MMS19 localizes to mitochondria and protects the mitochondrial genome from oxidative damage

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MMS19 localizes to mitochondria and protects the mitochondrial genome from oxidative damage

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ABSTRACT

MMS19 localizes to cytoplasmic and nuclear compartments involving in transcription and nucleotide excision repair (NER). However, whether or not MMS19 localizes to mitochondria where it plays a role in maintaining mitochondrial genome stability remains elusive. In this study, we provide the first evidence that MMS19 is localized in the inner membrane of mitochondria and participates in mtDNA oxidative damage repair. MMS19 knockdown led to mitochondrial dysfunctions including decreased mtDNA copy number, diminished mtDNA repair capacity and elevated level of mtDNA common deletion after oxidative stress. Immunoprecipitation-mass spectrometry analysis identified that MMS19 interacts with ANT2, a mitochondrial ATP metabolism-associated protein. ANT2 knockdown also resulted in a decreased mtDNA repair capacity post oxidative damage. Our findings suggest that MMS19 plays an essential role in maintaining mitochondrial genome stability.

Key words: MMS19, Mitochondria, ANT2, ROS, oxidative stress

Draft

1. Introduction

Methyl methanesulfonate sensitivity gene 19 (MMS19) is a highly conserved HEAT repeat protein that was first identified in *Saccharomyces cerevisiae* as being required for the removal of ultraviolet radiation (UV)-induced pyrimidine dimers (Gari et al. 2012). Like TFIIH components, MMS19 appears to be involved in both nucleotide excision repair (NER) and RNA polymerase II transcription (Lombaerts et al. 1997). Human MMS19 physically interacts with the XPB (Ssl2 homologue) and XPD (Rad3 homologue) subunits of TFIIH (Ito et al. 2010), and is assumed to be an upstream regulator of TFIIH activity, though the biological significance of these interactions remains unclear. MMS19 exerts its function in NER and transcription by interacting with the XPB and XPD helicases (Kou et al. 2008; Seroz et al. 2000). MMS19 protein contains three conserved regions, including domains A and B and a HEAT repeat domain C, and has been postulated to modulate the balance between transcription and DNA repair (Queimado et al. 2001). Mutants in eukaryotic MMS19 show a variety of phenotypes, including defects in

methionine synthesis, sensitivity to genotoxic stress induced by MMS (methyl methanesulfonate), and the presence of extended telomeres (Stehling et al. 2012).

Mitochondria are known as the 'power house' of the cell where ATP is generated by electron transport and oxidative phosphorylation across inner mitochondrial membrane, along which reactive oxygen species (ROS) as the by-product are formed continuously during normal cellular metabolism. ROS can result in multiple DNA lesions, such as abasic sites, oxidized DNA bases, and single- or double-strand breaks (Maynard et al. 2009). Since mitochondria DNA is lack of protective histones and is constantly exposed to free radical species, mtDNA is more sensitive than nuclear DNA in response to oxidative stress with persistent mtDNA damage and loss of mitochondrial function (Bohr 2002; Klaunig et al. 2011). Both mismatch repair (MMR) and base excision repair (BER) pathways are found in the nucleus and mitochondria. Although UV-induced nucleotide excision repair (NER) is observed to be defective in mitochondria, key nucleotide excision repair factor such as

CS proteins (CSA and CSB), Rad23a, and XPD have been localized in mitochondria (Liu et al. 2015; Melis et al. 2013). In addition, RecQL4, one of the five human RecQ helicases, is revealed to be located in mitochondria, and plays regulatory role in the maintenance of mitochondrial genome stability (Chi et al. 2012).

This is the first time we have identified that MMS19 is localized to the mitochondrial fractions and mtDNA deletion has been increased in MMS19-knockdown cells. Strikingly, capacity for oxidative mtDNA damage repair was markedly reduced in MMS19-knockdown cells. Similar to the reduced mtDNA repair capacity in MMS19-knockdown cells, oxidative mitochondrial DNA damage repair capacity in U2OS cells was also significantly changed after ANT2 knockdown. The siRNA-mediated knockdown of MMS19 or ANT2 led to mitochondrial dysfunction, which illustrated the significance of both MMS19 and ANT2 proteins for the mtDNA repair. Co-immunoprecipitation results revealed that SSBP1, a protein involved in mitochondrial DNA replication and molecular chaperone HSP70 directly interacted with MMS19, suggesting

that a potential mechanism in which MMS19 enters mitochondria by interacting with molecular chaperone HSP70. Furthermore, Our findings show that MMS19 plays an important role in the maintenance of mitochondrial genomic DNA stability through its interaction with mitochondrial proteins.

