J Bioenerg Biomembr (2012) 44:189–197
DOI 10.1007/s10863-012-9424-5

MINI-REVIEW

ATAD3, a vital membrane bound mitochondrial ATPase involved in tumor progression

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Published online: 9 February 2012
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Abstract ATAD3 (**AT**Pase family **A**AA **D**omain-containing protein **3**) is a mitochondrial membrane bound ATPase whose function has not yet been discovered but its role is essential for embryonic development. The ATAD3 gene has existed since the pluri-cellular organisms with specialized tissues and has remained unique until vertebrates. In primates and human, two other genes have appeared (called ATAD3B and ATAD3C versus ATAD3A the ancestral gene). ATAD3 knock-down in different non-transformed cell lines is associated with drastic changes in the mitochondrial network, inhibition of proliferation and modification of the functional interactions between mitochondria and endoplasmic reticulum. However, the analysis of the cellular properties of ATAD3A and ATAD3B in different human cancer cell lines shows on the contrary that they can present anti-proliferative and chemoresistant properties. ATAD3 may therefore be implicated in an unknown but essential and growth-linked mitochondrial function existing since pluri-cellular organization and involved in tumorigenesis.

Keywords ATAD3 · Mitochondria · Inner membrane · ATPase

Introduction

ATAD3 (**AT**Pase family **A**AA **D**omain-containing protein **3**) is a mitochondrial membrane ATPase which was first discovered in 2003 as a target gene of c-MYC (Zeller et al. 2003) and then as a protein of the mitochondrial inner membrane (Da Cruz et al. 2003).

ATAD3 belongs to the very large family of the AAA-ATPases (ATPases Associated with diverse cellular Activities). This family includes proteins with ATPase activity involved in many cellular processes such as transcription, replication, translation, proteolysis, vesicular transport and many others (Frickey and Lupas 2004). However, to date, the function of ATAD3 has not yet been discovered although all studies conducted so far reveal a very significant role. Indeed, its expression is ubiquitous, essential for embryogenesis, for the structure of the mitochondrial network and for the functional interactions between mitochondria and endoplasmic reticulum (Kamath and Ahringer 2003; Hoffmann et al. 2009; Gilquin et al. 2010a; Hubstenberger 2006). Therefore, ATAD3 participates in a vital mitochondrial function which remains to be determined.

Phylogeny

Exploring the emergence of ATAD3 in the genomes, it appears that no ortholog exists neither in yeast nor in prokaryotic organisms. A far ortholog is found in protists and yeast but it is about a protease whose characteristic domain is not present in ATAD3. The ATAD3 gene appears in higher eukaryotic organisms: in plants (Arabidopsis thaliana), nematodes (Caenorhabditis elegans), insects (Drosophila melanogaster), monkey (macaque) and human (see Fig. 1). A single copy of this gene is present in the haploid genomes of these higher eukaryotic organisms except for primates. In plants, the ATAD3 gene is present in three identical copies by the simple consequence of polyploidy. In primates and humans, ATAD3 was duplicated twice (contiguous duplications on chromosome 1 and mutations). As the ancestral form,

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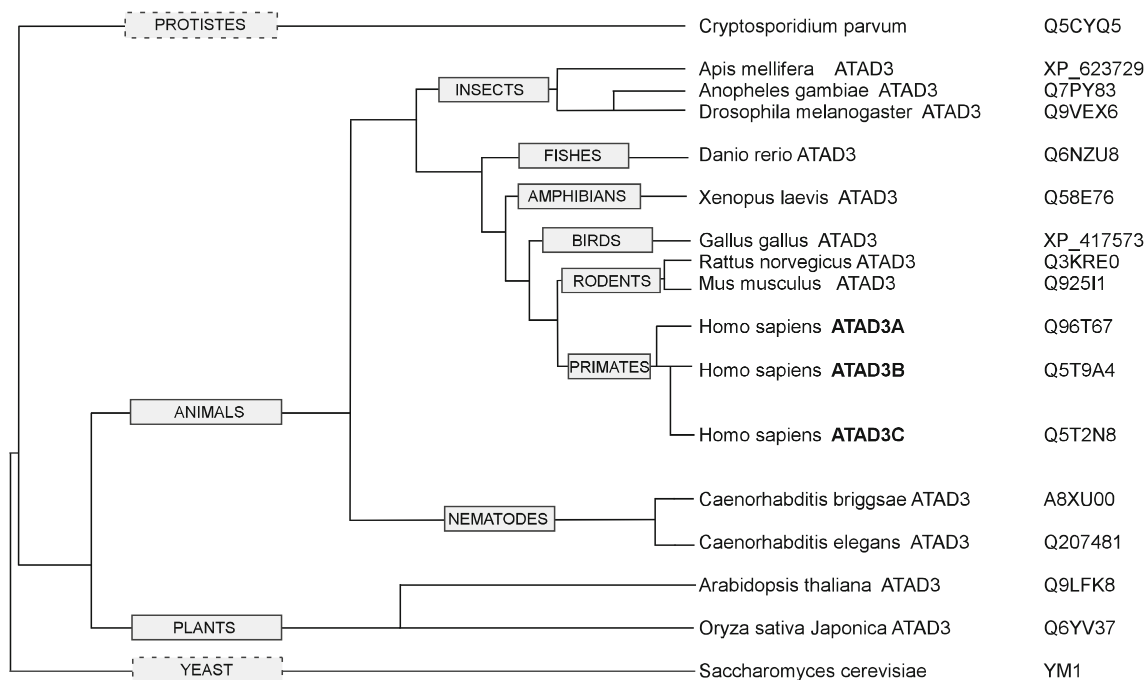

Fig. 1 ATAD3 phylogenetic tree: A far homologue is found in protists (Cryptosporidium) but the protease associated function is not present in ATAD3. From http://phylofacts.berkeley.edu/phyloscope or http://www.treefam.org/cgi-bin

one of the genes is called ATAD3A. The second gene is a very close paralogue, named ATAD3B, and the third gene, ATAD3C, presents an important deletion of the original coding sequence of ATAD3A producing a large N-terminal truncation (see Fig. 2).

