressed and presumably secreted) and monocyte chemotactic protein 1. In fact, extracellular treatment of AIMP1 activates monocyte and macrophages through mitogen-activated protein kinases (MAPKs) activation relayed by phospholipase Cγ, protein kinase C (PKC) and NF-*k*B [18,55,56], leading to increased expression of inflammatory molecules including tumor necrosis factor-α, interleukine-8 (IL-8), monocyte chemotactic protein-1, macrophage inflammatory protein-1 and IL1-β. AIMP1 increases IL-12 production through the activation of NF-*k*B in macrophage [57] and also in bone marrow-derived dendritic cells [58] (**Figure 2**). As IL-12 is known to have an important role in cell-mediated Th1 immune responses, AIMP1 may also function as a potential modulator of cell-mediated immunity through induction IL-12. Owing to the fact that IL-12 recently showed its effect on cancer immunotherapy, as well as on immune responses against infectious diseases [59], AIMP1 is expected to play a critical role in the development of the Th1 immune responses associated with cancer immunotherapy and protective immunity against intracellular pathogens [57,58].

Aside from the functions mentioned above, AIMP1 induces monocyte cell adhesion through the upregulation of intercellular adhesion molecule 1 (ICAM-1), which is an immunoglobulin-superfamily member widely expressed on the surface of vascular endothelium, monocytes, lymphocytes, and leukocytes [60-62]. ICAM-1 may mediate AIMP1-induced cell–cell adhesion through lymphocyte function-associated antigen-1 or leukocyte integrin Mac-1 [63]. Likewise, AIMP1 has been found to induce homotypic cell adhesion of monocytes by stimulating different MAPKs through phosphatidylinositol 3-kinase-dependent and -independent upregulation of ICAM-1 [55]. Because ICAM-1 promotes cell adhesion in a variety of processes such as inflammation and atherosclerosis [64], AIMP1 is probably implicated in such processes as well [55,56].

In contrast to secreted AIMP1 that boosts immune system, intracellular AIMP1 seems to play an opposite role in immune control (**Figure 2**). Some population of AIMP1 resides in endoplasmic reticulum (ER) to interact with gp96 [23], which is the ER-resident member of the hsp90 family [65]. Similar with AIMP1, heat-shock protein gp96 can be secreted out of ER, leading to activation and maturation of dendritic cells [66-68] by direct interaction with CD91 and toll-like receptor 2/4 of dendritic cells [69-71]. As a result, activated dendritic cells secrete pro-inflammatory cytokines and induce major histocompatibility class I and II [71,72]. Transgenic mice chronically expressing gp96 on cell surfaces show significant DC activation and systemic autoimmune disease phenotypes such as lupus [73]. As well, AIMP1-deficient mice show similar phenotypes as the gp96-expressing transgenic mice, suggesting their close functional linkage.

In addition, AIMP1-deficient mice show the increased cell surface localization of gp96, resulting in the same outcome as the transgenic mice overexpressing gp96 in the plasma membrane [23]. This phenomenon discloses characteristic phenotypes such as systemic inflammatory cell infiltration to various organs and deposition of autoimmune antibodies. Dendritic cells are involved in the pathogenesis of autoimmunity [74], and chronic maturation of tissue dendritic cells induces severe organ-specific autoimmune disease and systemic autoimmunity [75]. Because the association of AIMP1 with gp96 seems to be important for the suppression of autoimmune reaction and aberrant secretion of these two housekeeping proteins trigger abnormal immune response, controlling the intracellular association and extracellular secretion of the two proteins would provide

interesting therapeutic points for autoimmune diseases or even tumorigenesis.

5. AIMP1 in angiogenesis

AIMP1 seems to control angiogenesis process differently depending on its concentration (**Figure 2**) [15]. At low concentration, it prompts the migration of endothelial cells through extracellular signal-regulating kinase 1/2 (ERK1/2)-mediated induction of matrix metalloprotease 9. At high concentration, AIMP1 inhibits angiogenesis by the induction of Jun N-terminal kinase phosphorylation and caspase-3 activation. The dose-dependent biphasic activity of AIMP1 is reminiscent of other signaling molecules such as estrogen [76], statin [77], TGF-β1 [78] and thrombospondin-1 [79]. Because angiogenesis is a complex biological process, the mode of biphasic activity seems to be required for finer control in the regulation of the angiogenic process.

