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(54) Title: USE OF UBIQUITYLATION OF HISTONE 2A PROTEIN

(57) Abstract: Provided is the use of ubiquitylation of Histone 2A protein as a prognostic indicator of an aging related condition in a mammal.

USE OF UBIQUITYLATION OF HISTONE 2A PROTEIN

CROSS REFERENCE TO RELATED APPLICATIONS

None

FIELD OF THE DISCLOSURE

The present invention relates generally to the field of human health, and in particular to methods for the prophylaxis and treatment of aging related condition that are positively correlated with the ubiquitylation of Histone 2A protein.

BACKGROUND OF THE INVENTION

Aging and many age-associated diseases are linked to functional decline and damage to the proteome. Growing evidence associates aging with epigenetic, transcriptional, and proteomic alterations; yet how these processes contribute to adult physiology is only beginning to be revealed.

Accordingly, an interest exists for new biomarkers which may be used in the diagnosing, or providing a prognostic indicator of an aging related condition.

SUMMARY OF THE DISCLOSURE

The present disclosure provides a new biomarker for diagnosing, or providing a prognostic indicator of an aging related condition. Further, this disclosure is believed to be the first description of any the correlation between the ubiquitylation of Histone 2A protein and an aging related condition.

In the first aspect of present disclosure, the present disclosure provides a kit for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising an agent to measure the ubiquitylation of Histone 2A protein or functional equivalent thereof. According to the examples of present disclosure, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the kit described above may be used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In the second aspect of present disclosure, the present disclosure provides the usage of an agent to measure the ubiquitylation of Histone 2A protein (ubH2A) in the preparation of a kit

for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal. According to the examples of present disclosure, the present disclosure firstly demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the agent described above can be used in a kit to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In a still aspect of present disclosure, the present disclosure provides an agent to measure the ubiquitylation of Histone 2A protein for use in diagnosing, or providing a prognostic indicator of an aging related condition in a mammal. According to the examples of present disclosure, the present disclosure firstly demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the agent described above can be used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In a further another aspect of present disclosure, the present disclosure provides a method of diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising detecting the ubiquitylation of Histone 2A protein in a sample from said mammal, wherein an increase in the level of the ubiquitylation of Histone 2A protein in a cell of said sample, relative to the level of the ubiquitylation of Histone 2A protein in a normal cell indicates said sample is suffering from aging related condition. According to the examples of present disclosure, the method described above can be used to diagnose or provide a prognostic indicator of an aging related condition in a mammal effectively, and the inventors found that the higher level of the ubH2A, the more serious of the aging related condition.

In a further aspect of present disclosure, the present disclosure provides a method for screening an agent for the treatment of an aging related condition in a mammal comprising contacting a cell from a subject with the aging related condition with a candidate agent and determining whether the candidate agent reduces the level of the ubiquitylation of Histone 2A protein, wherein a reduction in the level of the ubiquitylation of Histone 2A protein indicates that the candidate agent is suitable for the treatment of the aging related condition. The present disclosure demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that the method described above may be used to screen an agent for the treatment of an aging related condition in a mammal effectively. According to the examples of present disclosure, that the said candidate agent which may decrease the ubiquitylation of Histone 2A protein to

a mammal may prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as aging related condition, in a mammal.

In a further aspect of present disclosure, the present disclosure provides a pharmaceutical composition for treating an aging related condition in a mammal comprising an agent to decrease the ubiquitylation of Histone 2A protein. The present disclosure firstly demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to treat an aging related condition. According to the examples of present disclosure, the said pharmaceutical composition may prevent, manage, treat or lessen disorders or diseases caused by ubH2A in a mammal.

In a further aspect of present disclosure, the present disclosure provides a usage of an agent to decrease the ubiquitylation of Histone 2A protein in the preparation of a pharmaceutical for treating an aging related condition in a mammal. The present disclosure firstly demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to prepare a pharmaceutical for treating an aging related condition in a mammal effectively. According to the examples of present disclosure, the said agent may be used in the preparation of a pharmaceutical which can preventing, managing, treating or lessening disorders or diseases caused by ubH2A in a mammal.

In a further aspect of present disclosure, the present disclosure provides the usage of an agent to decrease the ubiquitylation of Histone 2A protein for use in treating an aging related condition in a mammal. As said above, the present disclosure firstly demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to treat an aging related condition in a mammal effectively. According to the examples of present disclosure, the said agent may be used to prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as an aging related condition in a mammal.

In a further aspect of present disclosure, the present disclosure provides a method of treating an aging related condition in a mammal comprising administrating an agent to decrease ubiquitylation of Histone 2A protein to a subject in need of such treatment. The present

disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that administrating an agent which may decrease the ubiquitylation of Histone 2A protein to a mammal may prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as aging related condition.

In a further aspect of present disclosure, the present disclosure provides a cell model of an aging related condition with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof. The present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that a cell model with an increased ubiquitylation of Histone 2A protein compared with the wild type cell may represent that the said cell model having an aging related condition, thus the said cell model can be used as a research model with an aging related condition.

In a further aspect of present disclosure, the present disclosure provides an animal model of an aging related condition comprising a cell with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof. The present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an animal model with a cell which has an increased ubiquitylation of Histone 2A protein compared with the wild type cell may represent that the said animal model having an aging related condition, thus the said animal model can be used as a research model with an aging related condition.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows a quantification of $^{15}N\%$ in three tissues after pulse-chase labeling in an example of present disclosure,

Fig. 2 shows a quantification of $^{15}N\%$ in ubiquitylonme in another example of present disclosure,

Fig. 3 shows a quantification of ubH2A change in aging process in another example of present disclosure,

Fig. 4 show functions of ubH2A involved in the aging process in another example of present disclosure,

Fig. 5 shows a higher oxidation resistance ability is helpful maintaining longer life span for mutants in another example of present disclosure.

DESCRIPTION OF THE DISCLOSURE

The present disclosure is based at least in part on the present discoveries that the correlation between the ubiquitylation of Histone 2A protein and an aging process.

Histones are proteins that package DNA into nucleosomes. Histones are responsible for maintaining the shape and structure of a nucleosome. One chromatin molecule is composed of at least one of each core histones per 100 base pairs of DNA. There are five families of histones known to date; these histones are termed H1/H5, H2A, H2B, H3, and H4. H2A is considered a core histone, along with H2B, H3 and H4. Core formation first occurs through the interaction of two H2A molecules. Then, H2A forms a dimer with H2B; the core molecule is complete when H3-H4 also attaches to form a tetramer.

Histone H2A is composed of non-allelic variants. The term “Histone H2A” is intentionally non-specific and refers to a variety of closely related proteins that vary often by only a few amino acids. Notable variants include H2A.1, H2A.2, H2A.X, and H2A.Z. H2A variants can be explored using "HistoneDB with Variants" database, which can be obtained from the website of https://www.ncbi.nlm.nih.gov/research/HistoneDB2.0/index.fcgi/type/H2A/#msa_div_browse, or the website of http://www.uniprot.org/uniprot.

Growing evidence associates aging with epigenetic, transcriptional, and proteomic alterations; yet how these processes contribute to adult physiology is only beginning to be revealed. For long-lived histone proteins, chemical modifications through methylation and acetylation constitute age-modulated epigenetic changes that profoundly impacts adult lifespan. As noted, the level of select histone markings, e.g. tri-methylated histone H3 at lysine 27 (H3K27me3), may exhibit increased or decreased change with age in different model organisms or tissue types. Thus, identification of conserved aging hallmark in histone modification has potential implications for a common epigenetic mechanism that is operative in the aging process. Here using Drosophila, the inventors perform proteomic experiments with stable isotope switching method to systematically characterize long-lived proteome combined with antibody-enriched ubiquitylome analysis. The present data describes the first atlas of proteins with limited turnover in Drosophila head, muscle, and testis tissues, and further reveals an age-modulated ubiquitylation on long-lived Histone 2A protein (ubH2A), which represents a new hallmark

of aging that is evolutionarily conserved in Drosophila, mouse and human. Moreover, a reduction of ubH2A in mutant flies is associated with extended lifespan and enhanced ability to handle oxidative stress. Together, the present data reveals a new conserved hallmark of aging that links epigenetic modulation to adult lifespan.

A prominent feature of aging is a gradual decline in protein homeostasis network, particularly those of long-lived proteins that cannot be efficiently replenished by the protein quality control system. As a consequence, long-lived proteins may undergo aberrant chemical modification and damage leading to altered or loss of function over adult lifespan, and their identification is thus important for the present understanding of the aging process. Here, the inventors provide a comprehensive analysis of proteins with limited turnover in Drosophila somatic and reproductive tissues. Moreover, the inventors profile the ubiquitin-modified long-lived proteome in fly heads, and identify a new role of ubH2A as an epigenetic modulator of lifespan and potentially a conserved chromatin signature of aging.