2. Materials and methods

2.1. Cell culture and transfections

U2OS, 293F, HEK293 and HeLa S3 were incubated in Dulbecco' s modified Eagle' s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS, Hyclone) at 37°C in humidified incubator with 5% CO₂. U2OS cells were treated with 1 mM H₂O₂ for 1 hour at 37°C in FBS-free medium. The siRNA for MMS19: GCAAUGUACUGCCUUUACUTT, siRNA for ANT2: GAUCACUGCAGAUAAAGCAATT and siRNA control: UUCUCCGAACGUGUCACGUTT were synthesized by GenePharma (China). U2OS cells were transfected with Rfect siRNA transfection reagent

(BIO-TRANS, China). pcDNA3.0/HA-MMS19 was used to generate HeLa S3 stable cell lines with 600 µg/ml Hygromycin B.

2.2. Alkali extraction of mitochondria

mitochondria were treated with 0.1 M Na₂CO₃ (pH11.5), and then centrifuged at 16,000 g. Supernatants (S) were retained. The pellet (P) were washed once and then suspended in an suspension medium (Liu et al. 2015).

2.3. Long-range quantitative PCR (QPCR) assay

Total cellular DNA was extracted using Qiagen genomic DNA isolation kit. A novel quantitative PCR assay was used to measure H₂O₂-induced DNA damage and repair in a 13.5-kb fragment up stream of the nuclear β-globin gene and a 16.2-kb fragment from the mitochondrial genome. These primers:

5' -TAGAGGGACAAGTGGCGTTC-3' , 5' -CGCTGAGCCAGTCAGTGT-3'

5' -CACCCAAGAACAGGGTTTGT-3' , 5' -TGGCCATGGGTATGTTGTAA-3'

were used for relative quantitation with the Bio-Rad CFX96 Real-Time PCR Detection Systems. The following primers β -globin gene: 5' -CGAGTAAGAGACCATTGTGGCAG-3' and 5' -GCACTGGCTTAGGAGTTGGACT-3' ; mtDNA gene: 5' -TGAGGCCAAATATCATTCTGAGGGGC-3' and 5' -TTTCATCATGCGGAGATGTTGGATGG-3' were used for PCR. The PCR was done as (Liu et al. 2015).

2.4. Measurement of common deletion

Cells were grown in serum free medium and treated with 0.5 mM H_2O_2 with or without 5, 000 U/mL Catalase, then the genomic DNA was extracted. Measurement of common deletion (CD) was done by QPCR using the primers: 5' -ACCCCCATACTCCTTACACTATTCC-3' and 5' -AAGGTATTCCTGCTAATGCTAGGCT-3' . An 83 bp PCR fragment served as a standard (Kamenisch et al. 2010).

2.5. Isolation of mitochondria , Co-immunoprecipitation assay and protein identification by mass spectrometry

The large-scale mitochondrial fraction was performed as previously described (Graham 2001). HA-MMS19 stable expression The mitochondrial fraction in HeLa cells were collected and suspended in lysis buffer, and then incubated with anti-HA beads. After washing, HA peptides was added to release the MMS19 protein complex. The purified MMS19 complex was separated by SDS-PAGE, visualized by silver staining, and processed for mass spectrometry (Liu et al. 2015). The whole gel were cut into four pieces. Excised gel fragments were washed with acetonitrile and then dried. After dried gel fragments were incubated with dithiothreitol solution at 56°C, iodoacetamide solution was added. Gel fragments were washed and digested with trypsin (Promega) overnight. Extracted peptides were loaded into the column of C18 resin (ReproSil-Pur) and strong cation-exchange resin (Luna SCX 100A). LC-MS was done using LTQ Orbitrap MS (Thermo Fisher Scientific) and the MS data were analyzed using MaxQuant program following

(Yang et al. 2015). A FDR (False discovery rates) of 0.01 for proteins and peptides and a minimum peptide length of 8 amino acids were required.