Although it is not yet determined whether AIMP1 would work on all the types of endothelial cells, there are several lines of evidence suggesting its potential efficacy against cancer through the process of restricting neovascularization [15,80]. In a previous work, AIMP1 demonstrated its potential interaction with α-subunit of ATP synthase [81], previously identified as the binding target of the antiangiogenic factor, angiostatin [82,83]. As angiostatin inhibits vascularization through the suppression of ATP metabolism on the surface of endothelial cells, the interaction of AIMP1 with α-subunit of ATP synthase may render a similar effect on endothelial cell growth, resulting in the suppression of tumor vasculature. In physiological conditions, AIMP1 may work against cancer as a double-edged sword. First, it can activate cell-mediated immunity and suppress tumor vascularization, both of which can work together to smother cancer progression. In fact, systemic administration of AIMP1, either alone or in combination with cytotoxic anticancer drug, efficiently suppressed cancer progression and improved survival rate [84].

Interestingly, the genes encoding microRNAs, miR-15a and miR-16a, which are located at chromosome 13q14, are often deleted in pituitary adenomas [85]. Expression of miR-15a and miR-16a shows positive correlation with AIMP1 secretion but inversely related to tumor diameter and expression of RRS that anchors AIMP1 in multi-tRNA synthetase complex [85] but miR-15 and miR-16 were suggested to downregulate RRS expression through their association with the 3′-UTR region of RRS mRNA [86]. Thus, cancer-specific depletion of miR-15 or miR-16 would lead to the increase of RRS expression, which would inhibit AIMP1 secretion as it would hold AIMP1 in the multi-tRNA synthetase complex. Because extracellular AIMP1 would suppress tumorigenesis through its antiangiogenic and immune-activating activities, miR-15 and miR-16 would exert their tumor suppressive activity through its linkage to RRS that is physically in contact with AIMP1.

6. AIMP1 in wound repair

Owing to the fact that inflammation and angiogenesis constitute important parts of wound repair [87,88], it is reasonable to imagine that AIMP1 may play a role in this process (**Figure 2**). It is rapidly enriched in the wound site, causing secretion of tumor necrosis factor-α from recruited macrophages. Surprisingly, the secreted AIMP1 triggers proliferation of dermal fibroblasts in contrast to its antiproliferative activity in endothelial cells [47]. It likewise induces collagen production from the activated fibroblasts. Consistent with the observed activities, AIMP1-deficient mice showed severely retarded wound closure and reduced collagen density (**Figure 1**) [47]. The recovery of this phenotype by exogenous supplementation of purified AIMP1 suggests its potential use as a new wound-healing agent. Nonetheless, it is to be known whether it will result in a synergistic effect with the known growth factors used for wound healing agents such as fibroblast growth, epidermal growth or platelet-derived growth factors.

7. AIMP1 as a hormone for metabolism control

In addition to the functions mentioned above, AIMP1 also seems to work as a hormone for glucose homeostasis (**Figure 2**). Tissue blot analyses revealed that AIMP1 is highly enriched in various secretary organs such as salivary gland and pancreas [28]. In the pancreas, AIMP1 is particularly localized at the secretory vesicles of α cells in the pancreatic islets, together with glucagon. It is secreted to the blood under hypoglycemic condition and induces glucagon secretion from pancreatic α cell, recovering blood glucose level. In addition, AIMP1 also facilitates glucose supply to blood through the induction of glycogenolysis in liver, and the lipolysis of triglyceride in adipose tissue. AIMP1$^{-/-}$ mice display various glucose metabolism-related phenotypes; **Figure 1**, for instance, body growth is significantly retarded although it may have resulted from other unknown disorders. In addition, AIMP1$^{-/-}$ mice displayed reduced food intake, and the weight of major organs being involved in fuel metabolism. The reduction of body fat content shown in AIMP1$^{-/-}$ mice is also observed in glucagon receptor-null mice [89]. AIMP1$^{-/-}$ mice also show the dramatic reduction of glucose, fatty acid, glucagon, and insulin in the plasma levels, as compared with their wild type littermates under fasting conditions. Although the molecular mechanism of hormonal action is yet to be examined, the hormonal activity of AIMP1 could be used to rescue acute hypoglycemia, which is a critical concern for patients with diabetes. In addition, its lipolytic activity in adipose tissue can be explored as potential anti-obesity agent.