The recent reports of long-lived proteins in mouse, and rat indicate that proteins with extraordinary lifespan are more prevalent than previously appreciated. The present quantitative assessment shows that fly heads and muscles, which are primarily composed of post-mitotic cells in the adult stage, contain a significant amount of proteins with slow or limited turnover during aging. In contrast, the extent of long-lived proteins becomes relatively decreased in the fly testis of germline tissue where mitotically active cells are populating, suggesting that cell division coupled with new protein synthesis directly contribute to replenish the proteome.

The lifespan of fly is far more less than mammalian, however the lifespan of proteins is quiet similar, so large scale identification of long-lived proteins may result from the time point that the inventors choose to detect remaining ^{15}N signals left. Importantly, the long-lived proteome harbors functionally diverse proteins that regulate a myriad of biological events including nucleosome assembly, in which all histones were found to be long-lived and as well as mitochondria related biological process which is consistent with previously report that large set of mitochondria proteins are long-lived.

Although the long-lived proteome is now well-recognized in multiple model organisms, how these proteins are maintained and modified during aging remain poorly understood. For long-lived Nup93 and Nup153, it was shown that carbonyl groups can be added to the proteins and also PARylation markedly increased in aged mice's liver and muscle.

Ubiquitin modification is a critical PTM signal for target proteins. The present study extends the scope by profiling the interface between ubiquitylation and aging proteome. Remarkably, the present data reveals a significant preference for old proteins to remain ubiquitylated compared to that of newly synthesized proteins. This evidence may provide a direct support for a decline in UPS and protein turnover during aging as which would manifest a late-onset accumulation of ubiquitin-modified proteins. In addition, long-lived mitochondrial proteins also tend to be ubiquitylated. Since ubiquitin ligase Parkin can target substrates for both proteosomal and autophagic degradation, this finding may implicate a potential link between Parkin and mitochondrial protein quality control during aging.

Interestingly, whereas ubiquitin-modified protein turnover affects the stability of individual proteins, age-modulated ubiquitylation on histone may lead to global and site-specific chromatin remodeling, which in turn triggers a much broader effect in gene expression linked to altered cellular fitness. However, the exact role of ubH2A in limiting lifespan in Drosophila is still unclear. It has been shown that, in flies and humans, ubH2A resides in the promoter regions of Polycomb-regulated genes, including the Hox genes. Thus, it is possible that ubH2A is involved in transcriptional repression by introducing additional histone marking. Moreover, an emerging crosstalk between ubH2A and methylation on histone H3 at lysine 4 is also reported. Hence, more work will be required to determine the key epigenetic effects of ubH2A underlying fly lifespan modulation. Nevertheless, since the change of ubH2A defines a new, conserved aging hallmark in fly, mouse, and human, future investigation, including comparative analysis of ubH2A epigenome in the aging process may unveil common mechanisms that modulate age-associated physiological decline and disease.

A kit for diagnosing, or providing a prognostic indicator

In the first aspect of present disclosure, the present disclosure provides a kit for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising an agent to measure the ubiquitylation of Histone 2A protein or functional equivalent thereof. According to the examples of present disclosure, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the kit described above may be used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In some embodiments, the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHHKTK (SEQ ID NO:1).

MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHHKAK (SEQ ID NO:2).

MSGRGKQGGK ARAKAKSRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YMAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHKAKSK (SEQ ID NO:3).

MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHHKAK (SEQ ID NO:4).

MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY SERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGR VTIAQGGVLP NIQAVLLPKK TESHHKAK (SEQ ID NO:5).

MSGRGKQGGK ARAKAKSRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YMAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHKAKSK(SEQ ID NO:6).

MSGRGKQGGK ARAKAKTRSS RAGLQFPVGR VHRLLRKGNY SERVGAGAPV YLAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGR VTIAQGGVLP NIQAVLLPKK TESHHKAK(SEQ ID NO:7).

MSGRGKQGGK ARAKAKSRSS RAGLQFPVGR VHRLLRKGNY AERVGAGAPV YMAAVLEYLT AEILELAGNA ARDNKKTRII PRHLQLAIRN DEELNKLLGK VTIAQGGVLP NIQAVLLPKK TESHKAKSK(SEQ ID NO:8).

MSGPTKRGGK ARAKVKSRSS RAGLQFPVGR VHRLLRQGNY AQRIGAGAPV YLAAVLEYLT AEVLELAGNA ARDNKKTRIT PRHLQLAIRN DEELNKLLGR VTIAQGGVLP NIQAVLLPKK TESHKSQTK(SEQ ID NO:9).

SGRGKQGGKA RAKAKTRSSR AGLQFPVGRV HRLLRKGNYS ERVGAGAPVY LAAVLEYLTA EILELAGNAA RDNKKTRIIP RHLQLAIRND EELNKLLGRV TIAQGGVLPN IQAVLLPKKT ESHHKAK(SEQ ID NO:10).

LNKLLGRVTI AQGGVLPNIQ AVLLPKKTES HHKPKGK (SEQ ID NO:11).

The SEQ ID NO:1 shows the Q99878： Histone H2A type 1-J (Homo sapiens) peptide sequence. The SEQ ID NO:2 shows the Q96KK5： Histone H2A type 1-H (Homo sapiens) peptide sequence. The SEQ ID NO:3 shows the Q16777： Histone H2A type 2-C (Homo sapiens) peptide sequence. The SEQ ID NO:4 shows the A3KPC7： Histone H2A (Homo sapiens)peptide sequence. The SEQ ID NO:5 shows the Q8CGP6： Histone H2A type 1-H (Mus musculus) peptide sequence. The SEQ ID NO:6 shows the Q64523： Histone H2A type 2-C (Mus musculus) peptide sequence. The SEQ ID NO:7 shows the A3KPD0： Histone H2A(Mus musculus) peptide sequence. The SEQ ID NO:8 shows the Q149V4： Histone H2A(Mus musculus) peptide sequence. The SEQ ID NO:9 shows the Q8CGP4： Histone H2A (Mus musculus) peptide sequence. The SEQ ID NO:10 shows the A0AUV1： Histone H2A (Mus musculus) peptide sequence. The SEQ ID NO:11 shows the Q61668： Gene for histone H2a (Mus musculus) peptide sequence.

The inventors found that the ubiquitylation of Histone 2A protein on at least one of the said sites can used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal more effectively.

In some embodiments, the agent is an antibody specific to ubH2A. According to the examples of present disclosure, using an antibody specific to ubH2A to measure the ubH2A is more conveniently and more effectively.

In some embodiments, the antibody is monoclonal antibody. The inventors found that the said monoclonal antibody can recognize ubH2A more specifically.

In some embodiments, the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof. The inventors found that the said antibody can recognize ubH2A more specifically and more effectively.

In some embodiments, the aging related condition is at least one of the dementias, neurodegenerative diseases, neurological disorders, and age-related conditions. The inventors found that the kit can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the aging related condition is at least one of atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension , Alzheimer's Disease, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline. The inventors found that the kit can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal more effectively.

In some embodiments, the agent to measure the ubiquitylation is performed by means of Western-Blotting. The inventors found that Western blot assays can be used for measuring different histone, and the relative abundance of ubH2A can be normalized by Western blot assays.

The usage of an agent to measure the ubiquitylation of Histone 2A protein

In the second aspect of present disclosure, the present disclosure provides the usage of an agent to measure the ubiquitylation of Histone 2A protein in the preparation of a kit for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal. According to the examples of present disclosure, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the agent described above can be used in a kit to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In some embodiments, the ubiquitylation of Histone 2A protein is on the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

The inventors found that the ubiquitylation of Histone 2A protein on at least one of the said sites can used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal more effectively.

In some embodiments, the agent is an antibody specific to ubH2A. According to the examples of present disclosure, using an antibody specific to ubH2A to measure the ubH2A is more conveniently and more effectively.

In some embodiments, the antibody is monoclonal antibody. The inventors found that the said monoclonal antibody can recognize ubH2A more specifically.

In some embodiments, the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof. The inventors found that the said antibody can recognize ubH2A more specifically and more effectively.

In some embodiments, the aging related condition is at least one of the dementias, neurodegenerative diseases, neurological disorders, and age-related conditions. The inventors found that the kit can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the aging related condition is at least one of atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension , Alzheimer's Disease, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline. The inventors found that the kit can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the agent to measure the ubiquitylation is performed by means of Western-Blotting. The inventors found that Western blot assays can be used for measuring different histone, and the relative abundance of ubH2A can be normalized by Western blot assays.

An agent to measure the ubiquitylation of Histone 2A protein

In a still another aspect of present disclosure, the present disclosure provides an agent to measure the ubiquitylation of Histone 2A protein for use in diagnosing, or providing a prognostic indicator of an aging related condition in a mammal. According to the examples of present disclosure, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the agent described above can be used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal effectively.