These three genes are located side by side at the end of the short arm of chromosome 1 at the 1p36.33 locus. Since a single copy of the gene is present in chicken Gallus gallus, beef Bos taurus, mouse Mus musculus, rat Rattus norvegicus, dog Canis familiaris, cat Felis catus, pig Sus scrofa and dolphin Tursiops truncatus, it appears that these duplications occurred late in the species evolution, close to primates and humans.

The ATAD3B gene is indeed very similar to ATAD3A: the number of exons is identical (16) and the length of introns varies very little. Regarding the coding sequence, the strict identity of their protein sequences is 93% (see Fig. 2). The highest difference between ATAD3A and ATAD3B is found on the last exon, where a replacement mutation of the original ATAD3Ab Stop codon enables a 3′UTR sequence (not-translated in ATAD3A) to be translated in ATAD3B. For that reason, ATAD3B has a C-terminal extension of 62 amino acids compared to ATAD3A. This extension presents several potential phosphorylation sites (http://www.phosphosite.org) and a putative transmembrane sequence (see Fig. 2—http://www.enzim.hu/hmmtop). In addition, these two genes differ in their promoter structure (see below).

As regards to the third gene, ATAD3C, it possesses only the 12 last exons instead of the 16 of ATAD3A and ATAD3B. The exons encoding the first 70 amino acids of ATAD3A are not translated in ATAD3C because the translational initiation site is mutated, leaving the initiation to occur downstream (+210). For the rest of its structure, ATAD3C processes a 87% identity compared to ATAD3A (see Fig. 2).

Finally, the genes coding ATAD3 seem to present processes of differential excision-splicing about which we will discuss later.

The ATAD3 protein sequence is then highly conserved during evolution as reflected by the 39% identity existing between the human ATAD3 protein and its orthologue in plants. This strong conservation suggests that in higher eukaryotes, the ATAD3 protein has been involved in processes undergoing high selection pressure. Confirming this presumption, it has been shown that the invalidation of the ATAD3 ortholog in nematode C. Elegans is lethal during early embryonic development, at a stage where the massive mitochondrial biosynthesis is necessary (Kamath and Ahringer 2003; Hoffmann et al. 2009). Similarly, in drosophila, the invalidation of ATAD3 inhibits embryonic development at the pre-pupal stage (Gilquin et al. 2010a). We note here that ATAD3 was detected in all cell models, or all tissues studied to date (brain, heart, lung, liver, spleen, intestine, muscle; personal data).

The presence and absence of two alternatively spliced exons in ATAD3A and ATAD3B led others and us to define a nomenclature based on the respective protein sizes namely ***s*** for *small* and ***l*** for *long* (description on http://www.ensembl.

org/Homo_sapiens/Gene/Summary?g=ENSG00000197785 for ATAD3A and http://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000160072 for ATAD3B).

Proprieties *in silico*

The ATAD3 primary sequence allows us to distinguish two major functional areas corresponding to the two halves, the N- and the C-terminals of the protein (see Fig. 2). The C-terminal part of the protein constitutes the ATPase domain in which we can find the key motives involved in the binding and hydrolysis of ATP: sensor 1, sensor 2, arginine finger (Arg), Walker A (WA) and Walker B (WB) sites (Fig. 2 and (Frickey and Lupas 2004)). We note here that ATAD3 possesses the specific DEAD motif of the Walker B site (a.a. 411–414 for ATAD3As), specific for the RNA helicases subfamily. However, comparison of the ATAD3 with RNA helicases shows that this similarity is limited to the DEAD motif.

The N-terminal specific region of ATAD3 constitutes the specific domain of ATAD3. It contains one or some transmembrane domain(s) (some software finds up to three, Fig. 2 and (Hubstenberger et al. 2010)), a flexible region rich in proline (a.a 18–27 on ATAD3As), and a Coiled-coil region (a.a. 80–220 for ATAD3As) offering an interaction zone which could be important for the oligomerization of ATAD3 monomers and / or for interaction with partners (Gilquin et al. 2010a). We also know that the members of AAA-ATPases family, whose structure has already been clarified, form hexamers arranged in rings (Frickey and Lupas 2004).

We note also the presence of a glutamine-rich domain (Q domain a.a 89–166 for ATAD3As), conserved since Drosophila, but whose function remains unknown.

According to the in silico analysis of the primary sequence, none of the ATAD3A/B/C isoforms have mitochondrial addressing signal known by «cleavable N-terminal extremity» type. The targerting of ATAD3 to the mitochondria depends on a large central sequence including the major transmembrane sequence (Gilquin et al. 2010a; Hubstenberger 2006).

As regards to the predictable post-translational modifications, it is possible to detect several putative phosphorylation sites for PKC, PKA, GSK3, cdc2 CKI, CKII, DNAPK, RSK, Cdk5, PKG and p38MAPK INSR (NetPhosK 1.0 server). It was also detected two N6-acetyl-lysine in position 490 and 512 in the mouse ATAD3 protein (http://www.uniprot.org/uniprot/Q925I1).

We finally emphasize on the fact that splicing variants of ATAD3 exist for ATAD3A and ATAD3B genes (ATAD3Al/s and ATAD3Bl/s). These variants present two additional exons encoding a potential transmembrane sequence of hydrophobic alpha helix type present at the N-terminus (a.a 101–120 for ATAD3Al, Figs. 2 and 3).