8. AIMP1 structure and functional dissection

Although AIMP1 is recognized as cytosolic protein anchored to the multi-ARS complex, it seems to be present in other

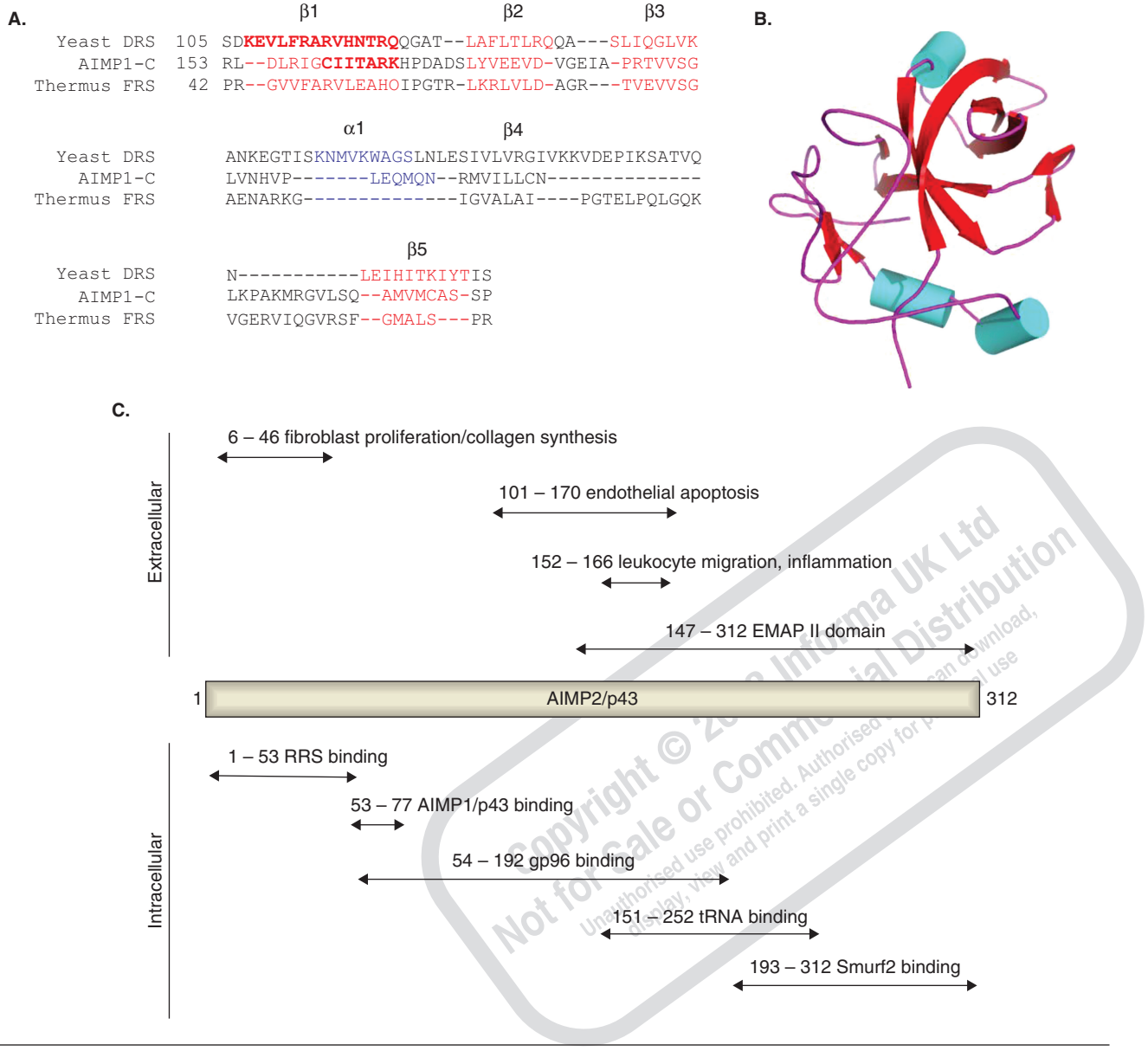

Figure 3. Structural feature of the C-terminal domain of AIMP1 and different activities along its polypeptide. A. AIMP1 C-terminal domain (aa 153 – 312) was aligned with the homologous regions of *Saccharomyces cerevisiae* aspartyl-tRNA synthetase (DRS) and *Thermus theromphilus* phenylalanyl-tRNA synthetase (FRS) with slight modification of the original data [92]. The α helical and β-sheet structures were marked with blue and red letters, respectively. **B.** Three-dimensional structure of the C-terminal domain of AIMP1 [92]. **C.** Different peptide regions of AIMP1 responsible for the indicated activities [91]. The peptide region of AIMP1 involved in the interaction with Smurf2, the negative regulator of TGF-β signal pathway, was recently identified [102].

intra- and extracellular locations playing diverse roles as described above [90,91]. Based on the caspase cleavage site, 312 aa human AIMP1 can be divided to the 146 aa N-terminal and the 166 aa C-terminal domains. Although the 3D structure of the whole protein is not yet determined, the crystal structure of the C-terminal domain structure was solved at high resolution [92,93]. As can be seen, the C-terminal domain containing OB fold shares a structural similarity with those of aspartyl-tRNA synthetase and phenylalanyl-tRNA synthetase of lower organisms (**Figure 3A** and **B**) [45,93]. The crystal structure for the EMAPII domain in three-stranded β-sheet (β1 – β3) and one-α helix (α1) was also identified to be structurally homologous with some chemokines [92], such as RANTES [94], human monocyte chemoattractant protein [95], and neutrophil-activating peptide-2 [96]. The 152 – 166 aa peptide containing RIGRIIT motif is also present in tyrosyl-tRNA synthetase (YRS), *Caenorhabditis elegans* MRS and *Saccharomyces cerevisiae* Arc1p. Although this peptide sequence of AIMP1 and YRS is thought to be

Table 1. The list of proteins with the potential to interact with AIMP1.

Gene	Full name	Database source	Ref.
ATP5A1	ATP synthase alpha subunit 1	–	[81]
BRCA2	Breast cancer 2, early onset	Rhodes2005	[99]
