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Noncanonical function of threonyl-tRNA synthetase regulates vascular development in zebrafish

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ABSTRACT

The canonical functions of Aminoacyl-tRNA synthetases (AARSs) are indispensable for protein synthesis. However, recent evidence indicates that some AARSs possess additional biological functions (noncanonical functions) related to immune responses and vascular development. Here, we identified a zebrafish *cq16* mutant presenting the disorganized vessels with abnormal branching of the established intersegmental vessels (ISVs) as well as aberrant patterning of the brain vascular network after 50 h post fertilization. The *cq16* mutant gene is responsible for encoding threonyl-tRNA synthetase (*tars*) with a missense mutation. The abnormal branching of ISVs was caused by the increased expression of vascular endothelial growth factor A (*vegfa*) in $tars^{cq16}$ mutant. Inhibition of Vegf signaling suppresses the abnormal vascular branching observed in $tars^{cq16}$ mutant. Furthermore, injection of human *TARS* mRNA potently reduced the vascular aberrant branching in $tars^{cq16}$ mutant, indicating a conserved function of *tars* in regulating angiogenesis between zebrafish and human. Therefore, we conclude that noncanonical function of *tars* regulates vascular development presumably by modulating the expression of *vegfa*.

1. Introduction

During embryonic development, endothelial cells assemble the tubular network of blood vessels that transport gases, nutrients, hormones, and metabolites throughout the vertebrate body. The network of blood vessels is one of the earliest structures to develop in a vertebrate embryo. It plays important roles in regulating tissue homeostasis and would healing and is involved in the pathology of numerous diseases including inflammation and cancer [1]. In recent years, zebrafish has become an important vertebrate model for studying vascular development. Zebrafish has a closed circulatory system and the molecular mechanisms controlling angiogenesis are highly conserved between zebrafish and mammals. As a model, zebrafish provide a unique advantage in the study of vascular development *in vivo*. Zebrafish is suitable for large scale forward genetic analysis, which makes it advantageous for screening vascular defect mutants to identify new genes that regulate vascular development. Additionally, transparent zebrafish embryo expressing fluorescent protein under the control of *flk1* or *fli1a* promoter allowed us to visualize and analyze the vascular development.

Although the vascular system is complex, almost all blood vessels arise by the sprouting of new capillaries from pre-existing vessels, a process termed angiogenesis. In the past 20 years, the molecular mechanisms controlling angiogenesis have attracted much attention. Vascular endothelial growth factor (Vegf) signaling pathway plays central roles in vascular formation and function in vertebrates [2]. Vegf signaling mainly includes four kinds of ligands and corresponding receptors, and the master regulator of new blood vessel sprouting is *Vegfa* [2,3]. *Vegfa* combines its tyrosine kinase receptor Vegfr2 to activate a variety of downstream signaling pathways. It also controls several processes in endothelial cells, such as proliferation, migration and survival. The change of the *Vegfa* expression has a direct effect on angiogenesis. Hence, Heterozygous $Vegfa^{+/-}$ mice exhibit severe vascular defects [4]. Similarly, deficient vascular assembly is observed in Vegfr2-null mice [5]. The expression of *Vegfa* is mainly regulated by the hypoxia. The transcriptional factors that were reported to regulate

Abbreviations: AARSs, Aminoacyl-tRNA synthetases; ISVs, intersegmental vessels; *tars*, threonyl-tRNA synthetase; *sars*, seryl-tRNA synthetase; hpf, hours post fertilization; *Vegfa*, Vascular endothelial growth factor A; HUVECs, human umbilical vein endothelial cells.

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Vegfa expression consist of HIF, stat3, Sp1/Sp3, AP etc. [6]. Thus, identification of new transcriptional factors for regulating *Vegfa* expression has an important role in revealing the molecular mechanisms regulating angiogenesis.

Aminoacyl-tRNA synthetases (AARSs) specially attach amino acids to their corresponding tRNA adaptors in an essential reaction of protein synthesis. In addition to their aminoacylation activities (canonical activity), recent evidence indicates that AARSs possess additional biological functions (noncanonical activities), including the regulation of angiogenesis, translation, inflammatory response and apoptosis [7]. For example, tyrosyl-tRNA synthetase (YARS) can stimulate endothelial cell proliferation and migration and regulate inflammatory responses [8,9]; tryptophanyl-tRNA synthetase (WARS) apparently binds VE-cadherin to inhibit angiogenesis [10]; glutamyl-prolyl-tRNA synthetase (EPRS) as an intergral component of the GAIT complexes inhibits angiogenesis through the translational silencing of *Vegfa* [11]. Of greater interest is that mutations in zebrafish *sars* gene encoding seryl-tRNA synthetase cause abnormal vascular sprouting in trunk and head [12,13]. Although YARS, WARS and EPRS can regulate angiogenesis in cell culture, only *sars* is reported to regulate vascular development *in vivo*. It is not fully understood whether other AARSs contribute to the establishment of vascular patterning in vertebrates.