Cellular localization and membrane topology

The mitochondrial localization of ATAD3 was first shown by a proteomic approach of the mouse liver mitochondrial inner membrane (Da Cruz et al. 2003). This result was then confirmed by immunofluorescence in HeLa cells (Schaffrik et al. 2006). Different biochemistry approaches were then used and showed that ATAD3 is strongly anchored to the mitochondrial inner membrane (Gilquin et al. 2010a; Hubstenberger 2006; He et al. 2007). Furthermore, it was shown that ATAD3 was found in a large multi-molecular complex associated with mitochondrial DNA (Wang and Bogenhagen 2006). He and his colleagues also showed that ATAD3 could interact with the mitochondrial DNA by its N-terminal (He et al. 2007). However, further analysis by chemical cross-linking showed that this interaction could be at long distance and could not involve the N-terminal (Bogenhagen et al. 2008). We observed as others that like outer membrane or inter-membrane space proteins, the N-terminal of ATAD3 is sensitive to trypsin attack in the context of the isolated mitochondria. The C-terminus which remains resistant to trypsin is thus probably in the matrix (Gilquin et al. 2010a; Bogenhagen et al. 2008). This shows that the N-terminal of ATAD3 is very probably not located in the mitochondria matrix (Fig. 3).

In order to clarify this problem, we carried out a back-titration ELISA and an immunofluorescence analysis on isolated human mitochondria (Hubstenberger et al. 2010). These experiments showed that the N-terminal part of ATAD3 is accessible to antibodies while the C-terminus is not. These experiments confirmed that the N-terminus is exposed to the outside of the mitochondrial inner membrane, either in the inter-membrane space, in the outer membrane or in the cytosol. The C-terminus is located in the matrix (Fig. 3) (Hubstenberger et al. 2010). The following work is now to determine the precise location of the N-terminal part of ATAD3. In fact, trypsin permeabilizing effects and low doses of detergent used do not enable one to unambiguously distinguish the three possible locations (cytosol, inter-membrane space or outer membrane). Recent experiments suggest that the N-terminus (a.a. 1–50 for ATAD3As) is embedded in the outer membrane and confirm that the C-terminus is located in the matrix (Gilquin et al. 2010a). The membrane insertion of the N-terminus of ATAD3As may also exist for ATAD3Al and ATAD3Bl, which contain this N-terminal part of 50 amino acids but not for ATAD3Bs which does not contain this sequence.

The problem is more complex if we consider the splicing variants of ATAD3A and ATAD3B (Hubstenberger et al. 2008). ATAD3Al and ATAD3Bs possess also an additional structural domain likely to form a transmembrane alpha helix (Fig. 2). This segment could allow the protein to join the cytosol or to cross again the inner membrane, and presents moreover phosphorylation sites (Fig. 3).

We finally note that ATAD3Bs/l, unlike ATAD3As/l, possess a potential transmembrane sequence at its C-terminus which could contribute to bring back the protein in the intermembrane space (Fig. 3).

However, we cannot exclude that these hydrophobic regions with transmembrane potential play a role in ATAD3 homo-polymerization, in interaction with partners or in the mechanisms of membrane insertion of ATAD3, without constituting *in fine* a transmembrane domain. Electron microscopy seems to be now an inescapable step to achieve this goal.

A more precise knowledge of the membrane topology of ATAD3 and of its variants seems now necessary to the pursuit of other investigations, particularly as regards to the search of partners.

ATAD3 expression in human

In primates and humans, the ancestral form of ATAD3 gene, named ATAD3A, has been duplicated and mutated twice to generate two paralogues named ATAD3B and ATAD3C. These three genes are located side by side at the end of chromosome 1 (locus 1p36.33).

As discussed above, the ATAD3B gene is very similar to ATAD3A (93% homology compared to ATAD3As). However, ATAD3B has a C-terminal extended to 62 amino acids where are mainly found potential phosphorylation sites and a presumed transmembrane domain (http://www.enzim.hu/hmmtop/, Figs. 2 and 3). The protein ATAD3C (87% homology with ATAD3As) lacks the first 70 a.a causing the loss of the coiled-coil structure. ATAD3C differs also in the last exon, giving it new potential phosphorylation sites. These three proteins could thus contribute to a common function presenting specificity in functioning (cellular localization, tissue expression, regulation, partners…).

The most notable observation concerns the promoters of these genes (http://www.bimas.cit.nih.gov). The putative promoter of the ancestral gene, ATAD3A, contains numerous regulatory elements linked to cell growth, such as C/EBP (CCAAT / enhancer binding protein, two binding sites), CBF/IRP/CREB (Core Binding Factor and Iron Regulatory Protein and cAMP Response Element Binding protein, 2 sites), GATA-1 (3 sites), Oct-1 (OCTAM-binding Protein, 1 site), and TFIID (1 site). The promoter of ATAD3B gene contains these same sites (CREB, GATA-1, Oct-1, and TFIID) and in addition, contains 5 Pit1 binding domains, two Myc-binding domains and a myogenin binding domain. The transcription factor Pit1 (growth hormone factor 1) is expressed during embryogenesis and in the adult (Simmons et al. 1990) and myogenin is also expressed during embryogenesis (Sassoon et al. 1989; Hinterberger et al. 1991; Pownall et al. 2002). Hence, these two factors could contribute to the specific expression of ATAD3Bl/s in embryonic tissues, particularly in muscle tissue.

Fig. 2 Alignment of human ATAD3A/3B/3C isoforms and structural areas (identity * and homology :). The ATPase region is represented by the light grey box (Walker sites dotted squared). The coiled-coil region is represented by the dark grey box. The three putative transmembrane domains are squared in black and numbered. The proline-rich region and the glutamine-rich region are squared in grey. From http://www.ebi.ac.uk/Tools/es/cgi-bin/clustalw2

We have shown that the relative expression of these genes varies significantly in humans (Hubstenberger et al. 2008). The ATAD3A gene is expressed ubiquitously, particularly in the brain. On the contrary, ATAD3B gene is not expressed in the adult brain, but expressed in human astrocytoma cell lines, in embryonic tissues and in the pituitary gland, an adult brain germinal zone (Hubstenberger et al. 2008; Schaffrik et al. 2006; He et al. 2007). It seems therefore that the ATAD3B gene is expressed in the tissues with high proliferative potential (embryo and adult germinal zones).

As regard to ATAD3C expression, no experimental data exits currently.

ATAD3 and cancers

Several articles have highlighted the link which exists between the ATAD3 expression and tumor progression [8, 9, 24, 27, 29 and Fig. 4]. In all studied cases, the tumor progression is associated with the ATAD3As or ATAD3Bl overexpression and with an increased resistance to apoptosis (chemoresistance).