MAPK14	Mitogen activated protein kinase 14	Rual2005	[100]
MAPK14	Mitogen activated protein kinase 14	HPRD:02619/04676	[41]
DARS	Aspartyl-tRNA synthetase	Rhodes2005	[99]
EIF1AX	Eukaryotic translation initiation factor 1A, X-linked	Rhodes2005	[99]
EIF2S1	Eukaryotic translation initiation factor 2, subunit 1 alpha	Rhodes2005	[99]
EPRS	Glutamyl-prolyl-tRNA synthetase	Rhodes2005	[99]
ERCC1	Excision repair cross-complementing rodent repair deficiency1	HomoMINT:12394	–
ERCC1	Excision repair cross-complementing rodent repair deficiency1	Rhodes2005	[99]
GARS	Glycyl-tRNA synthetase	Rhodes2005	[99]
GCLC	Glutamate-cysteine ligase, catalytic subunit	Rhodes2005	[99]
HARS	Histidyl-tRNA synthetase	Rhodes2005	[99]
IARS	Isoleucyl-tRNA synthetase	Rhodes2005	[99]
LIG1	Ligase I, DNA, ATP-dependent	Rhodes2005	[99]
LIG4	Ligase IV, DNA, ATP-dependent	Rhodes2005	[99]
MARS	Methionyl-tRNA synthetase	HPRD:08864	[41]
MARS	Methionyl-tRNA synthetase	Rhodes2005	[99]
NARS	Asparaginyl-tRNA synthetase	Rhodes2005	[99]
YBX1	Y box binding protein 1	Rhodes2005	[99]
POLR2G	RNA polymerase II polypeptide G	Rhodes2005	[99]
RARS	Arginyl-tRNA synthetase	HPRD:00142/04676	[44]
RARS	Arginyl-tRNA synthetase	Rhodes2005	[99]
RPA1	Replication protein A1, 70 kDa	Rhodes2005	[99]
RPA3	Replication protein A3, 14 kDa	Rhodes2005	[99]
MRPS12	Mitochondrial ribosomal protein S12	Rhodes2005	[99]
RPS11	Ribosomal protein S11	Rhodes2005	[99]
RPS23	Ribosomal protein S23	Rhodes2005	[99]
CLEC11A	C-type lectin domain family 11,member A	Rhodes2005	[99]
HSP90B1	gp96/heat-shock protein 90 kDa beta, member 1	–	[23]
JTV1	AIMP2/p38	–	[46]
MAD1L1	Mitotic arrest deficient-like 1	J_Lim2006	[101]
MAD1L1	Mitotic arrest deficient-like 1	HomoMINT:38129	–
PLA2G6	Phospholipase A2, group VI	HomoMINT:43418	–
CSDA	Cold shock domain protein A	Rhodes2005	[99]
YARS	Tyrosyl-tRNA synthetase	Rhodes2005	[99]
KHSRP	KH-type splicing regulatory protein	Rhodes2005	[99]
EIF1AY	Eukaryotic translation initiation factor 1A, Y-linked	Rhodes2005	[99]
ATG12	Autophagy related 12 homolog	Rhodes2005	[99]
SCYE1	AIMP1/small inducible cytokine subfamily E, member 1	HPRD:04676	[92]
SCYE1	AIMP1/small inducible cytokine subfamily E, member 1	Rual2005	[100]
SCYE1	AIMP1/small inducible cytokine subfamily E, member 1	HomoMINT:29540/4644	–

