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Noncanonical Activity of Seryl-Transfer RNA Synthetase and Vascular Development

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Abstract

Seryl-transfer RNA synthetase (Sars) is one of the 20 aminoacyl-transfer RNA synthetases that are enzymes essential for protein synthesis; however, the developmental function of Sars has not been elucidated. In zebrafish, impairment of zygotic Sars function leads to a significant dilatation of the aortic arch vessels and aberrant branching of cranial and intersegmental vessels. This abnormal vascular branching in sars mutants can be suppressed by a form of Sars that lacks canonical function, indicating that a noncanonical activity of Sars regulates vascular development. Inhibition or knockdown of vascular endothelial growth factor (Vegf) signaling, which plays pivotal roles in the establishment of the vascular network, suppresses the abnormal vascular branching observed in sars mutants. Here, we discuss the possible functional relationship between Sars function and Vegf signaling.

Introduction

Aminoacyl-transfer RNA (tRNA) synthetases (AARSs) catalyze the ligation of amino acids to their cognate tRNAs; this canonical function of AARSs is indispensable for protein synthesis. Recent evidence indicates that AARSs possess additional biologic functions (noncanonical activities), including the regulation of translation, inflammatory response, and apoptosis (Park et al. 2008), leading to the hypothesis that AARSs have acquired a diversity of novel functions. Notably, several AARSs are also involved in angiogenesis in different contexts (Table 1). For example, tyrosyl-tRNA synthetase (YARS) is cleaved by several endogenous enzymes, resulting in the production of an N-terminal fragment (Wakasugi and Schimmel 1999). This fragment is secreted and can stimulate endothelial cell proliferation and migration (Wakasugi et al. 2002a). On the other hand, an N-terminal truncated form of tryptophanyl-tRNA synthetase (WARS), whose production is induced by interferon- γ (IFN- γ), inhibits vascular endothelial growth factor (Vegf)-induced endothelial cell migration (Wakasugi et al. 2002b), suggesting that this fragment of WARS functions as an angiostatic factor. In addition, glutamyl-prolyl-tRNA synthetase (EPRS) inhibits angiogenesis through the translational silencing of Vegf-a (Ray and Fox 2007). Thus, although products from YARS, WARS, and EPRS loci can regulate angiogenesis in cell culture, it is not fully understood

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whether AARSs contribute to the establishment of vascular patterning in vertebrates. In this review, we discuss the novel noncanonical function of Seryl-tRNA synthetase (Sars) in vascular development.

Noncanonical Activity of Sars is Involved in Vascular Development

Because vascular patterning in vertebrates is established through a highly complex process, identification of new vascular regulators enables us to better understand the cellular and molecular mechanisms of vascular network formation and remodeling. Functional analyses of the zebrafish *adrasteia* (*adr*) mutant, in which *sars* is mutated, show that Sars is a novel regulator of vascular development (Jin et al. 2007, Fukui et al. 2009, Herzog et al. 2009). Zebrafish *adr/sars* mutants show a dilatation of aortic arch vessels (Herzog et al. 2009) and aberrant sprouting of cranial and intersegmental vessels at 3 days postfertilization (dpf) (Figure 1), whereas no clear difference in vascular development is observed at 2 dpf between wild-type siblings and *adr/sars* mutants (Fukui et al. 2009, Herzog et al. 2009). To confirm the contribution of zygotic *sars* in vascular development, we designed a splice blocking *sars* morpholino that specifically targets zygotic but not maternal transcripts. We found that knockdown of Sars by this morpholino caused vascular defects similar to those observed in *adr/sars* mutants (Fukui et al. 2009). Furthermore, wild-type *sars* messenger RNA (mRNA) injections rescued the intersegmental branching vessels in *adr/sars* mutants (Figure 1). Therefore, we concluded that loss of zygotic Sars function causes disorganization of vascular patterning.

One important question is why the *adr/sars* mutants develop during early embryogenesis without apparent morphologic phenotypes and specifically present vascular patterning defects at later stages. The *sars* transcripts are strongly detected in one-cell stage embryos (Fukui et al. 2009, Herzog et al. 2009). During the progression of embryogenesis, ubiquitous *sars* expression is decreased in *sars*^{ko095} mutants, presumably caused by nonsense-mediated decay of zygotic *sars* transcripts. We also found that Sars^{ko095} lacks aminoacylation/canonical activity (Figure 1). Complete loss of maternal and zygotic Sars function would abrogate protein synthesis and generally affect early embryogenesis. Thus, one likely explanation for the lack of early phenotypes in *adr/sars* mutant is that maternally supplied mRNA and protein initially compensate for the lack of zygotic Sars function.