The chromosomal localization of the three human genes ATAD3A, ATAD3B and ATAD3C (1p36-33) drew our attention because this region is known for many years to contain chemoresistance and tumor suppressor genes. Indeed, this chromosomal region could enable the discrimination of the two major glioma sub-types which are astrocytoma and oligodendroglioma (Smith et al. 1999). Oligodendroglioma presents a recurrent deletion of the short arm of chromosome 1 (1p), whereas astrocytoma do not have this genetic signature. Oligodendroglioma tumors are relatively benign, that is to say not too aggressive and chemosensitive, while astrocytomas are instead highly evolutive and chemoresistant glioma. It was then interesting to see if ATAD3A and ATAD3B genes could behave as anti-oncogenes or genes inducing chemoresistance in oligodendroglioma cells. To do this, we studied the expression of ATAD3As and ATAD3Bl and their over- and under-expression in oligodendroglial cell lines in order to measure the anti-proliferative and chemoresistance properties of ATAD3 (Hubstenberger et al. 2008).

We first noticed that ATAD3Bl was undetectable in cell lines derived from oligodendroglioma, whereas ATAD3As was slightly under-expressed, compared to cell lines derived

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ATAD3As_Human  MSWLFGINKGPKGEGAGPPPPLPPAQPGAEGGGDRGLGDRPAPKDKWSNFDPTGLERAAK 60
ATAD3A1_Human  MSWLFGINKGPKGEGAGPPPPLPPAQPGAEGGGDRGLGDRPAPKDKWSNFDPTGLERAAK 60
ATAD3Bs_Human  ------------------------------------------------------------
ATAD3B1_Human  MSWLFGVNKGPKGEGAGPPPPLPPAQPGAEGGGDRGLGDRPAPKDKWSNFDPTGLERAAK 60
ATAD3C_Human   ------------------------------------------------------------

                                                           5
ATAD3As_Human  AARELEHSR--YAKDALNLAQMQEQTLQLEQQSKLK------------------------ 94
ATAD3A1_Human  AARELEHSR--YAKDALNLAQMQEQTLQLEQQSKLKMRLEALSLLHTLVWAWSLCRAGAV 118
ATAD3Bs_Human  ------------------------------------MQLEALNLLHTLVWARSLCRAGAV 24
ATAD3B1_Human  AARELEHS--RYAKEALNLAQMQEQTLQLEQQSKLK------------------------ 94
ATAD3C_Human   ---------MSKDALNLAQMQEQTLQLEQQSKLK-------------------------- 25

ATAD3As_Human  ------------------------EYEAAVEQLKSEQIRAQAEERRKTLSEETRQHQARA 130
ATAD3A1_Human  QTQERLSGSASPEQVPAGECCALQEYEAAVEQLKSEQIRAQAEERRKTLSEETRQHQARA 178
ATAD3Bs_Human  QTQERLSGSASPEQVPAGECCALQEYEAAVEQLKSEQIRAQAEERRKTLSEETRQHQARA 84
ATAD3B1_Human  ------------------------EYEAAVEQLKSEQIRAQAEERRKTLSEETRQHQARA 130
ATAD3C_Human   ------------------------------------------------------------

ATAD3As_Human  QYQDKLARQRYEDQLKQQQLLNEENLRKQEESVQKQEAMRRATVEREMELRHKNEMLRVE 190
ATAD3A1_Human  QYQDKLARQRYEDQLKQQQLLNEENLRKQEESVQKQEAMRRATVEREMELRHKNEMLRVE 238
ATAD3Bs_Human  QYQDKLARQRYEDQLKQQQLLNEENLRKQEESVQKQEAMRRATVEREMELRHKNEMLRVE 144
ATAD3B1_Human  QYQDKLARQRYEDQLKQQQLLNEENLRKQEESVQKQEAMRRATVEREMELRHKNEMLRVE 190
ATAD3C_Human   -----------------QLVN-EDLRKQEESVQK-------------------------- 41

                                           2                        1
ATAD3As_Human  AEARARAKAERENADIIREQIRLKAAEHRQTVLESIRTAGTLFGEGFRAFVTDRDKVTAT 250
ATAD3A1_Human  AEARARAKAERENADIIREQIRLKAAEHRQTVLESIRTAGTLFGEGFRAFVTDRDKVTAT 298
ATAD3Bs_Human  TEARARAKAERENADIIREQIRLKASEHRQTVLESIRTAGTLFGEGFRAFVTDRDKVTAT 204
ATAD3B1_Human  TEARARAKAERENADIIREQIRLKASEHRQTVLESIRTAGTLFGEGFRAFVTDRDKVTAT 250
ATAD3C_Human   ---------------------------HRQTFLESIRAAGTLFGEGFRAFVTDRDKVTAT 74

                    1
ATAD3As_Human  VAGLTLLAVGVYSAKNATLVAGRFIEARLGKPSLVRETSRITVLEALRHPIQ-VSRRLLS 309
ATAD3A1_Human  VAGLTLLAVGVYSAKNATLVAGRFIEARLGKPSLVRETSRITVLEALRHPIQ-VSRRLLS 357
ATAD3Bs_Human  VAGLTLLAVGVYSAKNATAVTGRFIEARLGKPSLVRETSRITVLEALRHPIQ-VSRRLLS 263
ATAD3B1_Human  VAGLTLLAVGVYSAKNATAVTGRFIEARLGKPSLVRETSRITVLEALRHPIQ-VSRRLLS 309
ATAD3C_Human   VAGLTLLAVGVYSAKNATAVTGRYIEARLGKPSLVRETSRITVLEALRHPIQQVSRRLLS 134

