N-α-acetyltransferase 10 (NAA10) in development: the role of NAA10

Mi-Ni Lee¹, Hyae Yon Kweon¹ and Goo Taeg Oh¹

Abstract
N-α-acetyltransferase 10 (NAA10) is a subunit of N²-terminal protein acetyltransferase that plays a role in many biological processes. Among the six N-α-acetyltransferases (NATs) in eukaryotes, the biological significance of the N-terminal acetyl-activity of Naa10 has been the most studied. Recent findings in a few species, including humans, indicate that loss of N-terminal acetylation by NAA10 is associated with developmental defects. However, very little is known about the role of NAA10, and more research is required in relation to the developmental process. This review summarizes recent studies to understand the function of NAA10 in the development of multicellular organisms.

Introduction
N-α-acetyltransferase 10 (NAA10), the catalytic subunit of N-acetyltransferase A (NatA), a major N-terminal acetyltransferase complex, catalyzes the alpha (N-terminal) acetylation of nascent peptides as a cotranslational modification and epsilon (internal) acetylation of mature proteins (including itself) as a posttranslational modification. NAA10, which is conserved from yeast to humans and expressed in most cell types, is an important regulator in diverse biological processes, such as cell growth, differentiation, metastasis, apoptosis, and autophagy. In mammalian cells, most studies about the function of NAA10 are mainly focused on its relationship with cancer. Meanwhile, the biological significance of NAA10 is not as well understood, and further studies are needed in the context of a developing multicellular organism. Recently, NAA10 has been reported to play a critical role in development and human genetic diseases. According to these reports, several human genetic diseases have been shown to be associated with NAA10 mutations, thus highlighting the importance of NAA10 function during biological development. In this review, we mainly focus on NAA10 function during the development of multicellular organisms.

Expression of NAA10 during embryonic development
NAA10 is located on chromosome Xq28 in humans and X A7.3 in mouse, and is encoded by 8 exons. Alternative splicing of its mRNA produces several isoforms of NAA10. There are three mouse variants (mNaa10⁹⁸, mNaa10²²⁵, and mNaa10²³⁵) and two human variants (hNaa10¹³¹ and hNaa10²³⁵). The mNaa10²²⁵, mNaa10²³⁵, and hNaa10²³⁵ are the functional isoforms that contain the full N-acetyltransferase domain sequence, and they have been the most extensively studied and characterized among the aforementioned variants. Additionally, a homologous gene of NAA10, termed NAA11, has been identified. The human NAA11 is located on chromosome 4q21.23, and the sequence of hNAA11 protein is 81% identical to hNAA10, whereas mouse Naa11 is on chromosome 5E3. Previous studies showed the broad and ubiquitous expression of NAA10 in various cell types, including tumor cell lines and several tissue types, in the developmental stages of embryos and in adults. In general, dynamic changes in the spatiotemporal expression of distinct genes are shown during embryogenesis. The regulation of tissue- and stage-specific expression affects the development of different organs individually. On the
basis of this information, it is reasonable to suggest that the potential role of NAA10 varies depending on transcriptional levels in different tissues and embryonic stages during development. Interestingly, in mouse, according to expression atlas databases, the RNA expression level of mNaa10 for each organ is higher in the developmental stages of embryo than in the neonate\(^24,25\). For example, in kidney, liver and lung, the RNA level of mNaa10, which is very high in the stage from E12 to E14, decreased to half of this level in the neonate (Fig. 1a). A broad comparison of RNA levels between fetus and adult in humans indicated the same results as those found for mouse\(^24–27\) (Fig. 1b). However, there are other instances in which an increased or steady expression level is shown in some tissues at the postnatal stage, so we cannot form a definitive conclusion. Whether the changes in tissue- and stage-specific expression have an impact on development needs further investigation.

**N-terminal acetylation dependent functions of NAA10 during embryonic development**

Acetylation is one of the protein modifications that diversifies the function of genes in organisms. In particular, N-terminal acetylation occurs in 80~90% of eukaryotic proteins, and the molecular role of N-terminal acetylation affects various protein functions, including protein interaction, localization to the ER and degradation. To date, six distinct forms of NATs (NatA – NatF) have been identified in eukaryotes, and they are classified based on different subunit compositions\(^28,29\). Among others, NatA undertakes the majority of N-terminal acetylation, acetylating ~40% of the human proteome\(^10,28–31\). However, the distinct function of NatA has not yet been demonstrated during embryonic development. Nonetheless, abnormal phenotypes were observed in several species due to the absence or reduction of NAA10. These data are of great interest for understanding the role of NAA10 in development.