Blocking of protein synthesis by treatment with cycloheximide leads to a thinning of the blood vessels but not their aberrant sprouting (Fukui et al. 2009) or dilatation (Herzog et al. 2009), suggesting that a noncanonical activity of Sars, instead of its canonical activity, is involved in vascular development. This interpretation is strongly supported by the finding that the abnormal vascular branching phenotype of *sars* mutants is rescued by the forced expression of an enzymatically inactive form of *sars*, *T429A* (Figure 1) (Fukui et al. 2009).

Sars and Vegf Signaling

The Vegf signaling pathway plays central roles in blood vessel formation and function in vertebrates (Coults et al. 2005). Vascular endothelial growth factor stimulates endothelial cell proliferation and migration by activating Vegf receptor tyrosine kinases (Olsson et al. 2006). Notably, gain-of-function of Vegf signaling leads to ectopic branching of blood vessels (Gerhardt et al. 2003), a phenotype that resembles the abnormal vascular branching observed in zebrafish *sars* mutants. Treatment of *sars* mutant embryos with the Vegf receptor inhibitor SU5416 (Jia et al. 2004) suppressed the abnormal vascular branching defects (Fukui et al. 2009), as well as the vascular dilatation phenotype (Herzog et al. 2009). Furthermore, the aberrant vascular sprouting in *sars* mutants was effectively inhibited not only by knockdown of *vegf receptor* genes but also by knockdown of *vegf-a* (Fukui et al. 2009). Therefore, these

findings suggest that excessive Vegf signaling contributes to the abnormal vascular branching and vascular dilatation observed in *sars* mutants.

Sars appears to affect Vegf-mediated signal transduction in human endothelial cells (Figure 2). Human umbilical vein endothelial cells (HUVECs) cultured on a gel-like extracellular matrix substrate can be induced to form tubes upon exposure to Vegf (Davis and Camarillo 1996). When Sars expression was reduced in HUVECs by *Sars*-small interfering RNA transfection, the number of branchpoints in the network appeared to increase (Figure 2) (Herzog et al. 2009). Thus, knockdown of Sars enhances Vegf-induced endothelial sprouting. It has recently been reported that the *N*-terminal YARS fragment and the *N*-terminal truncated form of WARS possess cytokine-like activities (Table 1). Tumor necrosis factor- α stimulation produces the *N*-terminal YARS fragment in endothelial cells, and this YARS fragment activates Vegf signaling through the transactivation of Vegf receptor-2 (Vegfr2) (Greenberg et al. 2008). The *N*-terminal truncated form of WARS is produced by IFN- γ signaling in monocytes and keratinocytes. This WARS fragment inhibits Vegf-induced angiogenesis (Wakasugi et al. 2002b), and it binds to VE-cadherin on the surface of endothelial cells and subsequently inhibits Vegf-induced endothelial cell migration (Tzima et al. 2005). It is currently unclear whether Sars is secreted from endothelial cells and how Sars affects Vegf signaling. It is thus important to further investigate Sars function during vascular development.

Primary angiogenic sprouts from the dorsal aorta form the intersegmental vessels at 1 dpf. Because *sars* is strongly expressed in somites at 1 dpf, we examined whether Sars in somites affected vascular network formation. Transplantation analyses showed that wild-type donor cells incorporated into somitic tissue in *sars* mutants prevented the ectopic vascular sprouting around the transplanted area (Fukui et al. 2009). In contrast, when donor cells derived from *sars* mutants incorporated into somitic tissue in wild-type recipients, aberrant branching of the intersegmental vessels was induced around the transplanted cells. Thus, Sars expressed in somites can affect vascular patterning in a cell nonautonomous manner. It is noteworthy that *sars* is strongly expressed in somites at 1 to 2 dpf and that Vegf-*a* is produced in somites. We also found that the amount of *vegfr-1* transcript determined by quantitative real-time PCR was increased in *sars* mutants (Figure 2), whereas the expression of *vegfr-2*, *vegfr-3*, and *vegfr-4* was not (Fukui et al. 2009). Interestingly, this excessive of *vegfr-1* message was suppressed by mRNA injections of the enzymatically inactive *T429A sars* (Fukui et al. 2009), suggesting that the noncanonical activity of Sars negatively regulates *vegfr-1* expression during vascular development (Figure 2). It has been shown that IFN- γ induces the formation and activation of an IFN- γ -activated inhibitor of translation complex that includes EPRS, ribosomal protein L13a, glyceraldehyde 3-phosphate dehydrogenase, and NS1-associated protein 1 (Ray and Fox 2007), resulting in the translational silencing of Vegf-*a* expression (Table 1). It is very interesting that two distinct AARSs, Sars, and EPRS, negatively regulate the expression of Vegf-*a* during vascular development and the inflammatory response, respectively.