                                                    3    WA
ATAD3As_Human  RPQDALEGVVLSPSLEARVRDIAIATRNTKKNRSLYRNILMYGPPGTGKTLFAKKLALHS 369
ATAD3A1_Human  RPQDALEGVVLSPSLEARVRDIAIATRNTKKNRSLYRNILMYGPPGTGKTLFAKKLALHS 417
ATAD3Bs_Human  RPQDVLEGVVLSPSLEARVRDIAIATRNTKKNRGLYRHILLYGPPGTGKTLFAKKLALHS 323
ATAD3B1_Human  RPQDVLEGVVLSPSLEARVRDIAIATRNTKKNRGLYRHILLYGPPGTGKTLFAKKLALHS 369
ATAD3C_Human   RPQDVLEGVVLSPSLEARVRDIAIMTRNTKKNRGLYRHILLYGPPGTGKTLFAKKLALHS 194

                                                    WB
ATAD3As_Human  GMDYAIMTGGDVAPMGREGVTAMHKLFDWANTSRRGLLLFVDEADAFLRKRATEKISEDL 429
ATAD3A1_Human  GMDYAIMTGGDVAPMGREGVTAMHKLFDWANTSRRGLLLFVDEADAFLRKRATEKISEDL 477
ATAD3Bs_Human  GMDYAIMTGGDVAPMGREGVTAMHKLFDWANTSRRGLLLFMDEADAFLRKRATEEISKDL 383
ATAD3B1_Human  GMDYAIMTGGDVAPMGREGVTAMHKLFDWANTSRRGLLLFMDEADAFLRKRATEEISKDL 429
ATAD3C_Human   GMDYAIMTGGDVAPMGREGVTAMHKLFDWANTSRRGLLLFVDEADAFLRKRATEKISEDL 254

                       Sensor 1    Arg finger
ATAD3As_Human  RATLNAFLYRTGQHSNKFMLVLASNQPEQFDWAINDRINEMVHFDLPGQEERERLVRMYF 489
ATAD3A1_Human  RATLNAFLYRTGQHSNKFMLVLASNQPEQFDWAINDRINEMVHFDLPGQEERERLVRMYF 537
ATAD3Bs_Human  RATLNAFLYHMGQHSNKFMLVLASNLPEQFDCAINSRIDVMVHFDLPQQEERERLVRLHF 443
ATAD3B1_Human  RATLNAFLYHMGQHSNKFMLVLASNLPEQFDCAINSRIDVMVHFDLPQQEERERLVRLHF 489
ATAD3C_Human   RATLNAFLYRTGQHSNKFMLILASCHPEQFDWAINACIDVMVHFDLPGQEERARLVRMYL 314

                       Sensor 2
ATAD3As_Human  DKYVLKPATEGKQRLKLAQFDYGRKCSEVARLTEGMSGREIAQLAVSWQATAYASEDGVL 549
ATAD3A1_Human  DKYVLKPATEGKQRLKLAQFDYGRKCSEVARLTEGMSGREIAQLAVSWQATAYASEDGVL 597
ATAD3Bs_Human  DKCVLKPATEGKRRLKLAQFDYGRKCSEVARLTEGMSGREIAQLAVSWQATAYASKDGVL 503
ATAD3B1_Human  DKCVLKPATEGKRRLKLAQFDYGRKCSEVARLTEGMSGREIAQLAVSWQATAYASKDGVL 549
ATAD3C_Human   NEYVLKPATEGKRRLKLAQFDYGRKCLEIARLTEGMSCRKIAQLAVSWQATAYASKDGVL 374

ATAD3As_Human  TEAMMDTRVQDAVQQHQQKMCWLKAEGPGRGDEPSPS----------------------- 586
ATAD3A1_Human  TEAMMDTRVQDAVQQHQQKMCWLKAEGPGRGDEPSPS----------------------- 634
ATAD3Bs_Human  TEAMMDACVQDAVQQYRQKMRWLKAEGPGRGVEHPLSGVQGETLTSWSLATDPSYPCLAG 563
ATAD3B1_Human  TEAMMDACVQDAVQQYRQKMRWLKAEGPGRGVEHPLSGVQGETLTSWSLATDPSYPCLAG 609
ATAD3C_Human   TEAMMDACVQDFVQQHQQKMRWLKGERPGPEDEQPSS----------------------- 411

                          4
ATAD3As_Human  ---------------------------------------
ATAD3A1_Human  ---------------------------------------
ATAD3Bs_Human  PCTFRICSWMGTGLCPGPLSPRMSCGGGRPFCPPGHPLL 602
ATAD3B1_Human  PCTFRICSWMGTGLCPGPLSPRMSCGGGRPFCPPGHPLL 648
ATAD3C_Human   ---------------------------------------
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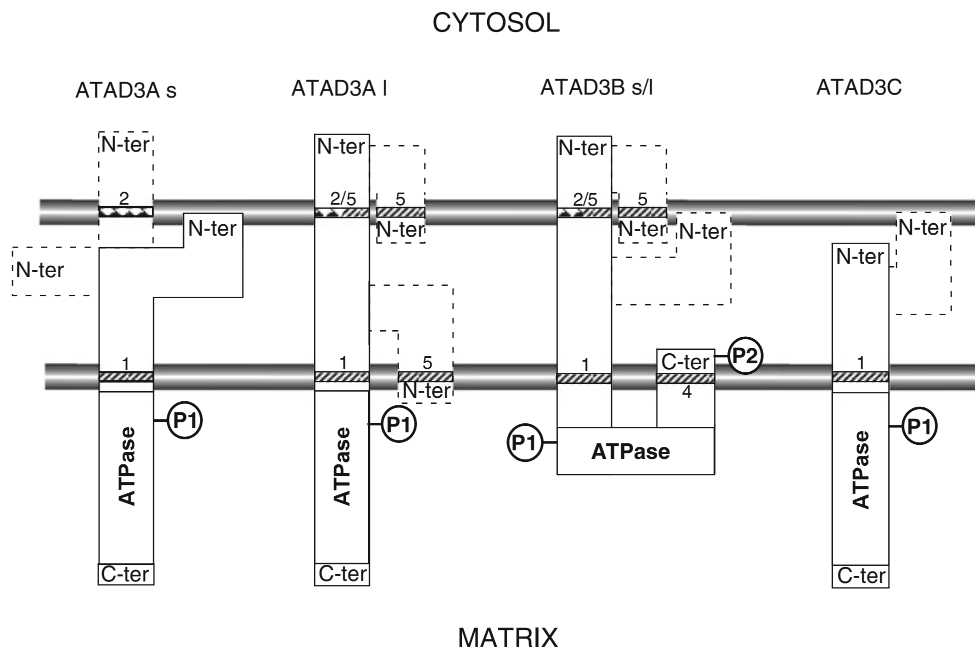