**Developmental roles in humans**

Recently, the importance of NAA10 catalytic activity in human development has emerged through the discovery of various NAA10 mutations in several pathological conditions (Table 1). Rope et al. reported the first human genetic disorder caused by a Ser37Pro mutation in NAA10 and termed the lethal X-linked Ogden syndrome\(^14,32\). Since this discovery, more human genetic disorders caused by NAA10 missense mutations, including Tyr43Ser, Arg83Cys, Phe128Leu/Ile, Val107Phe, and Arg116Trp, have been discovered\(^11,13,15,16\). Among a wide spectrum of malformations in NAA10-related syndromes, there are some common phenotypes with global developmental delay, cardiac anomalies, and intellectual disabilities, and the corresponding NAA10 mutations triggered a reduction in catalytic activity, suggesting that NATs are essential during early embryogenesis and that they possess important regulatory functions during tissue and organ development\(^11,33\).

Additionally, a splice mutation in the intron 7 splice donor site (c.471+2T → A) of NAA10 was implicated as a cause of Lenz microphthalmia syndrome\(^12\). Developmental abnormalities, such as microphthalmia or anophthalmia, developmental delay, intellectual disability, skeletal abnormalities and malformations of teeth, fingers, and toes, were observed. Gene expression array studies in patient fibroblasts displayed the dysregulation of several genes involved in embryonic, organ, tissue and skeletal and muscular system development, and above all, these changes were associated with the retinoic acid and Wnt signaling pathways, which are necessary for normal eye
| Organism scientific name | Homolog | Type of mutation | Protein effect/molecular mechanism                                                                 | Phenotype                                                                 | Ref. |
|--------------------------|---------|------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------|
| Human Homo sapiens       | NAA10   | Ser37Pro         | Impaired Nt-acetylation in vivo using COFRADIC, reduced catalytic activity for EEEI, DDDI, and SESS, inability to combine with Naa15, reduced degree of Nt-acetylation of THOC7 | Perinatal lethal disorder, hypotonia, global developmental delay, cryptorchidism, cardiac arrhythmias, skin laxity, dysmorphic features, hernias, large fontanels | 32   |
|                          |         | c.471+2T→A       | STRA6 expression significantly decreased, loss of TSC2 binding and a reduction of TSC2 stability     | Eye malformations, mild to severe developmental delay, defects in the skeletal and genitourinary systems, congenital bilateral anophthalmia, postnatal growth failure, skeletal anomalies, hypotonia, moderate-to-severe mental retardation | 12   |
|                          |         | Tyr43Ser         | Catalytically impaired in vitro, with approximately an 85% reduction in Nt-catalytic activity for EEEI, DDDI, and SESS | Intellectual disability, facial dysmorphism, scoliosis, long QT           | 11   |
|                          |         | Val107Phe/Arg116Trp | Reduction in catalytic activity for the EEEI and SESS (V107P, ~95%, R116W, ~15%)                     | Severe global developmental delay with postnatal growth, skeletal anomalies, truncal hypotonia with hypertonia of the extremities, minor facial features, behavioral anomalies | 13   |
|                          |         | Arg83Cys         | Interfere Ac-CoA binding, 60% reduction in Nt-catalytic activity                                    | Hypotonia, global developmental delay, dysmorphic features, autism spectrum disorder, epileptic encephalopathy, extrapyramidal signs, hypertension with left ventricular hypertrophy, thin corpus callosum, progressive white matter loss | 16   |
|                          |         | Phe128Leu/Ile    | Altered structure and reduced stability, dramatic reduction of Nt-catalytic activity                | Moderate to severe intellectually disabled, feeding difficulties, eye anomalies, hypotonia, developmental delay | 15   |