Conclusions and Perspectives

Although it is not well understood how AARSs have acquired noncanonical activities in addition to their aminoacylation function, accumulating evidence indicates that disruption of noncanonical functions of AARSs connects to various types of diseases, including neural pathologic conditions and cancer (Park et al. 2008). For example, point mutations in human YARS and *glycyl-tRNA synthetase* are associated with Charcot-Marie-Tooth (CMT) diseases, which are the most common heritable disorders of the peripheral nervous system (Jordanova et al. 2006, Seburn et al. 2006). Examination of the aminoacylation activities in the context of various CMT disease-causing mutations demonstrates that CMT disease can occur without loss of aminoacylation activity (Seburn et al. 2006, Nangle et al. 2007). Here, we reviewed evidence that a noncanonical activity of Sars is involved in vascular development. Sars may

influence Vegf signaling because the *vegf-a* message is increased in *sars* mutants, the vascular branching and dilatation phenotypes in *sars* mutants can be suppressed by blocking Vegf receptor function, and excessive branchpoints are observed in Vegf-induced vascular network formation after *Sars* knockdown in HUVECs (Figure 2). Further studies are required to clarify why loss of zygotic *Sars* causes two distinct phenotypes, excessive branching and dilatation, in different types of vessels. Because the abnormal vascular branching in *sars* mutants can be rescued by mRNA injections of human *Sars*, which is highly homologous to zebrafish *Sars* (Fukui et al. 2009), the noncanonical function(s) of *Sars* should be conserved between zebrafish and human. This raises the possibility that impairment of human *Sars* may be associated with vascular diseases and that the zebrafish *sars* mutants may represent a human disease model. In addition, because *Sars* affects Vegf signaling during zebrafish vascular development, *Sars* may be a promising target for neovascular diseases and cancers, where anti-Vegf therapies have shown some efficacy (Olsson et al. 2006). Further analysis of zebrafish and mammalian *Sars* should provide additional insights into the precise molecular mechanisms of vascular network formation and remodeling.

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Figure 1.

Noncanonical activity of Sars is involved in vascular development. (A-F) *Tg(fli1:egfp)* (A-C) wild-type and (D-F) *sars^{ko095}* mutant at 3 dpf. The process of vascular network formation was visualized by the EGFP expression in endothelial cells under the control of the *fli1* (an endothelial cell-specific gene) promoter. Aberrant branching (white arrowheads) was observed in the intersegmental vessels of *sars* mutants. (G) Involvement of noncanonical Sars activity in vascular development. Schematic representation of Sars constructs. Sars contains an N-terminal tRNA binding domain and a class II core catalytic domain. A stop codon in the *ko095* mutant allele of *sars* leads to a truncation in the catalytic domain (Sars ΔC). T429A is a Sars variant with a substitution from Thr to Ala at amino acid position 429 in a domain essential for binding to serine. The biologic activities of Sars constructs were previously reported (Fukui et al. 2009). Thus, both Sars ΔC and T429A lack aminoacylation activity, whereas WT Sars possesses aminoacylation activity. The abnormal vascular branching in *sars* mutants could be suppressed by mRNA injections of T429A to the same extent as WT *sars* injections, whereas *sars* ΔC injections failed to prevent the abnormal vascular branching in *sars* mutants.

Figure 2.

The potential role of Sars in vascular development. **(A)** *vegf-a* message is increased in *sars* mutants, presumably from an increase in *vegf-a* transcription or stabilization of *vegf-a* transcripts. The excessive *vegf-a* message can be suppressed by the expression of the enzymatically inactive T429A *sars*. **(B)** Knockdown of Sars in HUVECs leads to an increased number of branchpoints in Vegf-induced vascular network.

Table 1
Noncanonical activities of AARSs in vascular development

AARS	Functions	Reference
Sars	Disruption of <i>sars</i> leads to dilatation of the aortic arch vessels and aberrant branching of cranial and intersegmental vessels in zebrafish	Herzog et al. (2009)Fukui et al. (2009)
YARS	N-terminal fragment of YARS functions as an angiogenic factor for endothelial cells in culture	Wakasugi et al. (2002a)
WARS	N-terminal truncated form of WARS functions as an angiostatic factor for endothelial cells in culture	Wakasugi et al. (2002b)
EPRS	EPRS is involved in the IFN- γ -mediated translational silencing of VEGF-A in culture	Ray and Fox (2007)