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Research paper

Identification of an alternatively spliced nuclear isoform of human N-terminal acetyltransferase Naa30

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ABSTRACT

N-terminal acetylation is a highly abundant and important protein modification in eukaryotes catalyzed by N-terminal acetyltransferases (NATs). In humans, six different NATs have been identified (NatA-NatF), each composed of individual subunits and acetylating a distinct set of substrates. Along with most NATs, NatC acts co-translationally at the ribosome. The NatC complex consists of the catalytic subunit Naa30 and the auxiliary subunits Naa35 and Naa38, and can potentially Nt-acetylate cytoplasmic proteins when the initiator methionine is followed by a bulky hydrophobic/amphipathic residue at position 2. Here, we have identified a splice variant of human *NAA30*, which encodes a truncated protein named $Naa30_{288}$. The splice variant was abundantly present in thyroid cancer tissues and in several different human cancer cell lines. Surprisingly, $Naa30_{288}$ localized predominantly to the nucleus, as opposed to annotated Naa30 which has a cytoplasmic localization. Full-length Naa30 acetylated a classical NatC substrate peptide *in vitro*, whereas no significant NAT activity was detected for $Naa30_{288}$. Due to the nuclear localization, we also examined acetyltransferase activity towards lysine residues. Neither full-length Naa30 nor $Naa30_{288}$ displayed any lysine acetyltransferase activity. Overexpression of full-length Naa30 increased cell viability *via* inhibition of apoptosis. In contrast, $Naa30_{288}$ did not exert an anti-apoptotic effect. In sum, we identified a novel and widely expressed Naa30 isoform with a potential non-catalytic role in the nucleus.

1. Introduction

The majority of eukaryotic proteins are co-translationally acetylated at their N-terminal amino group (Arnesen et al., 2009; Van Damme et al., 2011b). Despite its prevalence, the biological importance of N-terminal acetylation (Nt-acetylation) remains largely unknown. This apparent irreversible protein modification was initially thought to protect proteins against degradation (Jornvall, 1975). However, later studies revealed that Nt-acetylation mediates protein folding (Maltsev et al., 2012; Bartels et al., 2014; Dikiy and Eliezer, 2014; Holmes et al., 2014), complex formation (Urbancikova and Hitchcockdegregori, 1994; Scott et al., 2011; Monda et al., 2013), subcellular targeting (Behnia et al., 2004; Setty et al., 2004; Murthi and Hopper, 2005; Forte et al., 2011), and may act as a degradation signal to regulate protein quality and stoichiometry (Hwang et al., 2010; Shemorry et al., 2013; Kim et al., 2014). Thus, Nt-acetylation is a multifaceted protein modification whose biological role depends on the substrate protein being targeted. Protein Nt-acetylation is characterized by the transfer of an acetyl moiety from acetyl-CoA (Ac-CoA) to the free α-amino group. The process is catalyzed by N-terminal acetyltransferases (NATs), whose activities are dictated by the identity of the N-terminal amino acid sequence (Varland et al., 2015). Six NATs, termed NatA to NatF, have been identified in humans (Drazic et al., 2016a). The NatC complex consists of the catalytic subunit Naa30 (Mak3/Nat12) and the auxiliary subunits Naa35 (Mak10/EGAP) and Naa38 (Mak31/Lsmd1) (Polevoda and Sherman, 2001; Starheim et al., 2009), and is estimated to Nt-acetylate 10% of all human protein species (Van Damme et al., 2011b; Aksnes et al., 2016). The substrate specificity profile of NatC comprises

Abbreviations: Ac-CoA, Acetyl coenzyme A; GNAT, GCN5-related *N*-acetyltransferase; KAT, lysine acetyltransferase; Naa30, N-alpha acetyltransferase 30; NAT, N-terminal acetyltransferase; Nt-acetylation, N-terminal acetylation; PTC, papillary thyroid carcinoma