Lee et al. Experimental & Molecular Medicine (2018) 50:87

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| Organism scientific name | Homolog | Symbol of mutation | Type of mutation | Protein effect/ molecular mechanism | Phenotype | Ref. |
|--------------------------|---------|--------------------|------------------|-------------------------------------|-----------|-----|
| Yeast *Saccharomyces cerevisiae* | ARD1, yNaa10 | ard1::HIS3 | Inserting a Barn HI fragment containing the HIS3 gene into the Barn HI site of plasmid YCpE18 that lies within the functional sequence of ARD1 | Defect in transcription of a-specific genes, Permit expression of the information resident at HML | Reduced viability, sensitive to heat shock and salt, fail to enter stationary phase, lack of glycerol accumulation, sporulation defect, poor mating, fail to undergo meiosis | 8, 9 |
|                           |         | nat1-5::LEU; ard1 | Mating nat1 and ard1 single mutant for nat1 ard1 double mutant | Impaired N-terminal activity (predicted); single mutants of nat1 (Naa15) and ard1 (Naa10) displayed identical phenotypes, no additional phenotypes are found in double mutant | Inable to sporulate, slow growth, reduced mating, inhibit sporulation, impaired resistance to heat shock, fail to G1 arrest, partial depression of HML, fail to accumulate storage | 4, 7 |
|                           |         | y[hNatA S37P] | A strain without yNatA and expression human NatA with a mutated hNaa10 S37P | Lack of proper complex formation with hNaa15 and reduced in vitro catalytic activity, decreased of Nt-acetylamine using COFRADIC, increase in the Hsp70 family proteins | Growth defect, sensitive to caffeine and cycloheximide, Impaired resistance to heat shock, decreased mating efficiency | 34, 35 |
|                           |         | yS39P | S39P mutation in homologous position to human Naa10 S37 | No obious effects | Growth retardation in vegetative stage, lethal, abortion of embryogenesis, drought-adapted root morphology | 35 |
| Plant *Arabidopsis*       | At5g13780, AtNAA10 | nna10-1 | T-DNA insertion-disrupting gene expression | Impaired N-terminal activity; Naa15 mutation also shared same phenotypes | Growth retardation in vegetative stage, lethal, abortion of embryogenesis, drought-adapted root morphology | 36 |
| Protozoan parasite *Trypanosoma brucei* | TbARD1 | amiNaa10 | Depleted-RNA silencing | Impaired N-terminal activity (predicted) | Impaired growth in bloodstream-form cells, reduced differentiation to insect-stage cells | 37 |
| Worm *Caenorhabditis elegans* | DAF-31 | daf-31(m655) | Remove 151 bp of promoter upstream of the ATG start codon and 242 bp of daf-31 coding region downstream of the ATG start codon | Regulates the transcriptional activity of DAF-16 (FOXO transcription factor) | Developmental larval arrest, fat accumulation, formation of dauer-like larvae under starvation conditions, decreased lifespan, no SDS-resistant, cannot resume development and reproduction after food re-providing | 38 |
|                           |         | daf-31 RNAi | RNAi knock down, reducing daf-31 mRNA | Decreased lifespan | | 39 |
|                           |         | daf-31 OE | Overexpression; full length dar-31 genomic DNA was cloned into pGEM-T | Increased lifespan in daf-2 mutant, enhancing reproduction | | |
|                           |         | vnc | vncDCL | Impaired N-terminal activity (predicted) | | |
development. Furthermore, TSC2 is known to be stabilized by NAA10-dependent N-terminal acetylation. C-terminal truncated mutation of NAA10 demonstrates both loss of NAA10-TSC2 binding and reduced TSC2 protein levels, thereby resulting in perturbed mTOR signaling. Given these findings, the N-terminal acetyltransferase activity of NAA10 plays a critical role in human development.