In this study, we identified a mutation in *tars* leading to ISV abundant sprouting in zebrafish and demonstrated that the noncanonical function of *tars* is involved in the regulation of vascular patterning by mediating Vegf signaling.

2. Materials and methods

2.1. Zebrafish strains

The Zebrafish (*Danio rerio*) of the AB genetic background *Tg(flk1:GFP)* transgenic line and *cq16* mutant line were raised and maintained under standard laboratory conditions according to institutional animal care and use committee protocols.

2.2. Genetic mapping

The *cq16* locus was defined by genotyping of *cq16* mutant embryos using simple sequence length polymorphic (SSLP) markers on chromosome 5. We finally identified a missense mutation in the zebrafish *tars* gene at 1505 bp (substitution from A to G), resulting in a glutamine converted to a Arginine at amino acid 502. To perform genotyping of the $tars^{cq16}$ mutation, the *tars* locus was amplified from the isolated genomic DNA by PCR using the following primers: *tars* sense, 5-GTCTTTTTCTGTGGTACAG-3; *tars* antisense, 5-GTTTCACTGAGTAAACACACC-3.

2.3. Microinjection of synthetic mRNAs

Total RNA was extracted using Trizol (Life Technologies) and reverse transcribed to cDNA using Omniscript reverse transcriptase kit (QIAGEN). Zebrafish *tars*, *sars* and human *TARS* cds fragment were amplificated and sub-cloned into the *PCS2+*, the mRNA expression vector. Capped mRNAs were synthesized by using the mMESSAGE mMACHINE (Ambion). Synthetic mRNAs were injected into the blastomere of 1- to 2-cell-stage embryos.

2.4. Treatment of vegfr inhibitor

The $tars^{cq16}$ mutant embryos were treated with Vegfr inhibitor SU5416 (0.8 μmol/L, Sigma) from 36 to 60 hpf, or 0.1% DMSO as control.

2.5. Whole-Mount in Situ Hybridization and quantitative real-time PCR

In situ hybridization and quantitative real-time PCR was performed as previously described [14]. The primers used for amplification were as previously described [12]. The synthetic probes were as follows: *tars* primers, 5-TGTGTGCCATTGAATAAGGA-3 and 5-CACCTTCATTATCAAGATAC-3.

2.6. Cells culture and immunostaining

Human umbilical vein endothelial cells (HUVECs, ALLCELLS) were raised according to standard protocol. Cell immunostaining was performed as previously described [15] using antibodies against TARS (1:100; Santa Cruz Biotechnology), green fluorescent protein (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). Antibody stained tissues were imaged using ZEN2010 software equipped on an LSM780 confocal microscope (Carl Zeiss).

2.7. CRISPR/cas9

The CRISPR/Cas9 was performed as described [16,17] and the *tars* target site sequence in Fig. 2G.

3. Results

3.1. Cq16 mutant exhibits abnormal vascular sprouting in trunk and head

In a recent N-ethyl N-nitrosourea mutagenesis screen, we identified an embryonic lethal mutant *cq16* that had defects in vascular patterning. By observing *Tg(flk1: GFP)* expression, we noticed no difference between wild type (WT) and *cq16* mutant at 40 hpf (Fig. 1A and B). However, by 50 hpf the *cq16* mutant started to exhibit ectopic ISV branching adjacent to the neural tube in the trunk (Fig. 1C and D). Subsequently, the abnormal ISV branching became more and more severe in the dorsal side of the horizontal myoseptum (Fig. 1E and F). Furthermore, we observed a pronounced dilatation of the aortic arch vasculature (Fig. 1G and H) and aberrant branching of the brain vascular network at 80 hpf (Fig. 1I and J). In bright filed, *cq16* mutant has a smaller head and thinner trunk than WT at 72 hpf (Fig. 1K). Although initially unaffected, circulation gradually diminishes and stops at 5 dpf. These results indicate that *cq16* is a vascular regulator.