2.6. Antibodies

The used antibodies were as follows: rabbit anti-HA (Santa Cruz), mouse anti-MMS19 (Santa Cruz), rabbit anti-ANT2 (Cell signaling), mouse anti-GAPDH (Millipore), mouse anti-PCNA (Santa Cruz), rabbit anti-Tubulin (Santa Cruz), rabbit anti-VDAC (Cell signaling), and mouse anti-SMAC (Cell signaling).

3. Results

3.1. MMS19 protein localizes to mitochondria

Cytoplasmic/nuclear localization of MMS19 have been reported previously; and MMS19 is involved both in NER and transcription (Lombaerts et al. 1997). The previous studies demonstrates that oxidative damage is much higher in mitochondrial DNA than in nuclear DNA (Barja and Herrero 2000). As mitochondria DNA is constantly

exposed to oxidative stress, MMS19 protein may be localized to mitochondria and participate in protecting mitochondria from oxidative damage. Western blot analysis was performed to detect the presence of MMS19 protein in the mitochondrial fractions isolated from HEK293 cells. Lack of detectable level of PCNA (nuclear protein marker) and Tubulin (cytoplasmic protein marker) in the lysis of mitochondrial fractions indicated that the mitochondrial fraction was free from nuclear and cytoplasmic contamination (Fig. 1A).

Alkali extraction assay was also performed to further demonstrate the mitochondrial location of MMS19 protein. When mitochondria samples were re-suspended to Alkali extraction, peripheral membrane proteins were obtained in the supernatant; integral membrane proteins were collected in the pellet (Fig. 1B). As expected, the outer membrane integral protein VDAC was primarily recovered in the pellet while the inter membrane space protein SMAC was largely recovered in the supernatant. MMS19 was distributed in both fractions, indicating that

MMS19 is located not only in the integral membrane, but partially in the soluble fraction as well (Bannwarth et al. 2012).

3.2. MMS19 deficiency leads to mtDNA CD and a defective mtDNA repair

Mitochondrial DNA mutations play a major role in human degenerative diseases and aging processes. The most frequently reported marker for mutations of the mtDNA in human is a 4,977 bp Common Deletion (CD) (Koch et al. 2001). A quantitative real-time polymerase chain reaction assay was established to detect the Common Deletion. As shown in Fig. 2A, MMS19-suppressed (siMMS19) and control (siControl) cells were treated with 1 mM H₂O₂ for 60 min and recover for 48 h. Our results showed MMS19 knockdown cells showed >2-fold higher increase of CD compared with control cells, suggesting that MMS19 protein is required to protect mtDNA from CD mutations coming from oxidative stress. Specifically, after 5,000 U/ml natural antioxidant catalase (an antioxidant agent) in company with H₂O₂

treatment the CD level in MMS19 knockdown U2OS cells were decreased in contrast to the level of controls. It means MMS19 deficiency could result in an elevated oxidative mtDNA damage that can be recovered by antioxidant catalase. As mentioned above, a long-range quantitative PCR amplification of 16.2 kb mtDNA was subsequently used for assessment of mtDNA repair efficiency (Yakes and Van Houten 1997). In this PCR reaction any unrepaired DNA will terminate chain elongation by DNA polymerase, thereby decreasing the efficiency of DNA amplification. The mtND1 PCR product (110 bp) was used to normalize differences in mtDNA input (Chi et al. 2012). The cells transfected with siControl and siMMS19 were treated with 1 mM concentration of H₂O₂ to detect repair capacity in mitochondria in response to oxidative stress. As shown in Fig. 2B, both siControl and siMMS19-transfected U2OS cells showed a markedly decreased level of mtDNA PCR product at early time points after H₂O₂ treatment. In contrast to control cells, which showed the restoration of full-length mtDNA PCR product at 24 h post-treatment, a markedly decreased level of mtDNA PCR product was found in

siMMS19-transfected U2OS cells even at 48 h post-treatment. These findings clearly illustrated that MMS19 is essential for the efficient repair of oxidative DNA damage in mitochondria. In contrast to increased mtDNA repair deficiency, no significant differences were observed in the copy number of nuclear β -globin gene either in knockdown of MMS19 U2OS cells or control after H₂O₂ treatment, suggesting that MMS19 is essential for the repair of oxidative stress in mitochondria but is dispensable for some of the nuclear genes such as β -globin.