Fig. 3 Models for membrane insertion of ATAD3 variants. According to current data, the ATPase part of ATAD3 is located in the mitochondrial matrix. For ATAD3As (s for small), which could have two helices or more, the N-terminal part is either in the cytosol or in the intermembrane space. For ATAD3Al (l for long) which clearly contains a second transmembrane helix (from a differential splicing), the N-terminal is either in the cytosol or returns in the matrix. For ATAD3B, the case is similar to ATAD3A except that the C-terminus could return to the intermembrane space. Finally, ATAD3C has probably the N-terminal part in the intermembrane space. The transmembrane sequences (numbered as in Fig. 3) in hatched correspond to sequences identified by all predictive softwares (DAS, TOPpred, TMpred and HMMTOP). The transmembrane sequences with dots (numbered as in Fig. 3) correspond to sequences identified by one or two predictive softwares (TMpred and HMMTOP). These last zones of hydrophobicity could correspond to transitory forms of membrane insertion. Major phosphorylation sites are indicated by P1 and P2

from astrocytoma. We showed that in an oligodendroglioma cell line (HS683), in addition to the 1p deletion (1pLOH), the second ATAD3B gene had disappeared by deletion, leaving only one ATAD3A copy from the 4 initial ATAD3A/B genes. This triple gene loss could be the genetic signature of the selective deletion of a tumor suppressor gene (heterozygosity loss).

Since ATAD3Bl is detectable in cell lines derived from astrocytoma but not in those derived from oligodendroglioma, it is now interesting to explore the expression of ATAD3Bl in biopsies of human glial tumors in order to see if ATAD3Bl could be a positive marker of astrocytoma compared to oligodendroglioma and healthy tissue adjacent to surgical resection or conversely a negative marker for oligodendroglioma against astrocytoma.

In vitro ectopic expression of ATAD3As and ATAD3Bl in cells derived from oligodendrogliomas induced also a notable inhibition of cell growth by increasing the duration of G1 phase of cell cycle) (Fig. 4, (Hubstenberger et al. 2008)). This observation suggests also that ATAD3 has the properties of an anti-oncogene in this model. Confirming this finding, a proliferative effect is observed by under-expression of ATAD3 with SiRNA in U373 cells, a cell line derived from astrocytoma. However, this observation may not be generalized because in the GHD-1 cells, cell line derived from carcinoma of the hypopharynx, the transfection of SiRNA induces an apoptotic effect associated with dysfunction of cytokinesis and endo-replication (Schaffrik et al. 2006). This is not contradictory, since apoptosis is intimately linked to processes of cell cycle progression. Moreover, in Hela cells, over-expression of ATAD3As showed a pro-apoptotic effect (Da Cruz et al. 2003). It is possible that in different cell lines derived from tumors, ATAD3 can be either dispensable or indispensable depending on the degree of differentiation or transformation. In HeLa and GHD-1 cells, over-expression of ATAD3s/l does not induce a significant slowdown of the cell cycle but here the relative over-expression of ATAD3 was not measured (Schaffrik et al. 2006).

In parallel, we have also shown that ectopic expression of ATAD3As and ATAD3Bl in a oligodendroglioma derived cell line (chemosensitive) induces in short- and long-term chemoresistance against different genotoxic agents (Hubstenberger et al. 2008). This observation was confirmed by the under-expression of ATAD3As and 3Bl in an astrocytoma derived cell line (chemoresistant) which makes them chemosensitive. The effect of ATAD3As and ATAD3Bl on short-term resistance suggests that ATAD3A and ATAD3B may protect cells

Fig. 4 Effects of under- and overexpression of ATAD3 in different cancer cells. Effects on proliferation, apoptosis and chemo- or radio-resistance of ATAD3 expression are recapitulated, describing the cellular model used, the studied isoform and the technique involved. Corresponding references are indicated

Cellular models	Techniques Studiedisoformes	Effects	References
COS Kidneycells-SV40/monkey	Surexpression transitoire ATAD3As/Bl	↘ proliferation	[8]
HS683 Oligodendroglioma/human	Surexpression transitoire/stable ATAD3As/Bl	↘ proliferation ↘ apoptosis	[8]
U373 Astrocytoma/human	Surexpression transitoire/stable ATAD3As/Bl	↘ proliferation	[8]
U373 Astrocytoma/human	SiRNA ATAD3As/Bl	↗ proliferation ↗ apoptosis	[8]
GHD-1 Hypopharynx carcinoma/human	SiRNA ATAD3Bs/Bl	↗ apoptosis	[9]
GHD-1 Hypopharynx carcinoma/human	Surexpression transitoire ATAD3Bs/Bl	noeffect	[9]
HeLa Cervixcancer/human	Surexpression transitoire ATAD3As	↗ apoptosis	[2]
A549 Lungcancer/human	SiRNA ATAD3As	↘ chemoresistance	[24]
H838 Lungcancer/human	SiRNA ATAD3As	↘ chemoresistance	[24]
U373 Astrocytoma/human	Surexpression ATAD3As/Bl	↗ chemoresistance	[8]
HS683 Oligodendroglioma/human	Surexpression ATAD3As/Bl	↗ chemoresistance	[8]
RAJI Lymphoma/human	Protéomiquedifférentielle ATAD3Bs/Bl	↗ radioresistance	[27]

against genotoxic agents by improving their rapid elimination (permeability, chemical modification and degradation). The long-term resistance suggests that ATAD3As and ATAD3Bl could increase the efficiency of the cell cycle arrest and DNA repair. Therefore, ATAD3 could be a target for optimizing the chemotherapeutic treatment of astrocytoma. Oligodendroglioma is a chemosensitive glioma which is characterized by loss of heterozygosity (LOH 1p), whereas chemoresistant astrocytoma is an aggressive tumor unaffected by the 1pLOH, we can imagine that within the population of glial precursor cells, the loss of ATAD3 genes could participate in their transformation into oligodendroglioma and contribute to their chemosensitive phenotype. Thus, ATAD3B is a putative marker which could enable the discrimination of oligodendroglioma from astrocytoma and surrounding healthy tissue (Hubstenberger et al. 2008). More recently and confirming these results, it has been shown that ATAD3A constitutes an anti-apoptotic marker in lung tumors (Fang et al. 2010) and a radioresistance marker in non-Hodgkin lymphoma and uterine cervical cancer (Jiang et al. 2009; Chen et al. 2011).