3.2. The gene responsible for cq16 encodes threonyl-tRNA synthetase with a missense mutation

The *cq16* mutant phenotype is similar to *sars* mutant [12,13] except that the abnormal ISV branching in *cq16* mutant appears about ten hours earlier than that in *sars* mutant. We sequenced the *sars* mRNA in *cq16* mutant but there was no difference between WT and mutant, suggesting that the gene responsible for *cq16* is not *sars*. To identify the gene responsible for this mutant, we performed the genome mapping of *cq16*. Finally, we found that there existed a A-to-G transition at 1505 bp in the *tars* gene of *cq16* mutant (Fig. 2A and B), which converted a glutamine to a arginine at amino acid 502 (Fig. 2C). Hence, the gene responsible for *cq16* is *tars* with a missense mutation.

We examined the expression of *tars* during embryo development by whole-mount *in situ* hybridization. The *tars* transcripts were maternally supplied, as evidenced by its expression in 1-cell stage (Fig. 2D), indicating that maternal *tars* may redeem the lack of functional zygotic *tars* in the progression of initial embryogenesis in $tars^{cq16}$ mutants. In the following days, *tars* was expressed

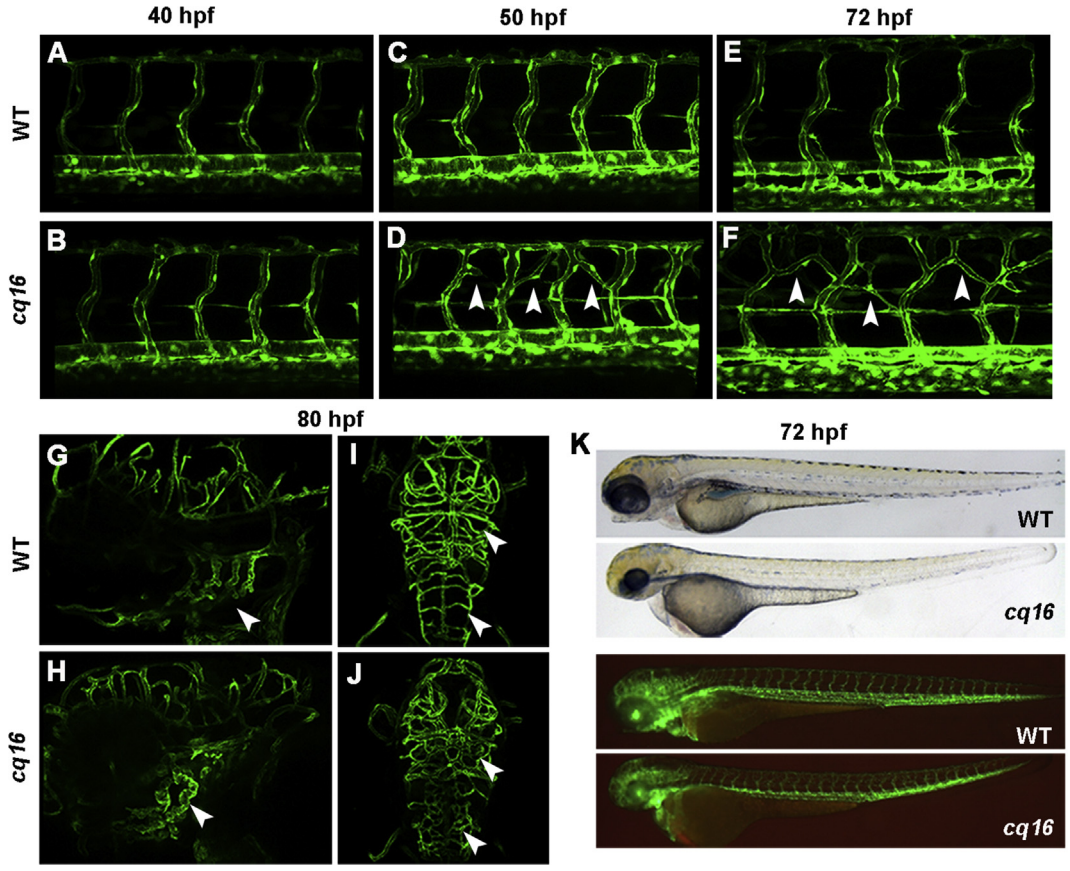

Fig. 1. *Cq16* mutant exhibits ectopic branching in the trunk and head vasculature. Blood vessels are visualized by GFP under the control of *flK1* promoter. (A–F). The abnormal ISV branching (arrowheads) in *cq16* mutants became time-dependently severe compared to those of WT. (G–J). Dilatation of the aortic arch vasculature (G, H) and aberrant branching of the brain vascular network (I, J) appear in *cq16* mutant at 80 hpf. (K). In bright filed, *cq16* mutant has a smaller head and thinner trunk than WT at 72 hpf.

ubiquitously in the embryo (Fig. 2D).