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N-termini starting with Met-His, Met-Ile, Met-Leu, Met-Lys, Met-Met, Met-Phe, Met-Trp, and Met-Val (Tercero et al., 1993; Polevoda et al., 1999; Kimura et al., 2000; Starheim et al., 2009; Van Damme et al., 2016). Confirmed NatC substrates, in which acetylation has proven important for function, includes the major coat protein (Gag) of the yeast dsRNA virus L-A (Tercero and Wickner, 1992), the tRNA methyltransferase Trm1-II (Murthi and Hopper, 2005), Grh1 (Behnia et al., 2007) as well as the ARF-like GTPases Arl3 (Behnia et al., 2004; Setty et al., 2004) and Arl8b (Starheim et al., 2009).

NatC was first described in the budding yeast *S. cerevisae* where its activity is necessary for growth on non-fermentable carbon sources (Wickner and Leibowitz, 1976; Dihanich et al., 1989; Tercero et al., 1992; Polevoda and Sherman, 2001). Thus, NatC seems to be required for proper mitochondrial function. This notion is supported by the finding that knockdown of human *NAA30* causes mitochondrial fragmentation and loss of mitochondrial membrane potential (Van Damme et al., 2016). Formation of a ternary complex has been considered a prerequisite for NatC activity in yeast (Polevoda and Sherman, 2001). This is not, however, always the case as Naa38 is not required for Golgi-targeting of Arl3 (Setty et al., 2004). Furthermore, the *A. thaliana* homologue AtMak3/AtNaa30 can functionally replace the entire yeast NatC complex (Pesaresi et al., 2003). AtNaa30 is essential for efficient photosynthesis. Loss of NatC activity leads to reduced synthesis of D1 and CP47, two core proteins of photosystem II, and thereby a substantial decrease of other thylakoid-associated proteins. AtNaa30 is thus necessary for proper chloroplast function (Pesaresi et al., 2003). Based on these findings, it was proposed that NatC mediates co-translational Nt-acetylation of crucial mitochondrial and chloroplast precursor proteins, which may be imperative for stability and/or organelle import (Tercero et al., 1993; Pesaresi et al., 2003). Moreover, depletion of hNaa30 induces scattering of the Golgi apparatus and altered localization of the trans-Golgi-associated GTPase ARFRP1, suggesting that NatC-mediated acetylation may affect the structural organization of the Golgi apparatus (Starheim et al., 2017). *NAA35* was identified as a potential longevity gene in a genetic study for factors mediating stress resistance in the nematode *C. elegans*. Warnhoff and colleagues showed that NATC-1/CeNaa35 is a downstream effector of the insulin/IGF-1 signaling pathway, and that loss-of-function mutation in CeNaa35 leads to increased resistance to heavy metals, heat, and oxidative stress (Warnhoff et al., 2014). Developmental studies in zebrafish revealed that NatC is essential for embryonic development (Wenzlau et al., 2006). Morpholino knockdown of EGAP/zNaa35 caused severe growth defects, including severely attenuated vessel formation, and premature death which was likely attributed to defective vasculature. Intriguingly, zNaa35 depletion was linked to a reduction of total and phosphorylated zTOR. NatC was thus suggested to have a role in TOR signaling and zebrafish embryonic development (Wenzlau et al., 2006). Perhaps related, depletion of the human NatC subunits results in reduced cell viability by inducing p53-mediated apoptosis (Starheim et al., 2009).