ATAD3, to a function

Today, data on ATAD3 are still quite few and some results are contradictory. However ATAD3 localization and its membrane topology are now better confirmed.

ATAD3 has been originally described twice as producing auto-immune responses in cancer patients (Gires et al. 2004; Geuijen et al. 2005). These results imply that ATAD3 could be present outside the cell, accessible to the immune system and to the antibodies. To date, achieved approaches have not

yet confirmed this location and ATAD3 has been visualized only in mitochondria. We can only exclude those different processes, such as a cleavage or a process of differential excision/splicing that could relocate ATAD3 in the extracellular compartment. However, the acquired autoimmune response against ATAD3 could result from dead or damaged cells produced by the anti-tumor treatment suffered by these patients, noting that ATAD3 is an abundant and relatively stable protein.

However, ATAD3 is now clearly considered as an inner-membrane mitochondrial protein. ATAD3 membrane topology is now nearly elucidated but doubts remain concerning the exact localization of the N-terminus (Gilquin et al. 2010a; Gilquin et al. 2010b; Fang et al. 2010). The C-terminus is located in the mitochondrial matrix and could be associated with the nucleoid (mitochondrial DNA and associated proteins) but this interaction may be indirect (Bogenhagen et al. 2008).

ATAD3A has been also shown to bind Frataxin (Correia et al. 2008), a mitochondrial protein from which mutations in exon 1 can cause Friedreich's ataxia (FRDA), a neurodegenerative disease. Frataxin interacts with mortalin/GRP75, an homologue of the yeast chaperone protein ssq1 which integrates the Iron-sulfur group into proteins imported into mitochondria (Shan et al. 2007). Another frataxin interactant is ISD11, a component of the Nfs/ICSU complex. These two proteins are also components of the Fe/S assembly complex (CSI) where Nfs, a cysteine disulfurase, removes sulfur from cysteine and converts it into alanine. These two proteins are essential for the biogenesis of the iron-sulfur group. These results suggest that Frataxin binds the complex Nfs/ICSU through ISD11 and that ATAD3 could be associated to this function.

In conclusion, we would insist on the role that ATAD3 could have in mitochondria-reticulum interactions as well as in inner-outer mitochondrial membranes interactions. Indeed, ATAD3 seems to be located at contact points where the outer membrane and the inner membrane of mitochondria are juxtaposed, and these contact points are in relation to contact areas between the endoplasmic reticulum and mitochondria (Gilquin et al. 2010a; Hubstenberger 2006; Fang et al. 2010). These contact areas are now considered to be the transit zones for many molecules such as neo-synthesized proteins, calcium and lipids such as phosphatidylserine and cholesterol. Also, the invalidation of ATAD3 which induces a fragmentation of the mitochondrial network and a loss of interaction between mitochondria and reticulum, induces a notable reduction of cholesterol flow between these compartments (Hoffmann et al. 2009; Gilquin et al. 2010a; Hubstenberger 2006; Gilquin et al. 2010b).

ATAD3 could therefore contribute to define structures capable of promoting mitochondrial inner-outer membranes as well as mitochondrial outer membrane-reticulum interactions, contributing to the transit of some molecules. ATAD3 could also, more generally, contribute to mitochondrial homeostasis by participating in a recycling/neosynthesis mechanism of mitochondrial constituents and this related to endoplasmic reticulum (Kornmann 2010; Kornmann and Walter 2010; Hayashi et al. 2009). However, we are not certain that there is a direct causality between invalidation of ATAD3 and modification of the mitochondrial network structure. This process could be indirect and associated with another mitochondrial dysfunction.

The function of this ATPase still remains an enigma although it is revealed essential for proper functioning of the mitochondrial network. Its abundance and its ubiquitous expression certainly reflect an important role in the cell and the search for its partners is now an essential question to discover its function. However, these approaches must be established pertinently because the high abundance of ATAD3, its membrane localization and its very basic pHi (pI=10) are major sources of artifacts. The study of a differentiation model is now necessary to know the action level of ATAD3 by precisely studying the cellular effects of its invalidation or over-expression.

Acknowledgements We are very grateful to Pr. Valdur SAKS who gave us a critical reading of the manuscript and also to Dr. Arnaud HUBSTENBERGER with whom a big piece of work was done on ATAD3. Shuijie LI is supported by Hubert Curien Program PhD fellowship. I'm sincerely and profoundly grateful to Yulia DIMITROVA for constant supports and fruitful discussions.

Conflict of interest The authors declare no conflict of interest.