Table 1. The list of proteins with the potential to interact with AIMP1 (continued).

Gene	Full name	Database source	Ref.
EFTUD2	Elongation factor Tu GTP binding domain containing 2	Rhodes2005	[99]
AIM2	Absent in melanoma 2	Rhodes2005	[99]
HOMER2	Homer homolog 2	HomoMINT:28433	–
HOMER1	Homer homolog 1	Rhodes2005	[99]
HMG20B	High-mobility group 20B	HomoMINT:44126	–
FARS2	Phenylalanyl-tRNA synthetase 2, mitochondrial	Rhodes2005	[99]
XPOT	Exportin, tRNA (nuclear export receptor for tRNAs)	Rhodes2005	[99]
HARSL	Histidyl-tRNA synthetase 2, mitochondrial	Rhodes2005	[99]
CCDC22	Coiled-coil domain containing 22	HomoMINT:44127	–
CCDC22	Coiled-coil domain containing 22	Rhodes2005	[99]
MRPL2	Mitochondrial ribosomal protein L2	Rhodes2005	[99]
MRPS17	Mitochondrial ribosomal protein S17	Rhodes2005	[99]
EIF5A2	Eukaryotic translation initiation factor 5A2	Rhodes2005	[99]
SMURF2	SMAD specific E3 ubiquitin protein ligase 2	–	[102]
MTMR9	Myotubularin related protein 9	HomoMINT:42342	–
GC11102	EIF1AD/eukaryotic translation initiation factor 1A domain containing	Rhodes2005	[99]

responsible for monocyte and leukocyte migration, as well as for inflammation response [97,98], MRS and Arc1p do not manifest a similar activity, suggesting that some other factors are necessary for cytokine activity. With this structural feature, AIMP1 has an affinity to nucleic acids including tRNAs [49]. Within the multi-ARS complex, it also binds to AIMP2 through its peptide spanning 53 – 77 aa, in addition to RRS, working as core protein for the assembly and stability of the whole complex. For the sequestration of gp96, it seems to use the peptide of 54 – 192 aa (**Figure 3C**) [23,46]. Refined deletion mapping further dissected the peptide regions based on their functional involvement (**Figure 3C**), namely the 6 – 46, 101 – 114, 114 – 192 aa regions are dedicated for fibroblast proliferation/collagen synthesis, endothelial cell apoptosis/caspase-3 activation and endothelial cell migration, respectively [91].