To confirm that *tars* is the gene responsible for *cq16*, we performed the rescue experiment. We injected the *tars* mRNA into $tars^{cq16}$ mutant and found the abnormal branching in $tars^{cq16}$ mutant was rescued to the WT pattern (Fig. 2E and F). We also knocked out *tars* gene in the WT fish by CRISPER/cas9. We designed the target site in exon1 and isolated 5bp deletion line predicted to produce a premature stop codon (Fig. 2G). The $tars^{cas9}$ mutants exhibited the same phenotype of $tars^{cq16}6$ mutants (Fig. 2H) which was also rescued by injection of *tars* mRNA (Fig. 2I). These results suggest that the abnormal ISV branching in $tars^{cq16}$ mutant appears to be ascribed to the loss of function of zygotic *tars*.

3.3. Noncanonical function of tars influences the vegfa expression

A well-known function of *tars* is to catalyze the ligation of threonine to its cognate tRNAs, resulting in threonyl-tRNA complexes necessary for translation of RNA into protein. However, As previously reported [12,13], blocking of protein synthesis by treatment with cycloheximide (CHX), a protein synthesis inhibitor, results in a thinning of the blood vessels, but not their ectopic ISV branching, suggesting that the noncanonical function of *tars*, instead of its canonical function, is involved in the regulation of vascular patterning.

Vegf signaling stimulates endothelial cell migration and proliferation by activating Vegf receptor tyrosine kinases [18]. Notably, gain-of-function of Vegf signaling leads to exuberant angiogenic sprouting [19], a phenotype that resembles the abnormal vascular branching observed in zebrafish $tars^{cq16}$ mutants. To explore whether *tars* regulates angiogenesis by mediating Vegf signaling, we assayed the expression of Vegf signaling molecules by quantitative real time PCR at 60 hpf, when the abnormal branching was obvious in $tars^{cq16}$ mutant. The expression of *vegfa* mRNA but not *vegfc*, *kdra/flk1*, or *vegfr3/flt4* was significantly increased in $tars^{cq16}$ mutants (Fig. 3A). Furthermore, we treated $tars^{cq16}$ mutant embryos from 36 to 60 hpf with the Vegf receptor inhibitor SU5416 and found that Inhibition of Vegf signaling abolished the ectopic ISV branching (Fig. 3B and C). So the abnormal branching of ISVs was caused by the increased expression of *vegfa* in $tars^{cq16}$ mutant. These data indicate that the noncanonical function of *tars* could regulate *vegfa* expression.

Recent evidence suggests that Sars is a transcriptional regulator for proper development of a functional vasculature by antagonizing c-Myc, a major transcription factor promoting VEGFA expression [20]. To clarify how *tars* influences the *vegfa* expression, we injected *sars* mRNA into the $tars^{cq16}$ mutant to explore whether *tars* adopts the way of *sars* to regulate the *vegfa* expression. However, *sars* mRNA was unable to reduce the abnormal branching in $tars^{cq16}$ mutant (Fig. 3D and E), indicating that the way *tars* used is different from that of *sars*.

3.4. The regulatory function of tars in vascular development is conserved between zebrafish and human

Aligning Tars protein sequence in five species shows that zebrafish Tars is highly homologous to mammalian TARS (Fig. 4A). Furthermore, Injection of highly homologous human *TARS* mRNA potently reduced the ectopic ISV branching in $tars^{cq16}$ mutant