In some embodiments, the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

The inventors found that the ubiquitylation of Histone 2A protein on at least one of the said sites can used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal more effectively.

In some embodiments, the agent is an antibody specific to ubH2A. According to the examples of present disclosure, using an antibody specific to ubH2A to measure the ubH2A is more conveniently and more effectively.

In some embodiments, the antibody is monoclonal antibody. The inventors found that the said monoclonal antibody can recognize ubH2A more specifically.

In some embodiments, the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof. The inventors found that the said antibody can recognize ubH2A more specifically and more effectively.

In some embodiments, the aging related condition is at least one of dementias, neurodegenerative diseases, neurological disorders, and age-related conditions. The inventors found that the agent can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the aging related condition is at least one of Alzheimer's Disease, atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline. The inventors found that the agent can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the agent to measure the ubiquitylation is performed by means of Western-Blotting. The inventors found that Western blot assays can be used for measuring different histone, and the relative abundance of ubH2A can be normalized by Western blot assays.

A method of diagnosing, or providing a prognostic indicator

In a further another aspect of present disclosure, the present disclosure provides a method of diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising detecting the ubiquitylation of Histone 2A protein in a sample from said mammal, wherein an increase in the level of the ubiquitylation of Histone 2A protein in a cell of said sample, relative to the level of the ubiquitylation of Histone 2A protein in a normal cell

indicates said sample is suffering from aging related condition. According to the examples of present disclosure, the method described above can be used to diagnose or provide a prognostic indicator of an aging related condition in a mammal effectively, and the inventors found that the higher level of the ubH2A, the more serious of the aging related condition.

In some embodiments, the said method further comprises treating the aging related condition by reducing level of the ubiquitylation of Histone 2A protein in a cell of said sample. The inventors found that the method described above may be provide a method of screening an agent which can prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as aging related condition.

In some embodiments, the level of the ubiquitylation of Histone 2A protein is reduced by site-specific mutagenesis of the Histone 2A protein. According to the examples of present disclosure, using the site-specific mutagenesis to specificity change the site of the ubiquitylation of Histone 2A protein without having a substantial effect on other site of the Histone 2A protein, can reduce the level of the ubiquitylation of Histone 2A protein. Therefore, Histone related diseases such as dementias, neurodegenerative diseases, neurological disorders, and age-related conditions, can be treated using the said site-specific mutagenesis method above.

In some embodiments, the site-specific mutagenesis is performed by means of Crispr-Cas9 method. According to the examples of present disclosure, using the Crispr-Cas9 method to specificity change the site of the ubiquitylation of Histone 2A protein without having a substantial effect on other site of the Histone 2A can reduce the level of the ubiquitylation of Histone 2A protein effectively. Therefore, Histone related diseases such as dementias, neurodegenerative diseases, neurological disorders, and age-related conditions, can be treated using the said site-specific mutagenesis method above.

In some embodiments, the level of the ubiquitylation of Histone 2A protein is reduced by inhibiting PRC1.The inventors found that inhibiting PRC1 can also reducing the level of the ubH2A,thus compounds which be used to inhibit PRC1 can be performed in preventing, managing, treating or lessening disorders or diseases caused by ubH2A, such as aging related condition.

In some embodiments, the ubiquitylation of Histone 2A protein is at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

The inventors found that the ubiquitylation of Histone 2A protein on at least one of the said sites can used to diagnose, or provide a prognostic indicator of an aging related condition in a mammal more effectively. Thus, according to the examples of present disclosure, the said method can diagnose or procide a prognostic indicator of an aging related condition more effectively.

In some embodiments, the detecting is performed with mass spectrometry. The inventors found that mass spectrometry (MS) can detect the said ubH2A more conveniently and effectively.

In some embodiments, the detecting is performed with an agent, wherein the said agent is an antibody specific to ubH2A. According to the examples of present disclosure, using an antibody specific to ubH2A to measure the ubH2A making the method more conveniently and more effectively.

In some embodiments, the antibody is monoclonal antibody. The inventors found that the said monoclonal antibody can recognize ubH2A more specifically. According to the examples of present disclosure, using the said monoclonal antibody to measure the ubH2A making the method more conveniently and more effectively.

In some embodiments, the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof. The inventors found that the said antibody can recognize ubH2A more specifically and more effectively .According to the examples of present disclosure, using the said monoclonal antibody to measure the ubH2A making the method more conveniently and more effectively.

In some embodiments, the agent to measure the ubiquitylation is performed by means of Western-Blotting. The inventors found that Western blot assays can be used for measuring different histone, and the relative abundance of ubH2A can be normalized by Western blot assays.

In some embodiments, the aging related condition is at least one of dementias, neurodegenerative diseases, neurological disorders, and age-related conditions. The inventors found that the said method can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal effectively.

In some embodiments, the aging related condition is at least one of Alzheimer's Disease, atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline. The inventors found that the said method can diagnose or provide a prognostic indicator of the aging related conditions described above in a mammal more effectively.

A method for screening an agent

In a further aspect of present disclosure, the present disclosure provides a method for screening an agent for the treatment of an aging related condition in a mammal comprising contacting a cell from a subject with the aging related condition with a candidate agent and determining whether the candidate agent reduces the level of the ubiquitylation of Histone 2A protein, wherein a reduction in the level of the ubiquitylation of Histone 2A protein indicates that the candidate agent is suitable for the treatment of the aging related condition. The present disclosure demonstrates the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that the method described above may be used to screen an agent for the treatment of an aging related condition in a mammal effectively. According to the examples of present disclosure, that the said candidate agent which may decrease the ubiquitylation of Histone 2A protein to a mammal may prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as aging related condition, in a mammal.

A pharmaceutical composition

In a further aspect of present disclosure, the present disclosure provides a pharmaceutical composition for treating an aging related condition in a mammal comprising an agent to decrease the ubiquitylation of Histone 2A protein. The present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to treat an aging related condition. According to the examples of present disclosure, the said pharmaceutical composition may prevent, manage, treat or lessen disorders or diseases caused by ubH2A in a mammal.

Usage of an agent

In a further aspect of present disclosure, the present disclosure provides the usage of an agent to decrease the ubiquitylation of Histone 2A protein in the preparation of a pharmaceutical for treating an aging related condition in a mammal. As said above, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to treat an aging related condition in a mammal effectively. According to the examples of present disclosure, the said agent may be used in the preparation of a pharmaceutical which can preventing, managing, treating or lessening disorders or diseases caused by ubH2A in a mammal.

Usage of an agent

In a further aspect of present disclosure, the present disclosure provides the usage of an agent to decrease the ubiquitylation of Histone 2A protein for use in treating an aging related condition in a mammal. As said above, the present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an agent which may decrease the ubiquitylation of Histone 2A protein may be used to treat an aging related condition in a mammal effectively. According to the examples of present disclosure, the said agent may be used to prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as an aging related condition in a mammal.

A method of treating an aging related condition in a mammal

In a further aspect of present disclosure, the present disclosure provides a method of treating an aging related condition in a mammal comprising administrating an agent to decrease ubiquitylation of Histone 2A protein to a subject in need of such treatment. The present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that administrating an agent which may decrease the ubiquitylation of Histone 2A protein to a mammal may prevent, manage, treat or lessen disorders or diseases caused by ubH2A, such as aging related condition.

A cell model

In a further aspect of present disclosure, the present disclosure provides a cell model of an aging related condition with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof. The present disclosure firstly demonstrate the correlation between

the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that a cell model with an increased ubiquitylation of Histone 2A protein compared with the wild type cell may represent that the said cell model having an aging related condition, thus the said cell model can be used as a research model with an aging related condition.

An animal model

In a further aspect of present disclosure, the present disclosure provides an animal model of an aging related condition comprising a cell with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof. The present disclosure firstly demonstrate the correlation between the ubiquitylation of Histone 2A protein and an aging related condition, then the person skilled in the art may understand that an animal model with a cell which has an increased ubiquitylation of Histone 2A protein compared with the wild type cell may represent that the said animal model having an aging related condition, thus the said animal model can be used as a research model with an aging related condition.

The following Examples are intended to illustrate but not limit the invention.

EXAMPLE

Methods

Fly culture

Flies were cultured in standard media at 25 ℃ with 60% humidity in a 12 hours light and 12 hours dark cycle. The WT (control) line used was 5905 (FlyBase ID FBst0005905, w1118). All fly lines used in this study have been backcrossed with 5905 for five consecutive generations for a uniform genetic background, to assure that phenotypes were not associated with any variation in background.

Switching stable isotope labeling of Flies for long-lived proteins

3d old male and female flies were crossed on media consisting of 10% (w/v) ^{15}N labeled *Saccharomyces cerevisiae* (Silantes GmbH, Germany), 150 mM sucrose, 6 mM methylparaben, and 0.5‰ propionic acid. Flies were maintained in a 12 hour light/12 hours dark cycle at 25 ℃ and 60% relative humidity. The parent flies were removed after 5 days. 11 days later, the newborn flies were collected and transferred to a ^{15}N diet for another 5-day culture. Then the flies were switched to a normal ^{14}N food until 30d and 60d old of age.