Developmental roles in other organisms

In several species, various developmental defects have also been observed due to a deficiency of NAA10 activity (Table 2). NAA10 was first discovered in yeast, where it was found to be crucial for cell growth and sporulation. The yNaa10 deleted strain, lacking NatA activity, is viable but exhibits a wide range of defects, including slow growth, de-repression of the silent mating type locus HML (Hidden MAT Left), temperature and salt sensitivity, and failure to enter G0 phase and sporulate. Recently, the yeast model of Ogden syndrome expressing hNaa10 S37P in yNaa10 deficient strains has also been shown to have impaired growth and resistance to stress and mating. Naa10 is required for Arabidopsis thaliana development, and thereby, Naa10 deficient mutations induce growth retardation and are lethal. Additionally, the loss of Naa10 in Trypanosoma brucei leads to mortality. In Caenorhabditis elegans, daf-31 (the ortholog of NAA10) is essential for larval development, metabolism and adult lifespan. Daf-31 mutants fail to properly enter the dauer stage, which is pivotal for C. elegans survival during starvation (when nutrients are limited). Furthermore, the mutants display developmental arrest under abundant nutrition and shift their metabolism to fat accumulation. The importance of Naa10 in normal development is also supported by a Drosophila melanogaster study in which two hypomorphic mutations of Naa10 led to pleiotropic oogenesis defects, including aberrant mitoses, defects in egg chamber encapsulation and nurse cell chromatin dispersion. Further, Naa10 deficient mutations have been shown to affect cell survival and proliferation and cause lethality. In a recent Danio rerio study, morpholino-mediated knockdown of Naa10 resulted in increased lethality, growth retardation and abnormal development, such as a bent axis, abnormal eyes, and bent tails, indicating the importance of Naa10 in early zebrafish development and viability.

Neuronal development

During brain development in mouse, Naa10 and Naa15 (the auxiliary subunit of NatA) are highly expressed in regions rich in proliferating and migrating cells, such as the ventricular zone, neocortex, olfactory bulb, and hippocampus. The expression of both genes is down-regulated as neurons differentiate and mitotic and
migratory activities subside. Then, once again, their expression increases during postnatal development in the hippocampus and during the neuronal dendritic development of Purkinje cells (PCs) in the cerebellum. This finding indicates that the regulation of expression of both genes is related to neuronal development, especially in the hippocampus and in the PCs of the cerebellum.

The acetylation of α-tubulin has been reported to be involved in regulating microtubule (MT) stability and dynamics, neuron polarization, and neurite branching and promotes vesicular transport on MTs in differentiated neurons \[^{42-46}\]. A recent study showed that the inhibition of Naa10 or acetyltransferase activity significantly reduced the dendritic extension of cultured neurons \[^{47}\]. Furthermore, Naa10 and Naa15 proteins co-localized with MTs in dendrites and induced the acetylation of tubulin in the brain fraction. Additionally, the dendritic arborization defect phenotype caused by the over-expression of HDAC6, a major deacetylase of α-tubulin, was rescued by the co-expression of Naa10. Therefore, the authors postulated that Naa10 counteracts HDAC6 by acetylating α-tubulin, thereby promoting MT stability for dendritic development. In spite of this fact, it is not clear that α-tubulin is the only distinct substrate of Naa10 that participates in dendritic development because the tubulin fractions contain both α/β-tubulin. Moreover, it is uncertain whether tubulin is acetylated by the N-terminal activity or lysine activity of Naa10. The lysine 40 residue of either tubulin is de-acetylated by HDAC6\[^{48-50}\], so lysine acetylation may be a potential catalytic function of α-tubulin. Characterizing the impact of whichever acetyl activity is involved in tubulin acetylation will help define a specific mechanism for neuronal development.

Melanogenesis

Naa10 knockdown in Zebra fish 2 days post-fertilization embryos showed less pigmentation, including abnormal development \[^{49}\]. Pigmentation in organisms is due to the deposition of the pigment melanin, which is produced by specialized cells called melanocytes. In the process that produces melanin, which is called melanogenesis, the most important molecule among melanocyte-stimulating hormones (MSHs) is the proopiomelanocortin (POMC)-derived peptide α-MSH \[^{21,22}\]. α-MSH is known to be N-terminally acetylated, and its stability and potency in stimulating pigment dispersion are increased by Nt-acetylation \[^{21,22}\]. So far, N-acetyltransferase enzyme acetylating α-MSH has not been identified yet. NatA is known to acetylate the amino acids starting with Ser, Ala, Thr, Val, Gly, and Cys \[^{28}\]. Based on this fact, the N-terminus of α-MSH, which starts with serine, demonstrates the possibility that α-MSH could be a potential target of NatA. In the future, more studies are needed to reveal the direct regulation of α-MSH and melanogenesis by Naa10.