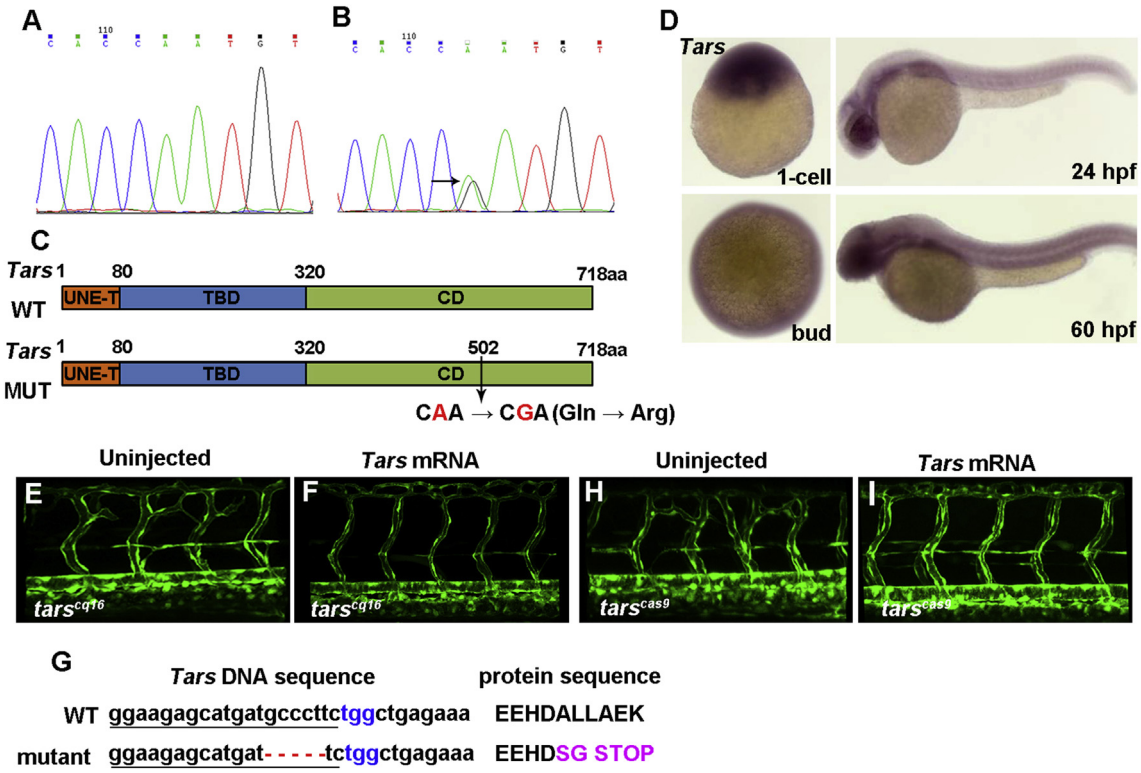

Fig. 2. The *cq16* mutant gene is *tars*. (A–C). The *tars* gene DNA sequence of WT (A) and *cq16* heterozygous (B) shows presence of a missense mutation in the zebrafish *tars* gene at 1505 bp (substitution from A to G), resulting in a glutamine converted to a Arginine at amino acid 502 (C), UNE-T: N-terminal appended domain; TBD: tRNA-binding domain; CD: catalytic domain. (D) Whole-Mount *in Situ* Hybridization of *tars* in zebrafish embryos shows *tars* transcript is maternally supplied and was expressed ubiquitously in the embryos. (E, F). The ectopic ISV branching in $tars^{cq16}$ mutants at 60 hpf are rescued by injection of zebrafish *tars* mRNA. (G). DNA and predicted protein sequences of WT and mutant *tars* allele. The *tars* target site is above line, blue indicates PAM, red dashed line indicates a deletion, and pink indicates altered amino acids. (H, I). CRISPR/Cas9-mediated *tars* knock-out fish exhibit the same phenotype with $tars^{cq16}$ mutants (H), which is also rescued by injection of zebrafish *tars* mRNA (I).