Pulse-SILAC labeling of Flies

5d old flies were started to culture with media consisting of 10% (w/v) 13C-lysine labeled Saccharomyces cerevisiae (Silantes GmbH, Germany), 150 mM sucrose, 6 mM methylparaben, and 0.5‰ propionic acid. Flies were maintained in a 12h light/12h dark cycle at 25 °C and 60% relative humidity. Head tissues were harvested after 5 days of 13C-lysine labeling.

MS analysis:

Sample preparation

Head, muscle and testis tissues were homogenized in ice-cold urea lysis buffer containing 8 M urea, 100 mM Tris-HCl, pH 8.5, 150 mm NaCl, and protease inhibitor (Pierce, USA). The homogenate was centrifuged using 14,000 g for 10 minutes, and the protein concentration of the supernatant was determined using the BCA protein assay (Pierce, USA). Proteins were digested with trypsin by a standard protocol of filter-aided sample preparation.

Basic Reversed Phase (RP) Chromatography

Off-line basic RP HPLC fractionation was performed on a XBridge BEH C18 column using an Agilent 1260 HPLC system. 500 μg or 8 mg of peptide was fractionated by using a 72 min basic RP HPLC gradient for aging proteome or ubiquitylome analysis respectively. Upon sample injection, 72 fractions were collected into 1.5ml Eppendorf tubes (Eppendorf, UK). The resulting small fractions were then pooled in a non-contiguous manner into 7 - 8 large fractions. The original fractions 1, 9, 17, 25, 33, 41, 49, 57 and 65 were combined to generate the first pooled fraction. The remaining seven fractions were created followed by a similar pooling strategy. Fractions were dried to completeness in a vacuum concentrator. Peptides from each fraction were subjected to LC-MS analysis or ubiquitylation enrichment using antibody recognizing K-GG remnant.

Basic Reversed Phase (RP) Chromatography

Off-line basic RP HPLC fractionation was performed on a XBridge BEH C_{18} column using an Agilent 1260 HPLC system. 500 μg or 8 mg of peptide was fractionated by using a 72 min basic RP HPLC gradient for aging proteome or ubiquitylome analysis respectively. Upon sample injection, 72 fractions were collected into 1.5ml Eppendorf tubes (Eppendorf, UK). The resulting small fractions were then pooled in a non-contiguous manner into 7-8 large fractions. The original fractions 1, 9, 17, 25, 33, 41, 49, 57 and 65 were combined to generate the first pooled fraction. The remaining seven fractions were created followed by a similar pooling strategy. Fractions were dried to completeness in a vacuum concentrator. Peptides

from each fraction were subjected to LC-MS analysis or ubiquitylation enrichment using antibody recognizing K-GG remnant.

Ubiquitylome enrichment

The peptide mixture about 1 mg each fraction were dissolved in 200 μl IP buffer (50 mM MOPS pH 7.2, 10 mM sodium phosphate, 50 mM NaCl, and 0.3% NP40). Total 80 μl of K-GG ubiquitin remnant motif antibody bead conjugate (PTM-1104 , PTM Biolabs, China) were washed three times with cold PBS, then were loaded into 8 homemade C_8 StageTips equally. Each fraction of resuspended peptides, were add to C_8 StageTips and flow past beads by centrifuge force of 200 g. IP buffer and IP buffer without NP40 were used to wash beads for 2 times separately to remove unspecific-binding peptides and following three times wash with H_2O by centrifuge force of 200 g. To elute peptides, 40 μl of 0.2% trifluoroacetic acid (TFA) were loaded to the tips and centrifuge at 100 g to collect peptides. The resulting peptides were dried to completeness.

LC-MS/MS

On-line LC-MS/MS analysis was performed on a tribrid Orbitrap fusion mass spectrometer coupled to a NanoLC-1000 HPLC system (Thermo Fisher Scientific, USA). The peptide mixture was separated by an in-house manufactured 15-cm fritless column packed with C_{18} resin (1.9-μm, Dr. Maisch GmbH, Germany) at a flow rate of 300 nl/min. Mass spectra were acquired in a data-dependent mode with one full scan in the Orbitrap (m/z: 350 –1800; resolution: 60000; AGC target value: 5×10^5), followed by MS_2 scan in the linear trap (32% normalized collision energy; AGC target value: 10000, maximal injection time: 50 ms or 200 ms for tissue lysate or ubiquitylation analysis respectively). The dynamic exclusion for precursor selection was set as 60 s.

Protein identification and ^{15}N quantification

60d aged flies' head, muscle and testis were used to identify long-lived proteins. Raw data of different fractions belong to one sample were searched together against Uniprot *Drosophila* database (download date: Feb 2016) using Integrated Proteomics Pipeline - IP2 (Integrated Proteomics Applications, San Diego, CA). http://www.integratedproteomics.com/), with 20 ppm and 400 ppm were set for precursor and fragment mass tolerance respectively. Trypsin was set as the enzyme, and maximum allowed cleavage was 1 and 2 for tissue lysate or ubiquitin enriched sample. Static modification was Carbamidomethyl (C) (+57.02 Da), while dynamic modification was oxidation of methionine (+15.99 Da) for all samples, and diGly of

lysine (+114.04 Da) only for ubiquitin-enriched samples. ^{15}N analysis was performed in parallel using the same parameters. ^{14}N and ^{15}N ratio of each peptide was calculated using CENSUS as described before. Two peptides were required for protein quantification, while only one ubiquitylated peptide was required to report a ubiquitylated protein.

Western blot assays for histone modification

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Chinese Academy of Sciences and are in accordance with the Guide for the Care and Use of Laboratory Animals of Chinese Academy of Sciences. The level of mono-ubiquitylated histone H2A was analyzed by western blot using dissected heads from 30d and 60d old flies. Aged male C57BL/6 mouse samples were obtained from East China Normal University, Shanghai, China. Human prefrontal cortex from 2 females and 12 males were obtained from Chinese Brain Bank Center (CBBC), Hubei, China. Equal amount of tissues were lysed in RIPA buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate and protease inhibitor (Pierce, USA) with motor pestle. Protein mixture was centrifuged at 16000 g, for 10 min at 4 °C to remove undissolved ceratine, and then separated on a NuPAGE 12% Bis-Tris gel (GeneScript, China). The separated proteins were then transferred to a polyvinylidene fluoride membrane (Millipore, USA). The respective primary antibodies were diluted 1:1000 (anti-ubH2A, 05-678, mouse host, Millipore, USA) for mouse brain samples, 1:2000 (anti-ubH2A, #8240, rabbit host, CST, USA) for *Drosophila* head and human brain samples, and 1:2000 (anti-H2A, 07-146, rabbit host, Millipore, USA) for *Drosophila*, mouse, and human samples, with overnight incubation at 4 °C. The HRP-conjugated secondary antibodies, anti-mouse (31430, Pierce, USA) and anti-rabbit (31460, Pierce, USA), were diluted 1:10000 and incubated for 1 hour at room temperature. The signal intensity was quantified by ImageQuant TL (GE Healthcare, UK). The relative abundance of histone modification was normalized to histone H2A signal intensity, and statistical analysis was using a student's *t*-test.

CRISPR-Cas9

CRISPR/Cas9 mutagenesis was performed as previously described. Two sgRNA plasmids for target gene were injected into fly embryo. Single-fly-PCR assays were used to screen for mutants. To do this, single fly was homogenized in 50 μl squashing buffer (10 mM Tris buffer (pH 8.5), 25 mM NaCl, 1 mM EDTA, 200 μg/ml Proteinase K), then incubated at 37 °C for 30 minutes, followed by 95 °C, 10 minutes for inactivation.

Screen primers for *Sce*^{c244} (F:5'-GTCGCTGTACGAGCTGCAGCGCAAG-3'(SEQ ID NO: 12);R:5'-CGATGTGCCCGAATGACCCGGATCC-3'(SEQ ID NO: 13)and *Su(z)2*^{c433} (F:5'-CGATCCAACCACTGTGGATTACTG-3' (SEQ ID NO: 14); R: 5'-GTAGGCAG TACCTCATCCTTGTAG-3' (SEQ ID NO: 15)) deletion respectively. The virgin females carrying the deletion were backcrossed into WT (5905) male flies for five consecutive generations for a uniform genetic background, to mitigate background effects.

Lifespan

Adult male flies were collected at the day of eclosion and maintained at 20 flies per vial at 25 ℃ with 60% humidity and a 12 hours light/12 hours dark cycle. Flies were transferred to new vials every other day and scored for survival.