Spermatogenesis

NAA11 (also known as Ardlb; ARD2), a homolog of NAA10, is predominantly expressed in mouse \[^{57}\] and human \[^{58}\] testis. Naa11 can reconstitute functional NAT in the presence of Naa15, where Naa11 is functionally equivalent to Naa10. Interestingly, NAA10 and NAA11 display opposite expression patterns during spermatogenesis. In the mouse, the expression of Naa11 is upregulated during meiosis, whereas Naa10 expression is downregulated. In contrast, Naa10 is expressed in pre-meiotic spermatogonia, which do not show Naa11 expression. Therefore, Pang et al. speculated that compensation for the loss of X-linked Naa10 occurs by expressing autosomal Naa11 due to the sex chromosome inactivation during male meiosis \[^{57,59}\]. In this sense, they also suggested that the NAT activity of Naa10 could play a pivotal role in mitotic spermatogonia and that the NAT activity of Naa11 is crucial for post-meiotic male germ cells \[^{59}\]. Furthermore, the differential expression of NAA10 and NAA11 was shown in the human promyelocytic NB4 cell line upon differentiation \[^{21}\]; NAA10 expression decreased with the induction of differentiation in NB4 cells, but the level of NAA11 remained unchanged, implying that Naa11 has a role in the cellular differentiation process and that Naa10 has a role in the cellular proliferation process. The differential expression pattern of Naa10/Naa11 suggests that Naa11 is complementary to Naa10 at least in the mice and that its biological role could be important in spermiogenesis or cellular processes \[^{59}\].

Lysine acetylation dependent functions of NAA10 during embryonic development

Naa10 is known to regulate cellular processes, and its effects are not only catalyzed through its major activity as a NAT but also through the N-ε-acetylation of several proteins \[^{3,60-73}\]. The N-ε-acetyl-activity of Naa10 requires auto-acetylation \[^{7,67}\]; this requirement is similar to that of other acetyltransferases, which acetylate themselves for their catalytic and functional activities \[^{74,75}\]. Recently, gain or loss of function studies in mice have demonstrated that Naa10 plays an important role in osteoblast differentiation and the early phases of bone formation \[^{3}\]. Overexpression of Naa10 in mice results in the delayed closure of calvarial fontanels and reduced bone density, osteoblast surfaces and mRNA levels of the osteoblastogenic genes in calvaria. In contrast, Naa10 deficient mice display calvarial and femoral bone development to a greater extent on postnatal day 3. Mechanically, Naa10 interacts with the RUNT domain of Runt-related transcription factor 2 (Runx2), which is the master regulator of
osteoblast development, and acetylates it at K225. The lysine acetylation of Runx2 restricts its transcriptional activity by interfering with CBFβ binding to Runx2. On the other hand, Runx2 stabilizes Naa10 in osteoblasts during bone morphogenic protein 2 (BMP-2)-induced differentiation through its inhibition of IKK-mediated phosphorylation and degradation of Naa10, which in turn inhibits Runx23 (Table 3).

In addition to Runx2, the lysine acetyl-activity of Naa10 has been reported to target proteins such as β-catenin (CTNNB1)60,61, Phosphoglycerate kinase 1 (PGK1)72,73, Hypoxia inducible factor 1α (HIF-1α)66–68, Myosin light-chain kinase (MLCK)62, Androgen receptor(AR)64,65, enzyme methionine sulfoxide reductase A (MSRA)63, SAM domain and HD domain containing protein 1 (SAMHD1)69, Heat shock protein 70 (Hsp70)70, Aurora kinase A (AuA)71, and Naa10 itself2,67. The lysine acet-yltransferase activity of NAA10 catalyzing these targets has not yet been directly implicated in development, but the targets are essential for developmental signaling pathways, such as Wnt/β-catenin signaling76–78, autophagy79–81, and the HIF-1 regulatory pathway82–84. Accordingly, there is a possibility that NAA10 could influence developmental processes via these signaling pathways. On the other hand, Magin et al. reported data on the lysine acetyl-activity of NAA10, demonstrating that NAA10 does not acetylate the lysine residues of MLCK, MSRA, or RUNX285. For the lysine acetyl-activity of NAA10, earlier reports demonstrated that the auto-acetylation of NAA10 at K136 was a critical step to generate an active form. For example, NAA10 K136R, a mutation in the auto-acetylation site, failed to acetylate β-catenin and did not succeed in the recruitment of β-catenin on the cyclin D1 promoter2. Therefore, the inability of NAA10 to acetylate the lysine residues of MLCK, MSRA and RUNX2 could be due to non-auto acetylated NAA10. There is a possibility that Magin et al. performed their assay with non-auto acetylated NAA10, thus losing lysine acetyl-activity in vitro. Nevertheless, more research is required to understand the role of lysine acetylation by Naa10.