Aberrant expression of NAT genes has been reported in various cancers (Kalvik and Arnesen, 2013). For instance, Mughal and colleagues described elevated *NAA30* expression levels in glioblastoma, a malignant brain tumor (Mughal et al., 2015). Knockdown of *NAA30* caused a significant decrease in sphere-forming ability and reduced viability of glioblastoma initiating cells, and also confirmed reduced levels of phosphorylated mTOR (Wenzlau et al., 2006). The same study showed that Naa30 was abundant near the perivascular space in glioblastoma tissue samples, which relates to findings in zebrafish (Mughal et al., 2015). A recent study investigating genetic causes of cerebral palsy identified a *de novo* mutation in *NAA35* (p.W532C) predicted to be pathogenic (McMichael et al., 2015). Whole-exome sequencing will undoubtedly uncover additional mutations in NatC genes, which may have disease-causing effect(s). The first NAT-gene to be directly linked to human disease was *NAA10*, encoding the catalytic subunit of the NatA complex. Dysfunctional NatA activity contributes to the pathogenesis of the lethal Ogden syndrome (Rope et al., 2011; Myklebust et al., 2015) and several related syndromic and non-syndromic intellectual disabilities (Casey et al., 2015; Popp et al., 2015; Saunier et al., 2016), as well as the Lenz microphthalmia syndrome (Esmailpour et al., 2014). Previous studies have reported splice variants of Naa10 and Naa15 with different subcellular localization and function (Gendron et al., 2000; Fluge et al., 2002; Arnesen et al., 2005; Seo et al., 2015). In the present study, we have identified and characterized a novel Naa30 isoform generated by alternative splicing of the primary transcript.

2. Materials and methods

2.1. Patient selection and tissue sampling

All tissue specimens were obtained from patients undergoing thyroid surgery at Haukeland University Hospital (Bergen, Norway) from 1991 to 2008 after written informed consent. The study was approved by the National Committee for Medical and Health Research Ethics, Norway, and the local regional ethics committee (REK Vest, Bergen, Norway, REK 2011/1119). All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki. Tumor and non-tumor samples from the surgical specimens were snap frozen in liquid nitrogen and stored at − 80 °C pending RNA extraction. The thyroid biopsies were classified on the basis of histological cell type as well as degree of differentiation (Myklebust et al., 2011). Patient sample information: nr 201; differentiated papillary thyroid carcinoma (PTC) from female, age 33, nr 240; differentiated PTC from female, age 55, nr 426; aggressive PTC from male, age 55 and nr 446; aggressive PTC from female, age 49.

2.2. Human cell lines used for cDNA synthesis

Human thyroid cell lines were derived from anaplastic carcinoma (8305C, ARO, CAL-62), follicular carcinoma (CGTH-W1, FTC-133), papillary carcinoma (BHT-101, B-CPAP, NPA, ONCO-DG1), and immortalized normal thyroid follicular cells (Nthy-ori 3.1, TAD-2) (Gromyko et al., 2010). The human non-thyroid cell lines were derived from breast adenocarcinoma (MCF-7), cervix carcinoma (HeLa S3), colorectal carcinoma (T84), embryonic kidney (HEK293), epidermoid carcinoma (A431), glioblastoma (U-87), neuroblastoma (SH-SY5Y), and primary peripheral blood mononuclear cells (PBMC). The non-thyroid cell line cDNA was a generous gift from Dr. Johannes Rack (Rack et al., 2014).

2.3. RNA extraction and cDNA synthesis

Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific) and cDNA was synthesized using Transcriptor Reverse Transcriptase (Roche) as previously described (Myklebust et al., 2011).

2.4. PCR analysis

The open reading frame of *NAA30* was amplified by nested PCR using the primer pair oTA270 and oTA271 in the first round and the primer pair oTA266 and oTA269 in the second round (Table S1). The PCR reaction parameters were as follows; First round: 95 °C, 5 min; 45 cycles of 95 °C, 30 s; 50 °C, 30 s; and 72 °C, 2 min, followed by 10 min extension at 72 °C. Second round: 94 °C, 5 min; 35 cycles of 94 °C, 35 s; 58 °C, 35 s; and 72 °C, 55 s, followed by 7 min extension at 72 °C. The PCR reaction mixture included 0.04 U TaKaRa Ex Taq DNA Polymerase, 1 × Ex Taq Buffer (with Mg^{2+}), 200 μM dNTPs, 0.2 μM of each primer, and 5% DMSO. 1 μl of cDNA was used as template in the first reaction (tot. 25 μl) while 2 μl PCR product served as template in the nested PCR reaction (tot. 50 μl). The amplified PCR products were Sanger sequenced by an in-house sequencing facility using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fischer Scientific).

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