GSH/GSSG measurement

One hundred fly heads were harvested and immediately frozen in liquid nitrogen. Tissues were homogenized using the homogenizer (Precellys 24, Bertin Technologies, Germany) with 300 μl ddH_2O. Then frozen at -80 ℃ for 30 minutes and thawed at room temperature, followed by 5 cycles of 30 seconds on and 30 seconds off sonication. Lysates were centrifuged using 10,000 g at 4 ℃ for 5 minutes. Supernatant was transferred to 10 kDa molecular weight cut-off spin filter (Millipore, USA), followed by centrifugation using 10,000 g at 4 ℃ for 30 minutes to remove proteins. Assays were assembled according to the instruction of manufacturer (S0053, Beyotime Biotechnology, China).Absorbance at 412 nm were detected by the Multiskan GO (Thermo Fisher Scientific, USA).

Climbing test

Ten male flies were transferred to an empty vial for 30 minutes adaption in dark. Flies were tapped to the bottom of the vial; the percentage of flies abled to climb up to a mark at 2 cm from the bottom within 5 seconds was scored. Ten biological replicates were done for each genotype at given age.

Oxidative stress

Male flies for each genotype were used, with 20 flies per vial. Prior to the test, flies were pre-treated with 1% Agar media for 6 hours before transferred to the media containing H_2O_2. Then, flies were transferred to vials containing a small piece of Kimwipe filter paper pre-soaked in 1.5 ml of 10% glucose and 2% H_2O_2 (Sinopharm Chemical Reagent, China). Dead flies were scored every 12 hours, and the Prism software (Graphpad) was used to generate the survival curve and statistical analysis.

RT-qPCR

Fifty heads were dissected and homogenized in a 1.5 ml tube containing 1 ml of Trizol Reagent (Thermo Fisher Scientific, USA). RNA isolation was followed in accordance with manufacturer's instruction. RNA was re-suspended in DEPC-treated RNase free water (Thermo Fisher Scientific, USA). TURBO DNA free kit was used to remove residual DNA contamination according to manufacturer's instruction (Thermo Fisher Sicentific, USA). 1 μg of total RNA was used for reverse transcription by random primers using SuperScript III First-strand synthesis system for RT-PCR (Thermo Fisher Scientific, USA). Analysis was performed using the QuantStudio 6 Flex real-time PCR system with SYBR selected master mix (Thermo Fisher Scientific, USA). The $2^{-\Delta\Delta CT}$ method was used for quantification upon normalization to the RP49 gene as internal control.

Primers for target gene GSTO1:

F: 5'-AGATCCCGTATCACAGCATCTAC-3'; (SEQ ID NO: 16).

R: 5'-GCTCGTCCAGATATTCACAAATC-3'. (SEQ ID NO: 17).

Primers for RP49: F: 5'-CCGCTTCAAGGGACAGTATCTG-3'; (SEQ ID NO: 18).

R: 5'-ATCTCGCCGCAGTAAACGC-3' (SEQ ID NO: 19).

PolyA-selected mRNA-seq

Head tissues were homogenized in a 1.5 ml tube containing 1 ml of Trizol Reagent (Thermo Fisher Scientific, USA). RNA isolation was followed in accordance with manufacturer's instruction. RNA was re-suspended in DEPC-treated RNase free water (Invitrogen, USA). TURBO DNA free kit was used to remove residual DNA contamination according to manufacturer's instruction (Invitrogen, USA). 1 μg of total RNA was used to generate sequencing library using VAHTS mRNA-seq v2 library Prep Kit for Illumina. The library quality was checked by Bioanalyzer 2100 (Agilent, USA). The quantification was performed by qRT-PCR with a reference to a standard library. The libraries were pooled together in equimolar amounts to a final 2 nM concentration. The normalized libraries were denatured with 0.1 M NaOH (Sigma, USA). Pooled denature libraries were sequenced on the illumina NextSeq 550 platforms with single end 100 bps. Sequencing reads were mapped to the reference genome dm6 with STAR2.3.0e by default parameter. The read counts for each gene were calculated by HTSeq-0.5.4e htseq-count with parameters "-m intersection-strict -s no". The count files were used as input to R package DESeq for normalization and the differential expression genes were set at a *p* value smaller than 0.05.

(http://bioinfogp.cnb.csic.es/tools/venny/index.html).

GO and pathway analysis

Biological pathway analysis for *Drosophila* LLPs and ELLPs were performed with David. Differential expression of mRNA genes was analyzed by ingenuity pathway analysis-IPA (QIAGEN, USA).

Data availability

The MS data have been deposited under the accession number IPX0001204000 at iProX and the Illumina RNA-seq data have been deposited in the Sequence Read Archive (SRA), using the NCBI portal, under the BioProject accession number PRJNA453196 and SRA accession number SRR7059190.

Results

Isotope Labeling Strategy Reveals Long-Lived Proteomes

To interrogate long-lived proteome, the inventors adapted the pulse-SILAM method (stable isotope labeling with amino acids in mammals) in *Drosophila* (Fig. 1a). Briefly, flies were fed with ^{15}N-labeled diet started *in utero*. For young animals at 5d post-eclosion, fly diet was switched from ^{15}N-labeled heavy form to natural ^{14}N-labeled light form, such that newly synthesized proteins during adult lifespan could incorporate ^{14}N, allowing the separation of the newer proteome from older proteome by mass spectrometry. Using this method, the inventors characterized the *Drosophila* age-dependent changes in proteomes. Head and muscle of non-dividing somatic tissues, and testis of mitotically active germ-line were isolated from animals of 5d, 30d and 60d of age. Mass spectrometry analysis of 5d old animals fed with ^{15}N-diet from *in utero* showed that ^{15}N-labeled peptides accounted for more than 99.7% of the total peptides analyzed (Supplementary Fig. 1a), suggesting a near-complete labeling efficiency. A total number of 3,074, 1,903, 3,034 proteins were quantitatively analyzed from all three aging time points in head, muscle and testis, respectively (Supplementary Fig. 1b and Supplementary data 1-3). The turnover of proteomes during aging is demonstrated with the synthesis of new proteins and decline in the older proteomes as shown by the reduction in the proportion of proteins with heavy ^{15}N labels (Fig. 1b). The relative ratios of ^{14}N and ^{15}N peptides of individual proteins between young and aged animals differed dramatically, thus reflecting their differential turnover rates (Fig. 1b). To map the proteome of long-lived proteins (LLP) in *Drosophila*, the inventors first defined long-lived proteins as those with at least 10% of ^{15}N-labeled forms remained at the age of day

60 compared to that of near 100% at 5d. Notably, this criterion was more stringent than previous studies in murine LLPs where 5% cutoff was used. This analysis showed that muscle proteome had the highest proportion of long-lived proteins, with 1,502 out of 1,903 (78.9%) proteins being LLPs; in comparison, 1,715 out of 3,074 (55.8%) proteins in the head proteome were LLPs (Supplementary Fig. 1c). In contrast, testis of *Drosophila* germline, where cells are mitotically active, had only 928 LLPs, accounting for 30.6% of identified testicular proteins. Gene Ontolgoy (GO) analysis found that LLPs shared by all three tissues were associated with energy generation (Supplementary Fig. 1d). While head-specific LLPs were involved in neurotransmission, LLPs in muscle and testis were preferentially enriched in metabolic processes (Supplementary Fig. 1e). Combined, the study of *Drosophila* aging proteomes by pulse-SILAM reveals the roles of LLPs in regulating energy metabolism and tissue specific function such as neurotransmission.

The inventors next clustered proteins according to the proportion of ^{15}N using the Fuzz C-means method. This analysis identified a cluster of proteins that retained more than 70% of ^{15}N forms at 60d, thus representing the extremely long-lived proteins (ELLPs) in *Drosophila* (Fig. 1c). Altogether, this study cataloged 276, 503, and 306 ELLPs in head, muscle, and testis, respectively (Fig. 1c). Among 16 ELLPs shared by all three tissues, 4 were laminin proteins (Supplementary Fig. 1f). This finding in *Drosophila* is consistent with a previous LLP study in rat, suggesting that laminin of the nuclear proteins are evolutionarily conserved ELLPs. Moreover, GO analysis of 149 ELLPs shared by both head and muscle indicated that nucleosome assembly was the most significantly enriched category (p=5.2e-9, Fisher's exact test) (Figure 1D). Strikingly, all previously reported long-lived murine histone proteins were found in this category, demonstrating the fact that histone proteins as ELLPs were also highly evolutionarily conserved (Fig. 1f). In addition, the inventors identified a new cohort of structure proteins as ELLPs, such as actin, tropomyosin and paramyosin in *Drosophila*.