3.3. Interaction between MMS19 and ANT family protein SSBP1, HSP70

To find an unidentified function of MMS19 in mitochondria, Co-immunoprecipitation was used to identify the MMS19 protein complex from mitochondria. Human MMS19 cDNA fused with a HA tag at the C terminus was stably expressed in HeLa S3 cells (Fig. 3A), and the intact mitochondria extract of which was used for sequential Co-immunoprecipitation with anti-HA beads (Fig. 3B). The MMS19

complex was separated by polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by silver staining (Fig. 3C). Mass spectrometry analysis (LC-MS/MS) was next performed to identify proteins interacted with MMS19 in mitochondria.

The mass spectrometry analysis of the MMS19 complex detected ANT1, ANT2, ANT3, SSBP1 and HSP70 proteins (Supplementary Table 1). ANT is an ADP/ATP translocase, function of which is to catalyze mitochondrial ADP/ATP exchange (Cho et al. 2017). SSBP1 is a mitochondrial protein involved in mitochondrial DNA replication. While HSP70 serves as molecular chaperone in assisting protein transport. To further validate the potential partners that interacts with MMS19 in mitochondria, co-immunoprecipitation (Co-IP) assay was used to identify protein–protein interactions. For this task, whole cell lysates from 293F human kidney cells expressing Flag-HA-ANT1, Flag-HA-ANT2 and Flag-HA-ANT3 was prepared. When cell lysates were incubated with anti-HA agarose beads, endogenous MMS19 was detected by anti-MMS19 antibody, and the tagged proteins were detected by HA

antibody, demonstrating the physical interaction between ANT family and MMS19 (Fig. 3D). Likewise, SSBP1 and HSP70 were also detected in protein complex of MMS19 (Fig. 3E).

3.4. ANT2 plays a role in mtDNA repair

Subsequently, we explored whether ANT2 was involved in oxidative mtDNA damage repair. U2OS cells were transfected with siControl and siANT2 . As mentioned above, long-range mtDNA PCR was used to quantify the capacity for mtDNA oxidative damage repair . After exposure to 1 mM H₂O₂ for 1 h, both control and ANT2 knockdown U2OS cells had a markedly decreased level of mtDNA PCR product at early time-points after H₂O₂ treatment. However, control cells showed the restoration of full-length mtDNA PCR product at 24 h, whereas cells with knockdown of ANT2 gene expression showed a very low-level of PCR product, even at 48 h post-treatment (Fig. 4). Similar as MMS19, ANT2 deficiency also results in remarkably attenuating mtDNA oxidative

damage repair compared to control cells, but it is dispensable for some nuclear gene such as β -globin.

4. Discussion

Mitochondria are essential cellular organelles in every single living eukaryote, which provide energy in the form of ATP and important metabolic intermediates to a number of cellular functions including lipid biogenesis. In addition, mitochondria are important for other key processes including calcium homeostasis, iron-sulfur cluster biogenesis, and heme production (Van Houten et al. 2016). Mitochondrial DNA is constantly exposed to oxidative stress. Most intracellular reactive oxygen species (ROS) are produced in mitochondria; mtDNA is more susceptible than nuclear DNA to oxidative damage. ROS can generate a large number of DNA lesions, including oxidized DNA bases, abasic sites, and single- and double-strand breaks. Many of these ROS-induced DNA lesions show mutagenic or cytotoxic effects due to mispair of bases, which may give rise to mutations upon DNA replication (Gredilla et al.

2010). The steady-state levels of oxidatively induced mtDNA lesions can be several fold higher than nuclear DNA (Hamilton et al. 2001). MMS19 has been reported as an upstream regulator of TFIIH activity, which is indispensable for both NER and RNA polymerase II transcription (Kou et al. 2008).

Early studies demonstrate that MMS19 plays specific physiological role based on its sub-cellular distributions. In nuclear, human MMS19 interacts with two components of TFIIH: XPB and the Rad3 homolog XPD, so exerting its function in DNA repair and transcription. In cytoplasm, MMS19 interacts with XPD forming MMXD complex independently of TFIIH and is proposed to play a role in mitotic spindle formation and chromosome segregation, an abnormality of which could contribute to the pathogenesis in some cases of xeroderma pigmentosum (XP) and XP combined with Cockayne syndrome. However, whether or not MMS19 protein locations and functions in mitochondria remains unclear. Western blotting analysis of the lysis of cellular fractions showed the presence of the MMS19 protein in the mitochondrial fraction, which

demonstrated that MMS19 protein was co-localized with mitochondria. Moreover, alkali extraction experiments demonstrated that MMS19 was present in both the membrane-containing pellet and soluble fraction of mitochondria.