### Acetylation independent functions of NAA10 during embryonic development

Several recent studies have suggested that NAA10 is able to interact directly with other proteins and enhance or inhibit the activity of its partner in an acetylation-independent manner86. The signaling pathways of NAA10 and its binding partners have been actively investigated for cell growth and function in cancer research, but reports on acetylation-independent roles of NAA10 during development are very partial as described below.

#### Table 3 Summary of NAA10 mutations in mouse

| Organism | scientific name | Homolog | Symbol of mutation | Type of mutation | Protein effect/ molecular mechanism | Phenotype |
|----------|----------------|---------|--------------------|------------------|-------------------------------------|-----------|
| Mouse    | Mus musculus   | Naa10   | TgNaa10             | Overexpression of Naa10, 2 different founders #10 were used | Blocks the Runx2–CBFβ interaction by acetylating Runx2 at K225 | Delayed calvarial bone development |
|          |                |         | Naa10 KO           | Remove E3 containing the start codon and Exon2-4 containing N-acetyltransferases, NLS and the Acetyl-CoA binding domain | Enhance the the Runx2–CBFβ interaction | Delayed calvarial bone development |
|          |                |         | Naa10f/Y; EIIa-Cre | Insertion of loxp into the intron 1 and 6, and the Neo binding domain | Disrupts its binding to the imprinted allele at ICRs/DMRs and Dnmt1 recruitment | Partial embryonic lethality, growth retardation, brain disorders, maternal effect lethality, defective genomic imprinting |

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Genomic imprinting regulation in embryonic development

Genomic imprinting is an epigenetic process controlled by DNA methylation, and it plays a vital role in normal development. During embryonic development, the establishment of appropriate imprinting is accomplished by the intact regulation of Dnmt3 and Dnmt1 DNA methyltransferases. Lee et al. reported that Naa10 deficiency mice exhibited developmental defects, including partial embryonic lethality, postnatal growth retardation, brain disorders, and maternal effect lethality, resembling the phenotypes caused by the dysregulation of genomic imprinting (Table 3). The authors described that despite questions about how Naa10 selectively binds to the imprinted allele, mechanistically, Naa10 binds to the unmethylated GCXGXG in the imprinting control region (ICR)/differentially methylated region (DMR) of the imprinted allele and then recruits Dnmt1 for methylation in the S phase. In addition, by showing the disrupted DNA binding activity of clinical NAA10 mutations (S37P, V107F, and R116W) to ICR, a potential connection between NAA10 mutation-associated syndromes and defects in DNA methylation and genomic imprinting was presented. Together, these results suggested that normal DNA methylation and genomic imprinting is regulated by appropriate ICR binding of Naa10 and Dnmt1 recruitment during development. Previously, Lee et al. demonstrated that Naa10 acetyl activity does not mediate Naa10-Dnmt1 binding and does not stimulate the activity of Dnmt1. However, even though Naa10 acetyl activity is dispensable for stimulating Dnmt1 activity, we could obtain an insight from their recent study that showed that the N-terminal acetyl-activity of Naa10 is required for binding to ICRs/DMRs. The authors showed that reduced DNA binding to clinically relevant mutations (S37P; defects in Naa15 binding domain for NatA complex, V107F; mutation in the acetyltransferase domain, R116W; putative interference with Ac-CoA binding) are associated with N-terminal acetyltransferase activity, which is not related to the DNA-binding domain at the C terminus of Naa10. Given these results, there is a possibility that the enzyme activity of Naa10 directly or indirectly influences ICR binding. For example, Naa10 may combine with other proteins to acetylate the N-terminal residue, and then, these proteins may interact with ICRs/DMRs together. Interestingly, unlike other reports in other species, including humans, Naa10 deficient mice exhibited only a few phenotypes, such as embryonic lethality and growth retardation. Moreover, some of the Naa10 deficient mice developed to term. The authors suggested some reasons for the decreased penetrance of various phenotypes. First, they suggested that this finding was a general effect of imprinting disorders caused by the deficiency of genes that maintain genomic imprinting during global DNA demethylation in zygotes. Second, the remaining DNA methylation occurred at different regions in each individual mouse, which may contribute to stochastic phenotypes. Third, the dysregulation of other imprinted genes, such as Igf2, Ascl2, and Grb10, and the effects of unidentified non-imprinted genes or protein acetylation by Naa10 deficiency are suggested. In spite of these descriptions, there are still issues to be resolved. It would have been a more complete study if the maternal effects leading to the death of embryos and neonatal mice were explained and if a histological analysis in not only placenta but also whole embryos was performed to determine the precise effects of embryonic lethality. Therefore, more elaborate mechanisms remain to be determined and more extensive mouse studies are needed.