Ubiquitylation is over-represented in older proteins than their younger forms

Given that ubiquitylation plays a critical role in regulating protein turnover, the inventors interrogated how ubiquitylation might be involved in aging proteome. To address this question, the inventors combined the pulse-SILAM with antibody-enriched ubiquitylation that allowed the quantitative measurement of ubiquitylome on both old and newly synthesized proteins. In fly heads from 60d old animals, 5,317 unique ubiquitylation sites in 2,068 proteins were identified, in which 2734 unique ubiquitylation sites in 988 proteins were

quantified (Supplementary data 4). While 451 quantified proteins contained only one ubiquitylation site, 537 quantified proteins were found to have multiple sites with 37 quantified proteins harboring even more than 10 modifications (Supplementary Fig. 2).

The inventors next examined whether ubiquitylation might differentially modify old versus newly synthesized proteins. Analysis of peptide-spectrum matches (PSMs) determined that while ^{15}N-labeled old forms accounted for 28% of the entire proteome at 60d, they represented 59% of the ubiquitylated proteome (Fig. 2a). Thus, older proteomes showed increased modification by ubiquitylation. Consistently, majority of ubiquitylated proteins (~64%) displayed a higher percentage between $^{15}N/^{14}N$ forms compared to their normal ratio in the aging proteome as revealed by the above pulse-SILAM experiments (p=2.2e-16, Mann-Whitney-Wilcoxon test) (Fig. 2b). Thus, ubiquitylation of individual proteins were also comparatively increased in the older proteins than their young counterparts.

UbH2A is a highly conserved aging hallmark

The inventors next characterized a group of individual long-lived proteins with significant ubiquitylation. This group included myosin heavy chain, voltage-dependent anion-selective channel, sodium/potassium transporting ATPase, and notably histone proteins (Fig. 2c and Supplementary Table 1). Interestingly, the inventors noted a significant enrichment of ubquitylated histone 2A at lysine 118 (ubH2A) and multiple ubiquitylated histone 2B (Fig. 2d-e).

Since H2A119K in mammals (K118 in fly) is a conserved site for histone mono-ubiquitylation, the inventors asked whether this modification might be also changed with age in different species (Fig. 3a). The inventors first examined the level of ubH2A by western blot and revealed an increase in aged *Drosophila* (Fig. 3b). The inventors then analyzed young and aged mice (32 weeks and 104 weeks, respectively) and found increased accumulation of ubH2A in the brain of aged mice as compared to that of young animals (Fig. 3c). More strikingly, the abundance of ubH2A was substantially increased in the prefrontal cortex samples from older human (Fig. 3d). Combined, these data demonstrate that age-modulated increase of ubH2A is a highly conserved hallmark of aging.

Table 1

Proteins	Identified sites	#total PSM	^{15}N% ratio for ub peptide	Description
E1JHJ4	K1894	48	0.92	Myosin heavy chain
Q94920	K217	48	0.85	Voltage-dependent
Q94920	K52	46	0.80	anion-selective channel
A0A0B4LGZ7	K332	40	0.95	Uncharacterized protein
P13607	K54	40	0.80	Sodium/potassium-transporting ATPase
A1Z7S3	K616	30	0.46	Rab32
Q9W350	K587	29	0.04	C11
Q9W418	K63	29	0.05	GH17761p
P84051	K118	26	0.83	Histone H2A
M9PGW0	K351	24	0.63	Innexin

Reducing ubH2A promotes adult healthy lifespan in *Drosophila*

To understand the biological significance of ubiquitin-modified long-lived proteins, the inventors chose to explore how H2A ubiquitylation could impact adult lifespan in *Drosophila*. As noted, studies have implicated *Sce* and *Su(z)2* in modulation of H2A ubiquitylation. The inventors generated site-specific null mutants of Sce^{c244} and $Su(z)2^{c433}$ using CRISPR/Cas9 mutagenesis (Supplementary Fig. 3a-b). Lifespan analysis demonstrated that animals deficient in sce^{c244} lived longer than WT (Fig. 4a), coupled with a reduction of ubH2A (Fig. 4b). Since the effects of epigenetic genes are usually dose-dependent, the inventors further analyzed adult survival using Sce^{c244}, $Su(z)2^{c433}$ trans-heterozygous double mutants. Remarkably, double mutants demonstrated more striking effects on life-extension and ubH2A-reduction (Fig. 4a-b). This evidence indicates that reducing ubH2A couples with an effect to promote adult longevity.

Biological roles of histone modification link chromatin accessibility to gene expression, and specifically, upregulation in the level of ubH2A has been implicated in gene repression. The inventors then asked whether transcriptional change of particular genes might account for ubH2A-dependent regulation on aging. Using dissected head tissues, the inventors generated RNA-seq datasets for polyA-selected mRNAs on WT and Sce^{c244}, $Su(z)2^{c433}$ trans-heterozygous double mutants that gave rise to the stronger life-extension phenotype than single mutants (Fig. 4a). Interestingly, pathway analysis and qRT-PCR experiments revealed that the glutathione-related pathways, GSTO1, in particular, was significantly upregulated in double mutants as compared to age-matched WT animals (Fig. 4c, d). Furthermore, the inventors examined the glutathione, an important indicator of the cellular redox state. These data showed that GSH/(GSH+GSSG) ratio in mutants was significantly higher than that in WT (Fig. 4e), suggesting improved cellular redox potential in long-lived Sce^{c244}, $Su(z)2^{c433}$

double mutants.

The inventors then asked how this ability of enhanced redox potential in *Sce*^{c244} single mutants and *Sce*^{c244}, *Su(z)2*^{c433} double mutants was translated into fitness and stress resistance in aging. To illuminate this, the inventors characterized age-related phenotypes. Analysis of climbing ability demonstrated that mutants had better locomotion than age-matched WT (Fig. 5a). To examine the sensitivity to oxidative stress, we utilized hydrogen peroxide (H_2O_2), a source of reactive oxygen species (ROS). We observed substantially enhanced resistance to oxidation in *Sce*^{c244} single mutants and *Sce*^{c244}, *Su(z)2*^{c433} double mutants than WT (Fig. 5b). Taken together, these data implicate that a reduction of ubH2A by gene mutation couples longevity with enhanced ability to handle stress, a property reflecting improved adult fitness. While the invention has been described through illustrative examples, routine modifications will be apparent to those skilled in the art, which modifications are intended to be within the scope of the invention.

The inventors claim:

1. A kit for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising an agent to measure the ubiquitylation of Histone 2A protein or functional equivalent thereof.

2. The kit of claim 1, wherein the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

3. The kit of claim 1, wherein the agent is an antibody specific to ubH2A.

4. The kit of claim 3, wherein the antibody is monoclonal antibody.

5. The kit of claim 3, wherein the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof.

6. The kit of claim 1, wherein the aging related condition is at least one of the dementias, neurodegenerative diseases, neurological disorders, and age-related conditions.

7. The kit of claim 1, wherein the aging related condition is at least one of atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Alzheimer's Disease, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline.

8. The kit of claim 1, wherein the agent to measure the ubiquitylation is performed by means of Western-Blotting.

9. Usage of an agent to measure the ubiquitylation of Histone 2A protein in the preparation of a kit for diagnosing, or providing a prognostic indicator of an aging related condition in a mammal.

10. The usage of claim 9, wherein the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

11. The usage of claim 9, wherein the agent is an antibody specific to ubH2A.

12. The usage of claim11, wherein the antibody is monoclonal antibody.

13. The usage of claim 11, wherein the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof.

14. The usage of claim 9, wherein the aging related condition is at least one of the dementias, neurodegenerative diseases, neurological disorders, and age-related conditions.

15. The usage of claim 9, wherein the aging related condition is at least one of Alzheimer's Disease, atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline.

16. The usage of claim 9, wherein the agent to measure the ubiquitylation is performed by means of Western-Blotting.

17. An agent to measure the ubiquitylation of Histone 2A protein for use in diagnosing, or providing a prognostic indicator of an aging related condition in a mammal.

18. The agent of claim 17, wherein the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

19. The agent of claim 17, wherein the agent is an antibody specific to ubH2A.

20. The agent of claim19, wherein the antibody is monoclonal antibody.

21. The agent of claim 19, wherein the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof.

22. The agent of claim 17, wherein the aging related condition is at least one of dementias, neurodegenerative diseases, neurological disorders, and age-related conditions.

23. The agent of claim 17, wherein the aging related condition is at least one of Alzheimer's Disease, atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Lewy body dementia, vascular dementia, Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline.

24. The agent of claim 17, wherein the agent to measure the ubiquitylation is performed by means of Western-Blotting.

25. A method of diagnosing, or providing a prognostic indicator of an aging related condition in a mammal comprising detecting the ubiquitylation of Histone 2A protein in a sample from said mammal, wherein an increase in the level of the ubiquitylation of Histone 2A protein in a cell of said sample, relative to the level of the ubiquitylation of Histone 2A protein in a normal cell indicates said sample is suffering from aging related condition.

26. The method of claim 25, further comprising treating the aging related condition by reducing level of the ubiquitylation of Histone 2A protein in a cell of said sample.