The stability of mtDNA is constantly challenged by the endogenous production of mitochondrial ROS (mtROS), which are generated during normal electron flux through the mitochondrial electron transport. Unlike nuclear DNA, mtDNA is located in the proximity of mtROS generation-sites which are located within complex I and III of the electron transport chain (Muller et al. 2004). In the study, we observed that MMS19 knockdown led to a significantly increased CD level, and also a substantially lower capacity for repairing the mtDNA oxidative damage. Although a molecular explanation of how MMS19 affects so many different processes has remained elusive, this study indicates that MMS19 deficiency is likely to result in defective repair of oxidative damage in the mitochondrial DNA, leading to a higher mutation rate of mtDNA. MMS19 deficiency selectively affects the oxidative DNA damage

repair capacity of mtDNA but not nuclear genes such as β -globin, suggesting that MMS19 is a critical factor for repair machinery of oxidative DNA damage operating in mitochondria.

We next searched for other patterns that may corroborate with MMS19 protein in the repair of mitochondrial oxidative DNA damages by Co-immunoprecipitation together with liquid chromatography-mass spectrometry analysis on mitochondrial fraction. Results showed that MMS19 interacted with ANT family, SSBP1 and HSP70, respectively, which was further validated by Co-IP analysis. ANTs play an essential role for energy metabolism of eukaryotic cell by transporting ADP and ATP through mitochondrial inner membrane (Cho et al. 2015). We observed that MMS19 physically interacted with ANT1, ANT2 and ANT3 in mitochondria. Similar to its interacting partner, a significantly decreased capacity for repairing mitochondrial oxidative DNA damages was also observed when ANT2 expression in mitochondria was suppressed. SSBP1 (also known as mtSSB) is distinct from nuclear SSB, exists in eukaryotic mitochondria, and plays an important role in mtDNA replication,

recombination, and repair by binding to single-stranded mtDNA (Farr et al. 1999; Jiang et al. 2016). The interaction between MMS19 and SSBP1 suggested MMS19 indirectly involved in oxidative DNA damage repair capacity of mtDNA through SSBP1. Mass spectrometry analysis also showed that MMS19 physically interacted with HSP70, suggesting a potential mechanism in which MMS19 enters mitochondria by interacting with molecular chaperone HSP70. Taken together, our findings uncover strong evidence that MMS19 is involved in mtDNA repair for oxidative damage and plays an essential role in maintaining mitochondrial genome DNA stability.

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FIGURE LEGENDS

Fig. 1 : The localization of MMS19 protein in mitochondria by western blotting coupled with alkali extraction method on isolated mitochondrial fraction. (A) Endogenous MMS19 from both

mitochondrial and nuclear fractions of HEK293 cells was determined by western blotting. Presence of MMS19 protein in mitochondrial fraction was detected. VDAC was used as a mitochondrial marker. PCNA was used as a nuclear marker, and Tubulin as a marker for contamination by cytosol. (B) Mitochondria from HEK293 cells were subjected to alkali extraction. VDAC and SMAC were used to identify behaviors of well defined mitochondrial proteins that were integral membrane protein and soluble, respectively. Both the integral membrane protein and soluble protein fraction were isolated and checked by western blotting.

‘M’ indicates extracted mitochondria sample without other treatment.

The data demonstrated the MMS19 localization in both the inner mitochondrial membrane and mitochondrial inner matrix compartment.

Fig. 2 : MMS19 protein is critical in MMS19-regulated mtDNA repair. (A) The relative quantities of CD was measured by real-time quantitative PCR in MMS19 knockdown and control U2OS cells at 48 h after 1 mM H₂O₂ treatment in the presence or absence of 5,000 U/ml of

Catalase. The relative levels of CD were assessed using Bio-Rad CFX Manager v3.0. Samples were collected at 0 h (Con) and 48 h recovery with or without catalase (H_2O_2 or H_2O_2 +catalase) post treatment. (B) Long-range QPCR results of full-length mitochondrial DNA (mt-FL) in siControl and siMMS19 U2OS cells at 0, 6, 12, 24, 48 h after 1 mM H_2O_2 treatment for 1 h. The 110 bp mtND1 PCR product was used as a control to normalize the input of the mtDNA template amount in each sample. Nuclear β -globin gene was used as the repair efficiency marker for nuclear DNA damage.