In this paper, we collected all the known and predicted molecular partners of AIMP1, which are obtained from the published reports and available databases (**Table 1**) and then generated the predicted linkage map of AIMP1 (**Figure 4**). Although many of the AIMP1-linked proteins in this diagram need to be experimentally validated, this proposed interaction network of AIMP1 implies that there are still more functions to be found from this factor. In particular, heavy connection to nuclear factors suggests its potential role in nuclear processes. Based on these functions and diverse molecular interactions, AIMP1 may be one of the protein hubs working at systems level serving as a coordinator of diverse cellular processes.

9. Expert opinion

Although all human proteins could be pathologically associated with a certain disease as long as they have biological functions, they would not necessarily make druggable targets or agents in practice for various shortcomings. Among several criteria required for a 'druggable' condition, functional specificity of the target protein is highly valued to avoid undesirable side effect or toxicity and for the convenience of activity control. However, even if we find a protein that seems to be specifically dedicated to a single activity, its functional specificity would be diluted through the complex network as at least one of the proteins connected to this protein is multi-linked to other biological processes. Thus, it would be somewhat unrealistic to expect that a drug would give only the expected activity from its target site. Even those that are thought to be dedicated to a specific function may actually have more functions as we further investigate them. In this respect, AIMP1 provides a good example. Although there was little knowledge on its function of AIMP1 several years ago, it is now expected to be a hub protein whose functions are linked to extremely diverse biological processes as illustrated here. Considering the complexity of our body system, we may now have to reconsider whether it is realistic or practical to chase the proteins with just a single function. Instead, we may want to explore multi-functional proteins more strategically to maximize the efficacy of the desired function whereas minimizing the side effect.