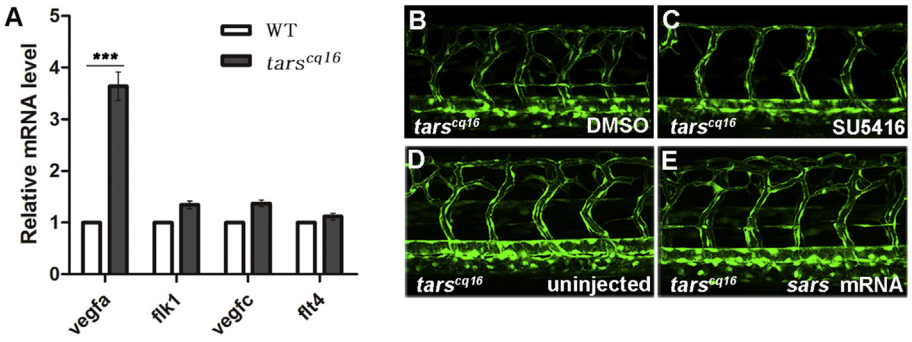

Fig. 3. *Tars* influences Vegf signaling by repressing the expression of *vegfa*. (A). Quantitative real-time PCR shows the *vegfa* mRNA level in $tars^{cq16}$ mutant is significantly upregulated compared to that of WT. (mean ± s.e.m, $^{***}P < 0.0001$, two tailed t-test, error bars indicate s.e.m). . (B, C). The ectopic ISV branching in $tars^{cq16}$ mutants at 60 hpf are reduced by treatment with SU5416. (D, E). There is no difference in vascular branching between uninjected (D) and *sars* mRNA injected mutants (E).

(Fig. 4B and C), suggesting that the regulatory function of *tars* in vascular development might be conserved between zebrafish and human. In general, AARSs are present in the cytoplasm for protein synthesis. However, we demonstrated nuclear localization of endogenous TARS protein in human umbilical vein endothelial cells (HUVECs) (Fig. 4D), indicating that *tars* may be another transcriptional regulator for development of a functional vasculature.

4. Discussion

Here we identified one AARSs, *tars*, that regulates vascular development throughout its noncanonical function. Previous studies have shown that TARS is secreted from endothelial cells in response to TNF-α and VEGF, and potently stimulates endothelial cell migration and angiogenesis *in vitro* [21]. Moreover, Inhibition of Tars with borrelidin derivative BC194, a potent inhibitor of TARS, in zebrafish suppresses angiogenesis [22]. A strong association between the tumor expression of TARS and advancing stage of epithelial ovarian cancer provides evidence that TARS is associated with angiogenesis in ovarian cancer [23]. However, no *in vivo* direct evidence shows that noncanonical function of *tars* regulates vascular development in vertebrates. In this present study, a mutation in zebrafish *tars* leads to abnormal branching of ISVs as well as aberrant vascular patterning of the brain, suggesting that loss of