Fig. 3 : Characterization of MMS19 protein complex in mitochondria. (A) Establishment of stable HeLa S3 cell lines overexpressing HA-MMS19. (B) Experimental procedure for purification of MMS19 protein complex in mitochondria. (C) Silver staining of MMS19 protein complexes purified from mitochondrial extracts. As a control, a mock purification was performed with mitochondrial extracts from non-transfected HeLa S3 cells. Purified polypeptides were

processed for Liquid chromatography-tandem mass spectrometry. (D)

Endogenous MMS19 was observed in the ANT pull-down complex. (E)

Exogenous MMS19 was observed in the SSBP1 pull-down complex.

Exogenous HSP70 was observed in the MMS19 pull-down complex.

Fig. 4 : Result of long-range QPCR in siControl and siANT2 U2OS

cells. Long-range QPCR results of full-length mitochondrial DNA (mt-FL)

at 0, 6, 12, 24, 48 h after treatment with 1 mM H₂O₂ for 1 h. Equal amount

of mtDNA template DNA in each sample was normalized by mtND1.

Supplementary Table 1. The data of proteins interacted with

MMS19 in mitochondria by using Co-immunoprecipitation-LC-MS/MS.

Fig. 1 : The localization of MMS19 protein in mitochondria by western blotting coupled with alkali extraction method on isolated mitochondrial fraction.

90x57mm (300 x 300 DPI)

Figure2

Fig. 2 : MMS19 protein is critical in MMS19-regulated mtDNA repair.

90x109mm (300 x 300 DPI)

Fig. 3 : Characterization of MMS19 protein complex in mitochondria.

160x74mm (300 x 300 DPI)

Figure4

Fig. 4 : Result of long-range QPCR in siControl and siANT2 U2OS cells.

90x109mm (300 x 300 DPI)

Accession	Description	Σ Coverage	Σ # Proteins	Σ # Unique Peptides	Σ # Peptides	Σ # PSMs
P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7 - [ADT2_HUMAN]	28.19	3	4	8	9
	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications
	DFLAGGVAAAISK	1	1	1	P05141	
	QIFLGGVDKR	1	1	1	P05141	
	GIIDCVVR	1	2	1	P05141	C5(Carbamido methyl)
	YFPTQALNFAFKDK	1	5	2	P12236;P05141	
	YFPTQALNFAFK	1	5	2	P12236;P05141	
	AAFYGIYDTAK	1	1	1	P05141	
	VKLLQVQHASK	1	4	2	P12236;P05141	
	GMGGAFVLVLYDEIKK	2	3	2	P12236;P05141	M2(Oxidation)
Q04837	Single-stranded DNA-binding protein, mitochondrial OS=Homo sapiens GN=SSBP1 PE=1 SV=1 - [SSBP_HUMAN]	39.86	3	5	5	7
	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications
	QATTIADNIIFLSDQTK	3	1	1	Q04837	
	SGDSEVYQLGDVSQK	1	3	1	Q04837	
	VGQDPVLR	1	3	1	Q04837	
	QATTIADNIIFLSDQTKEKE	1	1	1	Q04837	
	NPVTIFSLATNEMWR	1	3	1	Q04837	M13(Oxidation)
P12236	ADP/ATP translocase 3 OS=Homo sapiens GN=SLC25A6 PE=1 SV=4 - [ADT3_HUMAN]	18.46	3	1	5	6
	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications
	DFLAGGIAAAISK	1	2	1	P12236	
	YFPTQALNFAFKDK	1	5	2	P12236;P05141	
	YFPTQALNFAFK	1	5	2	P12236;P05141	
	VKLLQVQHASK	1	4	2	P12236;P05141	
	GMGGAFVLVLYDELKK	2	3	2	P12236;P05141	M2(Oxidation)
H0Y5S7	Heat shock protein HSP 70-beta (Fragment) OS=Homo sapiens GN=HSP70AB1 PE=4 SV=1 - [H0Y5S7_HUMAN]	9.86	11	1	1	3
	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group Accessions	Modifications
	GVVDSDELPLNISR	3	11	1	H0Y5S7	