References

Zeller KI, Jegga AG, Aronow BJ, O'Donnell KA, Dang CV (2003) An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. Genome Biol 4(10):R69

Da Cruz S, Xenarios I, Langridge J, Vilbois F, Parone PA, Martinou JC (2003) Proteomic analysis of the mouse liver mitochondrial inner membrane. J Biol Chem 278(42):41566–71

Frickey T, Lupas AN (2004) Phylogenitic analysis of AAA proteins. J Struct Biol 146:2–10

Kamath RS, Ahringer J (2003) Genome-wide RNAi screening in Caenorhabditis elegans. Methods 30:313–21

Hoffmann M, Bellance N, Rossignol R, Koopman WJ, Willems PH, Mayatepek E, Bossinger O, Distelmaier FC (2009) elegans ATAD-3 is essential for mitochondrial activity and development. PLoS One 4(10):e7644

Gilquin B, Taillebourg E, Cherradi N, Hubstenberger A, Gay O, Merle N, Assard N, Fauvarque MO, Tomohiro S, Kuge O, Baudier J (2010a) The AAA+ATPase ATAD3A controls mitochondrial dynamics at the interface of the inner and outer membrane. Mol Cell Biol 30(8):1984–96

Hubstenberger A (2006) MSBP, une protéine identifiée comme une cible de la S100B, impliquée dans la distribution subcellulaire des mitochondries. Thèse de l'Université J. Fourier 2006. http://tel.archives-ouvertes.fr/docs/00/17/52/59/PDF/Arnaud_Hubstenberger.pdf

Hubstenberger A, Labourdette G, Baudier J, Rousseau D (2008) ATAD3A and ATAD3B are distal 1p-located genes differentially expressed in human glioma cell lines and present in vitro anti-oncogenic and chemoresistant properties. Exp Cell Res 314:2870–2883

Schaffrik M, Mack B, Matthias C, Rauch J, Gires O (2006) Molecular characterization of the tumor-associated antigen AAA-TOB3. Cell Mol Life Sci 63(18):2162–2174

He J, Mao CC, Reyes A, Sembongi H, Di Re M, Granycome C, Clippingdale AB, Fearnley IM, Harbour M, Robinson AJ, Reichelt S, Spelbrink JN, Walker JE, Holt IJ (2007) The AAA+protein ATAD3 has displacement loop binding propeties and is involved in mitochondrial nucleoid organization. J Cell Biol 176(2):141–146

Wang Y, Bogenhagen DF (2006) Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. J Biol Chem 281 (35):25791–802

Bogenhagen DF, Rousseau D, Burke S (2008) The layered structure of human mitochondrial DNA nucleoids. J Biol Chem 283(6):3665–3667

Hubstenberger A, Merle N, Charton R, Brandolin G, Rousseau D (2010) Topological analysis of ATAD3A insertion in purified human mitochondria. J Bioenerg Biomembr 42(2):143–50

Simmons DM, Voss JW, Ingraham HA, Holloway JM, Broide RS, Rosenfeld MG, Swanson LW (1990) Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergistic interactions with other classes of transcription factors. Genes Dev 4 (5):695–711

Sassoon D, Lyons G, Wright WE, Lin V, Lassar A, Weintraub H, Buckingham M (1989) Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis. Nature 341(6240):303–307

Hinterberger TJ, Sassoon DA, Rhodes SJ, Konieczny SF (1991) Expression of the muscle regulatory factor MRF4 during somite and skeletal myofiber development. Dev Biol 147(1):144–156

Pownall ME, Gustafsson MK, Emerson CP Jr (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryons. Annu Rev Cell Dev Biol 18:747–783

Smith JS, Alderete B, Minn Y, Borell TJ, Perry A, Mohapatra G, Mohapatra G, Hosek SM, Kimmel D, O'Fallon J, Yates A, Feuerstein BG, Burger PC, Scheithauer BW, Jenkins RB (1999) Localization of common deletion regions on 1p and 19q in human glioma and their association with histological subtype. Oncogene 18:4144–4152

Correia AR, Pastore C, Adinolfi S, Pastore A, Gomes CM (2008) Dynamics, stability and iron-binding activity of frataxin clinical mutants. FEBS J 275(14):3680–90

Shan Y, Napoli E, Cortopassi G (2007) Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum Mol Genet 16(8):929–41

Gires O, Münz M, Schaffrik M, Kieu C, Rauch J, Ahlemann M, Eberle D, Mack B, Wollenberg B, Lang S, Hofmann T, Hammerschmidt W, Zeidler R (2004) Profile identification of disease-associated humoral antigens using AMIDA, a novel proteomics-based technology. Cell Mol Life Sci 61(10):1198–207

Geuijen CA, Bijl N, Smit RC, Cox F, Throsby M, Visser TJ, Jongeneelen MA, Bakker AB, Kruisbeek AM, Goudsmit J, de Kruif JA (2005) proteomic approach to tumour target identification using phage display, affinity purification and mass spectrometry. Eur J Cancer 41(1):178–87

Gilquin B, Cannon BR, Hubstenberger A, Moulouel B, Falk E, Merle N, Assard N, Kieffer S, Rousseau D, Wilder PT, Weber DJ, Baudier J (2010b) The calcium-dependent interaction between S100B and the mitochondrial AAA-ATPase ATAD3A and the role of this complex in the cytoplasmic processing of ATAD3A. Mol Cell Biol 30(11):2724–36

Fang HY, Chang CL, Hsu SH, Huang CY, Chiang SF, Chiou SH, Huang CH, Hsiao YT, Lin TY, Chiang IP, Hsu WH, Sugano S, Chen CY, Lin CY, Ko WJ, Chow KC (2010) ATPase family AAA domain-containing 3A is a novel anti-apoptotic factor in lung adenocarcinoma cells. J Cell Sci 123(7):1171–80

Kornmann B (2010) Le complexe ERMES : une connexion haut débit entre le réticulum endoplasmique et les mitochondries. Médecine/Science (2)

Kornmann B, Walter P (2010) ERMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology. J Cell Sci 123:1389–93

Hayashi T, Rizzuto R, Hajnoczky G, Su TP (2009) MAM: more than just a housekeeper. Trends Cell Biol 19(2):81–8

Jiang Y, Liu X, Fang X, Wang X (2009) Proteomic analysis of mitochondria in Raji cells following exposure to radiation: implications for radiotherapy response. Protein Pept Lett 16 (11):1350–9

Chen TC, Hung YC, Lin TY, Chang HW, Chiang IP, Chen YY, Chow KC (2011) Human papillomavirus infection and expression of ATPase family AAA domain containing 3A, a novel anti-autophagy factor, in uterine cervical cancer. Int J Mol Med 28 (5):689–96. doi:10.3892/ijmm.2011.743