Vasculogenesis

Xu et al. demonstrate a potential role of Naa10 in vasculogenesis and neurogenesis through the regulation of UNC-5 Homolog B (UNC5B) and Netrin-1 (NTA1). UNC5B and its ligand NTA1 function as essential genes in morphogenesis of the vascular system or nervous system and the negative regulation of Naa10. Knockdown of Naa10 induced morphological changes (shuttle-shaped membrane protrusion) and increased the ability for tube formation in both H1299 lung cancer cell lines and immortalized mouse endothelial cell lines. Additionally, the expression of UNC5B and NTA1 increased in Naa10 knockdown H1299 cells, and negative regulation was also verified in the mouse caudal half region of E10 embryos (when exuberant vasculature developed at that stage). However, a direct mechanism explaining how Naa10 regulates NTA1 and its receptor UNC5B has not been determined. Nevertheless, it has been reported that Naa10 physically binds with RelA/p65, an NFkB transcription factor and a negative regulator of NTA1. Therefore, the authors speculate that Naa10 physically interacts with p65, thereby negatively regulating NTA1 and its receptor UNC5B.

Conclusion and perspectives

In this review, we summarized the functions of NAA10 that are enzyme activity-dependent and -independent during the development of several organisms. Most studies have focused on the N-terminal activity of NAA10, whereas some findings have been related to lysine activity and the independent role of NAA10. Our overall understanding of the function of NAA10 in developmental processes suggests the following insights. First, depending on which acetyl-activity is acting and how it affects development, the corresponding physiological responses could be very different. NatA is presumed to act on a large number of substrates involved in a multitude of different cellular processes. As NatA targets many substrates, it could be expected to affect diverse cellular processes and...
eventually lead to developmental processes in many different organs. On the other hand, since the known targets of lysine acetylation by NAA10 mediate important signaling pathways during development, more intensive and accurate mechanistic studies of these signaling pathways could reveal the role of NAA10 in development. It is also important to understand how the regulation of NAA10 itself is managed during the developmental stage. For example, NAA10 auto-acetylation activates its lysine catalytic activity, and active IKKβ degrades NAA10 by phosphorylation.1 Furthermore, NAA10 acts as a coactivator of DNMT1 without its enzymatic activity. Likewise, given that NAA10 carries out a wide spectrum of functions, the cellular-, tissue- and developmental stage-specific effects of NAA10 need to be discovered. Second, interestingly, some individuals normally survive and grow with decreased or mutated NAA10, which suggests the possibility of a homologous gene that might compensate for the function of NAA10. This suggestion is supported by the existence of NAA11, which has been identified as a homologous gene of NAA10, and the reciprocal expression between NAA10 and NAA11 during spermatogenesis. However, we are unable to answer whether NAA11, whose expression appears to be limited to the testis, can cover the entire function of the ubiquitously expressed NAA10 protein. Therefore, an open question about the existence of another homologous gene to NAA10 in different tissues remains. The goal of future studies will be to elaborate on the cellular/molecular specific activity and distinct pathways of NAA10. Above all, in vivo studies are necessary to analyze the definite biological effects of NAA10.

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Conflict of interest

The authors declare that they have no conflict of interest.

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