27. The method of claim 26, wherein the level of the ubiquitylation of Histone 2A protein is reduced by site-specific mutagenesis of the Histone 2A protein.

28. The method of claim 27, wherein the site-specific mutagenesis is performed by means of Crispr-Cas9 method.

29. The method of claim 26, wherein the level of the ubiquitylation of Histone 2A protein is reduced by inhibiting PRC1.

30. The method of claim 25, wherein the ubiquitylation of Histone 2A protein is on at least one of the following sites:

119K of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 or 9,

118K of SEQ ID NO: 10,

26K of SEQ ID NO: 11.

31. The method of claim 25, wherein the detecting is performed with mass spectrometry.

32. The method of claim 25, wherein the detecting is performed with an agent, wherein the said agent is an antibody specific to ubH2A.

33. The method of claim 32, wherein the antibody is monoclonal antibody.

34. The method of claim 32, wherein the antibody is at least one of 05-678 Millipore, #8240 CST and derivatives thereof.

35. The method of claim 32, wherein the agent to measure the ubiquitylation is performed by means of Western-Blotting.

36. The method of claim 25, wherein the aging related condition is at least one of dementias, neurodegenerative diseases, neurological disorders, and age-related conditions.

37. The method of claim 25, wherein the aging related condition is at least one of Alzheimer's Disease, atherosclerosis and cardiovascular disease, cancer, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, Lewy body dementia, vascular dementia, Huntington's Disease,

Parkinson's Disease, amyotrophic lateral sclerosis, schizophrenia, depression, Mild Cognitive Impairment, Age-Related Cognitive Decline.

38. A method for screening an agent for the treatment of an aging related condition in a mammal comprising contacting a cell from a subject with the aging related condition with a candidate agent and determining whether the candidate agent reduces the level of the ubiquitylation of Histone 2A protein, wherein a reduction in the level of the ubiquitylation of Histone 2A protein indicates that the candidate agent is suitable for the treatment of the aging related condition.

39. A pharmaceutical composition for treating an aging related condition in a mammal comprising an agent to decrease the ubiquitylation of Histone 2A protein.

40. Usage of an agent to decrease the ubiquitylation of Histone 2A protein in the preparation of a pharmaceutical for treating an aging related condition in a mammal.

41. An agent to decrease the ubiquitylation of Histone 2A protein for use in treating an aging related condition in a mammal.

42. A method of treating an aging related condition in a mammal comprising administrating an agent to decrease ubiquitylation of Histone 2A protein to a subject in need of such treatment.

43. A cell model of an aging related condition with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof.

44. An animal model of an aging related condition comprising a cell with increased ubiquitylation of Histone 2A protein compared with the wild type cell thereof.

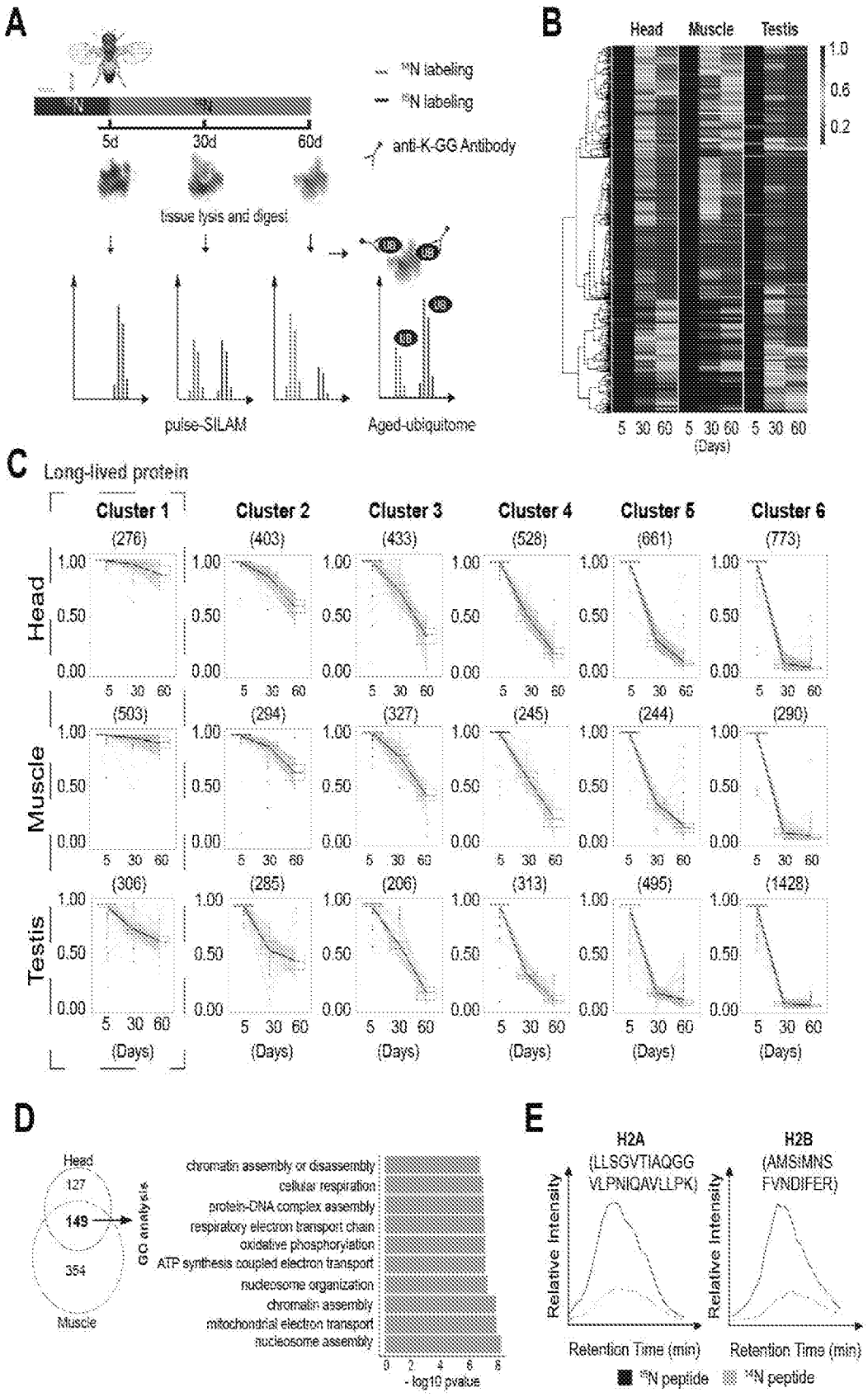
A
14N labeling
15N labeling
anti-K-GG Antibody
5d
30d
60d
tissue lysis and digest
pulse-SILAM
Aged-ubiquitome
B
Head
Muscle
Testis
1.0
0.6
0.2
5 30 60 5 30 60 5 30 60
(Days)
C
Long-lived protein
Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
(276)
(403)
(433)
(528)
(661)
(773)
(503)
(294)
(327)
(245)
(244)
(290)
(306)
(285)
(206)
(313)
(495)
(1428)
Head
Muscle
Testis
1.00
0.50
0.00
5 30 60
(Days)
D
Head
127
149
354
Muscle
GO analysis
chromatin assembly or disassembly
cellular respiration
protein-DNA complex assembly
respiratory electron transport chain
oxidative phosphorylation
ATP synthesis coupled electron transport
nucleosome organization
chromatin assembly
mitochondrial electron transport
nucleosome assembly
0 2 4 6 8
- log10 pvalue
E
H2A
(LLSGVTIAQGG
VLPNIQAVLLPK)
H2B
(AMSIMNS
FVNDIFER)
Relative Intensity
Retention Time (min)
14N peptide
15N peptide