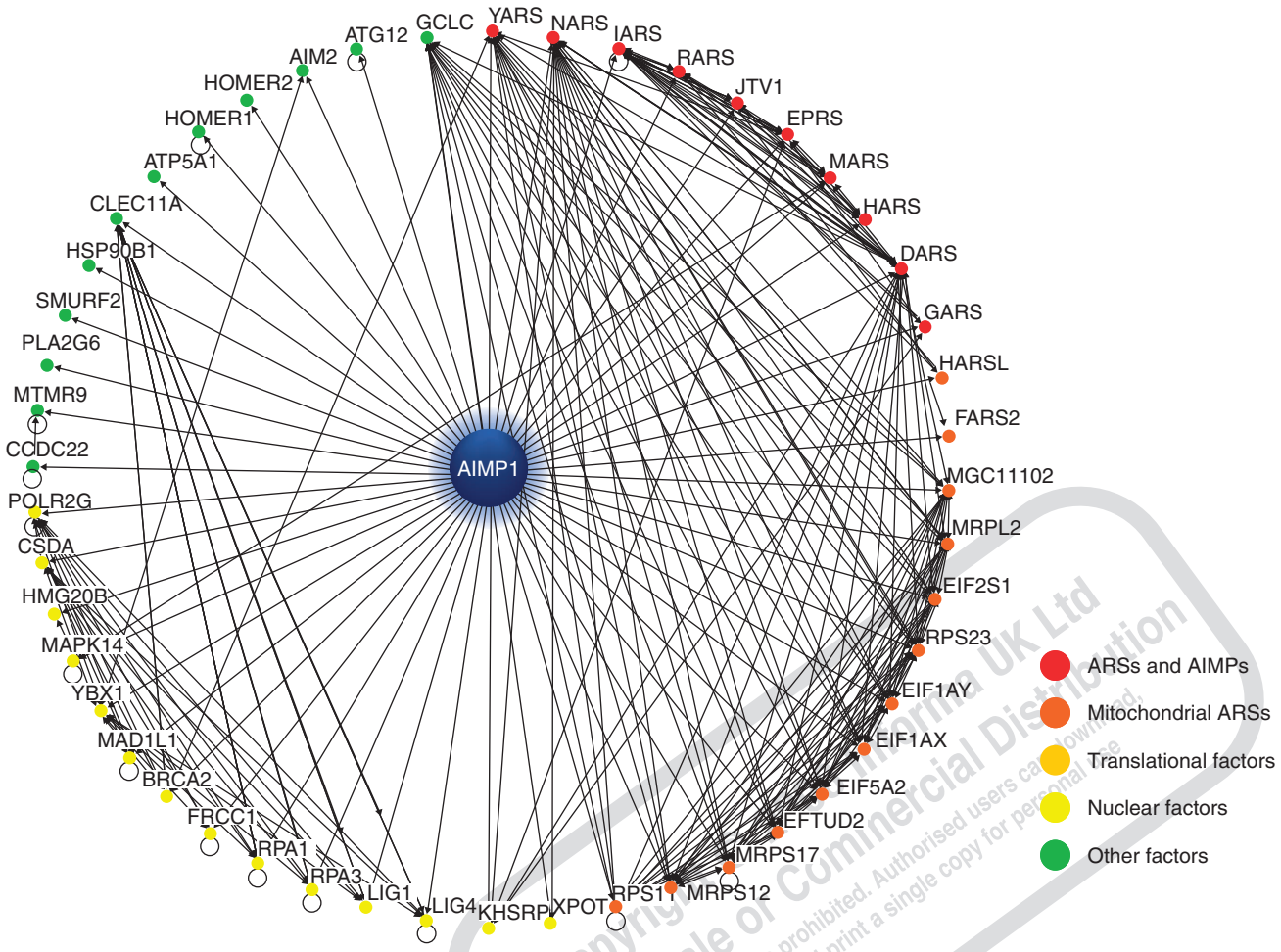

Figure 4. Interaction network of AIMP1 with its potential target proteins. The suggested AIMP1-associated proteins were collected from the databases of HPRD [103], Rhodes 2005 [99], HomoMINT [104], Rual2005 [100] and other reports describing the molecular interactions including AIMP1/p43. The linkages of AIMP1 in this diagram do not necessarily mean direction interaction with AIMP1 and most of the suggested AIMP1-interacting partners should be experimentally validated except for a few cases whose interactions were proven such as RRS (arginyl-tRNA synthetase), AIMP2/p38, gp96 and Smurf2. The detailed information for each target is shown in **Table 1**. The dynamic graphic view of AIMP1-interaction network can be also obtained at http://pharmdb.org.
AIMP: ARS-interacting multi-functional proteins; ARS: Aminoacyl-tRNA synthetase.

Protein network at physiological level seems to have hierarchical structure. In this structure, some proteins would be positioned at the top level in the signaling network, being involved in multiple pathways, whereas others would be located in the downstream of the network, dedicated to specific roles. As a drug touches downstream targets, its action could be more specific but its impact may be weak or limited. In contrast, a multi-functional target protein may be involved in each individual pathway less strongly and specifically but its total impact on whole body system could be stronger. This would be particularly true in the case of cancer targets. Because anticancer drugs need to kill or suppress cancer cells, they may prefer the targets that would efficiently disrupt the viability of cancer cells although keeping the normal cells intact. In practice, it is not easy to eradicate cancer cells by inhibiting a single cancer-associated target as biological systems are pretty resilient to disturbance. For this reason, many drug companies are designing the combination therapy to generate synergy among the different drug targets. If we explore a target working at multiple directions against cancer, it would give an effect similar to the combination therapy hitting multiple targets simultaneously. In this regard, the multi-functionality of AIMP1 can be considered as advantage for therapeutic usage. For instance, AIMP1 can effectively suppress cancer by the combination of its angiostatic and immune-stimulating activities. Similarly, antiAIMP1 could give synergistic effect on wound repair by boosting fibroblast

proliferation, collagen production and the generation of keratinocyte growth factor simultaneously.

As the development of 'omics' increases, many hidden functions and unexpected connections among the known proteins are continuously unveiled. Considering much less number of protein-coding genes than expected, one can even predict that multi-functionality of proteins would be more dominant than single functionality. If this is really the case, it would become more difficult to chase 'selectivity' of a protein function as they are all interconnected. Thus, it is about time to accept the reality of our body system, which consists of complex networks of proteins with multiple functions and design drugs based on systems perspectives. If we cannot avoid the side effect resulting from the multi-directional network surrounding a target, we should begin to look into the highly effective target proteins working at systems level.

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Declaration of interest

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