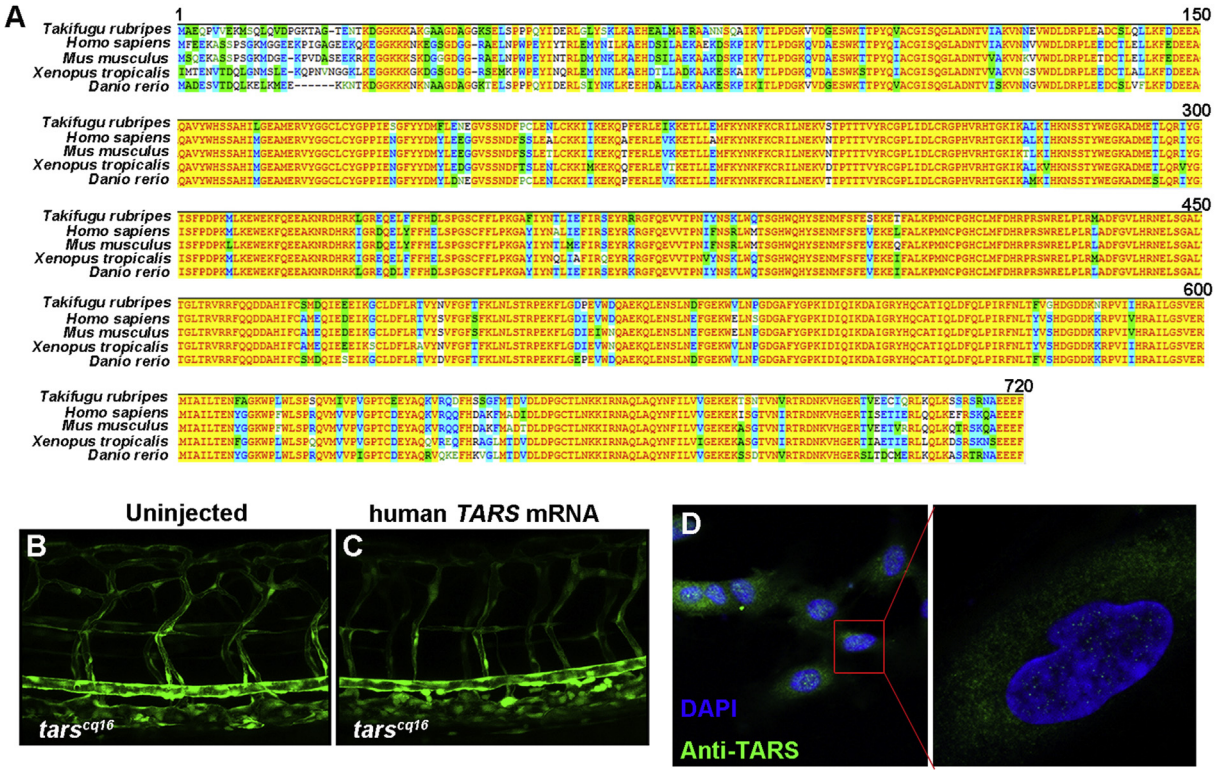

Fig. 4. It is conserved that *tars* regulates vascular development between zebrafish and human. (A). Aligning five species tars protein sequence shows highly homologous. (B, C). Injection of Human *TARS* mRNA can potently reduce the ectopic ISV branching in *tars*^{cq16} mutants, suggesting that the regulatory function of tars in vascular development is conserved between zebrafish and human. (D). Immunofluorescence of TARS in HUVECs shows part of TARS protein locates in nucleus.

tars function stimulates angiogenesis. Furthermore, we found that it was noncanonical function of *tars* that influenced angiogenesis by modulating the expression of *vegfa*. In addition, we demonstrated that part of TARS protein was distributed to the nucleus, indicating that TARS in the nucleus may directly regulate the transcription of *vegfa*. Further analysis is required to clarify how *tars* regulates the *vegfa* expression.

Recent evidence suggests that a unique domain (named UNE-S) at the C-terminus of SARS harbours nuclear localization signal directing SARS to the nucleus where it attenuates VEGFA expression [24]. However, the mutation site is not located in N-terminal appended domain (named UNE-T) but catalytic domain of TARS protein. One possibility is that the mutation may cause failure of TARS protein to bind promoters or other proteins. However, *Sars* mRNA is unable to abolish or reduce aberrant sprouting in *tars*^{cq16} mutants, indicating that TARS in nucleus may have its own unique way to regulate *vegfa* expression.

The *vegfa* mRNA is increased in *tars*^{cq16} mutants and the aberrant vascular branching phenotypes in *tars*^{cq16} mutants can be suppressed by blocking Vegf receptor function, indicating that *tars* may influence Vegf signaling. Since the abnormal vascular branching in *tars*^{cq16} mutants can be rescued by injection of human *TARS* mRNA, the noncanonical function of Tars should be conserved between zebrafish and human. This raises the possibility that impairment of human TARS may be associated with vascular diseases and that the zebrafish *tars*^{cq16} mutants may represent a human disease model. In addition, since *tars* affects Vegf signaling during zebrafish vascular development, *tars* may be a promising target for cancers and neovascular diseases. Further analysis of zebrafish and mammalian Tars should provide additional insights into the precise molecular mechanisms of vascular formation and remodeling.

Conflicts of interest

The authors disclose no conflicts.

Author contributions

L.L. and Z.C. designed the experimental strategy, analyzed data, and wrote the manuscript. Z.C. performed all the experiments except plasmid construction. H.W. provided the *cq16* mutant line. X.M. performed plasmid construction.

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References

[1] P. Carmeliet, Angiogenesis in health and disease, Nat. Med. 9 (2003) 653–660.
[2] L. Coultas, K. Chawengsaksophak, J. Rossant, Endothelial cells and VEGF in vascular development, Nature 438 (2005) 937–945.
[3] M. Lohela, M. Bry, T. Tammela, K. Alitalo, VEGFs and receptors involved in angiogenesis versus lymphangiogenesis, Curr. Opin. Cell Biol. 21 (2009) 154–165.
[4] P. Carmeliet, V. Ferreira, G. Breier, S. Pollefeyt, L. Kieckens, M. Gertsenstein, M. Fahrig, A. Vandenhoeck, K. Harpal, C. Eberhardt, C. Declercq, J. Pawling, L. Moons, D. Collen, W. Risau, A. Nagy, Abnormal blood vessel development

and lethality in embryos lacking a single VEGF allele, Nature 380 (1996) 435–439.
[5] F. Shalaby, J. Rossant, T.P. Yamaguchi, M. Gertsenstein, X.F. Wu, M.L. Breitman, A.C. Schuh, Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice, Nature 376 (1995) 62–66.
[6] G. Pages, J. Pouyssegur, Transcriptional regulation of the Vascular Endothelial Growth Factor gene–a concert of activating factors, Cardiovasc. Res. 65 (2005) 564–573.
[7] S.G. Park, P. Schimmel, S. Kim, Aminoacyl tRNA synthetases and their connections to disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11043–11049.
[8] K. Wakasugi, P. Schimmel, Two distinct cytokines released from a human aminoacyl-tRNA synthetase, Science 284 (1999) 147–151.
[9] K. Wakasugi, B.M. Slike, J. Hood, K.L. Ewalt, D.A. Cheresh, P. Schimmel, Induction of angiogenesis by a fragment of human tyrosyl-tRNA synthetase, J. Biol. Chem. 277 (2002) 20124–20126.
[10] K. Wakasugi, B.M. Slike, J. Hood, A. Otani, K.L. Ewalt, M. Friedlander, D.A. Cheresh, P. Schimmel, A human aminoacyl-tRNA synthetase as a regulator of angiogenesis, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 173–177.
[11] P.S. Ray, P.L. Fox, A post-transcriptional pathway represses monocyte VEGF-A expression and angiogenic activity, EMBO J. 26 (2007) 3360–3372.
[12] H. Fukui, R. Hanaoka, A. Kawahara, Noncanonical activity of seryl-tRNA synthetase is involved in vascular development, Circulation Res. 104 (2009) 1253–1259.
[13] W. Herzog, K. Muller, J. Huisken, D.Y. Stainier, Genetic evidence for a noncanonical function of seryl-tRNA synthetase in vascular development, Circ. Res. 104 (2009) 1260–1266.
[14] H. Lu, J. Ma, Y. Yang, W. Shi, L. Luo, EpCAM is an endoderm-specific Wnt derepressor that licenses hepatic development, Dev. Cell 24 (2013) 543–553.
[15] J. He, H. Lu, Q. Zou, L. Luo, Regeneration of liver after extreme hepatocyte loss occurs mainly via biliary transdifferentiation in zebrafish, Gastroenterology 146 (2014) 789–800 e788.
[16] N. Chang, C. Sun, L. Gao, D. Zhu, X. Xu, X. Zhu, J.W. Xiong, J.J. Xi, Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos, Cell Res. 23 (2013) 465–472.
[17] A. Hruscha, P. Krawitz, A. Rechenberg, V. Heinrich, J. Hecht, C. Haass, B. Schmid, Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish, Development 140 (2013) 4982–4987.
[18] A.K. Olsson, A. Dimberg, J. Kreuger, L. Claesson-Welsh, VEGF receptor signalling - in control of vascular function, Nature Rev. Mol. Cell Biol. 7 (2006) 359–371.
[19] H. Gerhardt, M. Golding, M. Fruttiger, C. Ruhrberg, A. Lundkvist, A. Abramsson, M. Jeltsch, C. Mitchell, K. Alitalo, D. Shima, C. Betsholtz, VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia, J. Cell Biol. 161 (2003) 1163–1177.
[20] Y. Shi, X. Xu, Q. Zhang, G. Fu, Z. Mo, G.S. Wang, S. Kishi, X.L. Yang, tRNA synthetase counteracts c-Myc to develop functional vasculature, eLife 3 (2014) e02349.
[21] T.F. Williams, A.C. Mirando, B. Wilkinson, C.S. Francklyn, K.M. Lounsbury, Secreted threonyl-tRNA synthetase stimulates endothelial cell migration and angiogenesis, Sci. Rep. 3 (2013) 1317.
[22] A.C. Mirando, P. Fang, T.F. Williams, L.C. Baldor, A.K. Howe, A.M. Ebert, B. Wilkinson, K.M. Lounsbury, M. Guo, C.S. Francklyn, Aminoacyl-tRNA synthetase dependent angiogenesis revealed by a bioengineered macrolide inhibitor, Sci. Rep. 5 (2015) 13160.
[23] T.L. Wellman, M. Eckenstein, C. Wong, M. Rincon, T. Ashikaga, S.L. Mount, C.S. Francklyn, K.M. Lounsbury, Threonyl-tRNA synthetase overexpression correlates with angiogenic markers and progression of human ovarian cancer, BMC Cancer 14 (2014) 620.
[24] X. Xu, Y. Shi, H.M. Zhang, E.C. Swindell, A.G. Marshall, M. Guo, S. Kishi, X.L. Yang, Unique domain appended to vertebrate tRNA synthetase is essential for vascular development, Nat. Commun. 3 (2012) 681.