Fig.1

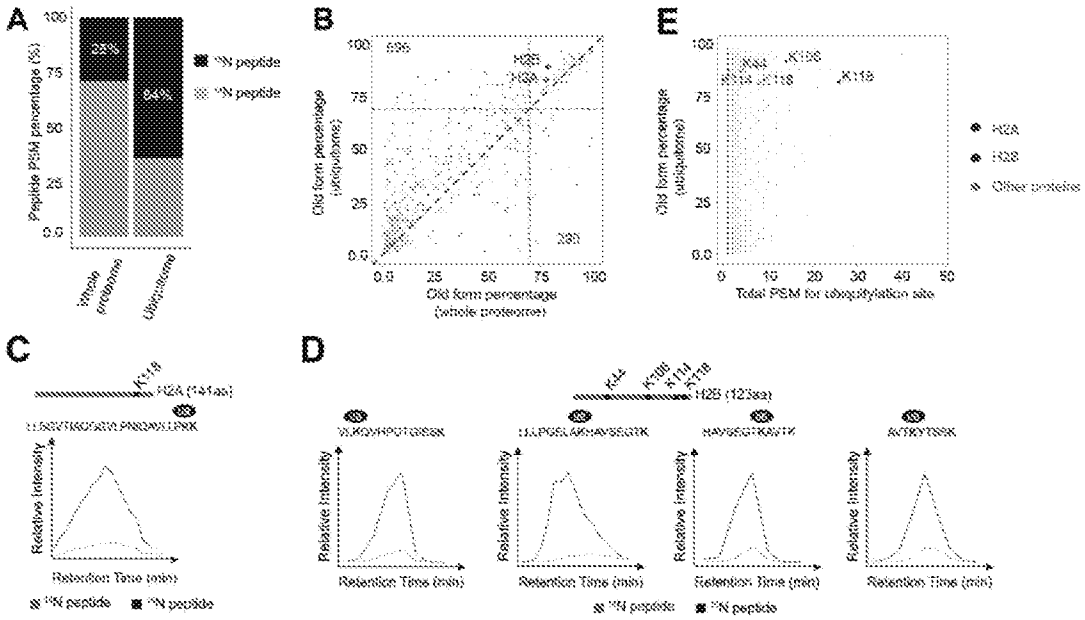
A
B
E
C
D
Peptide PSM percentage (%)
Whole proteome
Ubiquitome
Old form percentage (ubiquitome)
Old form percentage (whole proteome)
Total PSM for ubiquitylation site
H2A
H2B
Other proteins
Relative Intensity
Retention Time (min)

Fig.2

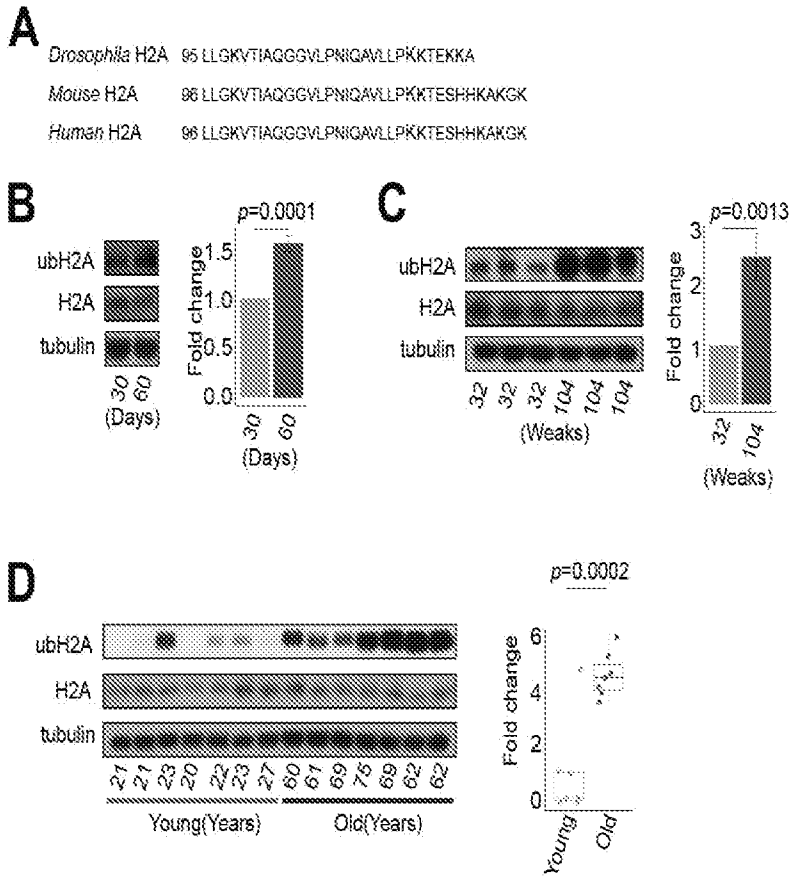
A
Drosophila H2A 95 LLGKVTIAGGGVLPNIQAVLLPKKTEKKA
Mouse H2A 96 LLGKVTIAQGGVLPNIQAVLLPKKTESHHKAKGK
Human H2A 96 LLGKVTIAGGGVLPNIQAVLLPKKTESHHKAKGK
B
ubH2A
H2A
tubulin
(Days)
p=0.0001
Fold change
C
p=0.0013
(Weaks)
D
p=0.0002
Young(Years)
Old(Years)
Young
Old

Fig.3

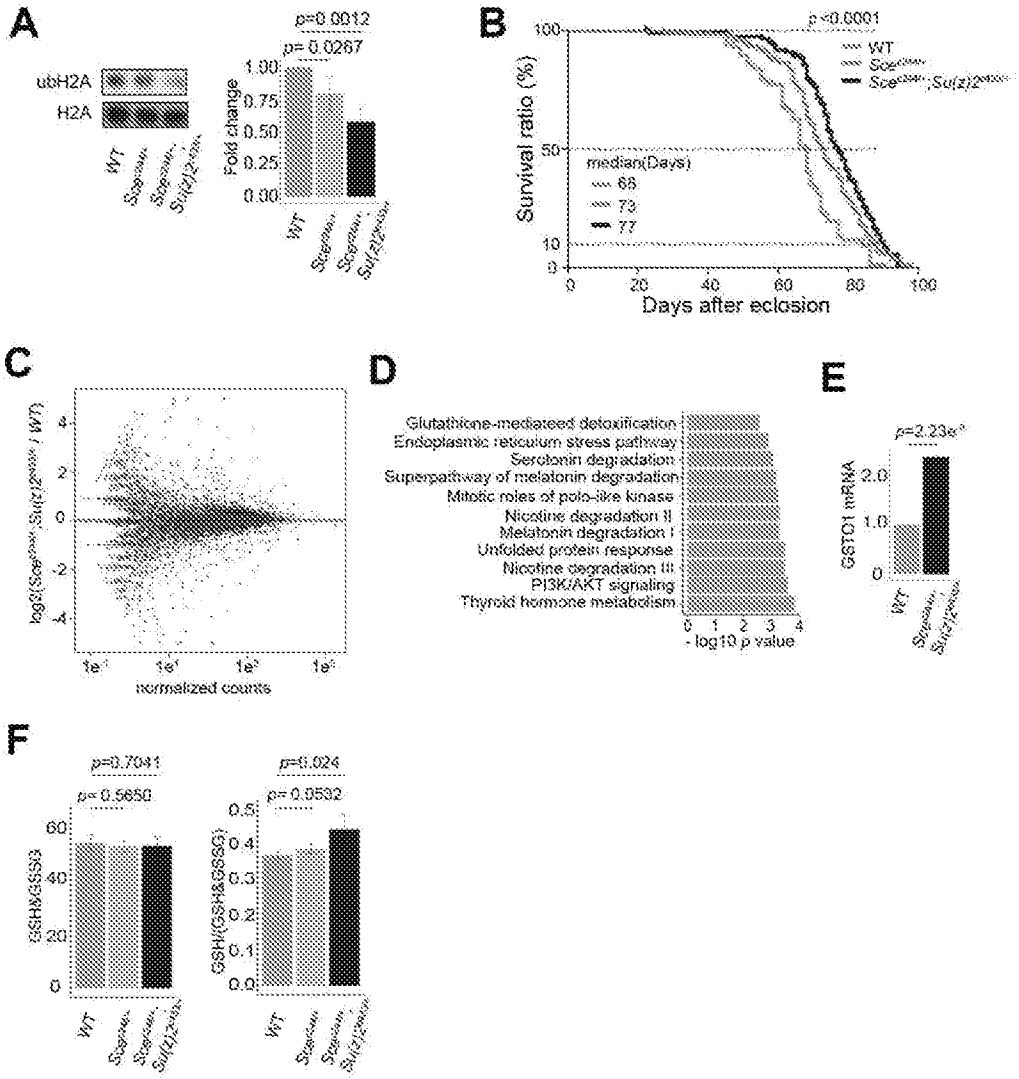
A
p=0.0012
p= 0.0267
ubH2A
H2A
Fold change
1.00
0.75
0.50
0.25
0.00
WT
B
p <0.0001
WT
Survival ratio (%)
100
50
10
0
median(Days)
66
73
77
0
20
40
60
80
100
Days after eclosion
C
log2(
normalized counts
1e-1
1e1
1e3
1e5
D
Glutathione-mediateed detoxification
Endoplasmic reticulum stress pathway
Serotonin degradation
Superpathway of melatonin degradation
Mitotic roles of polo-like kinase
Nicotine degradation II
Melatonin degradation I
Unfolded protein response
Nicotine degradation III
PI3K/AKT signaling
Thyroid hormone metabolism
0 1 2 3 4
- log10 p value
E
p=2.23e-5
GSTO1 mRNA
2.0
1.0
0
WT
F
p=0.7041
p= 0.5650
GSH/GSSG
60
40
20
0
p=0.024
p= 0.0532
GSH/(GSH&GSSG)
0.5
0.4
0.3
0.2
0.1
0.0
WT

Fig.4

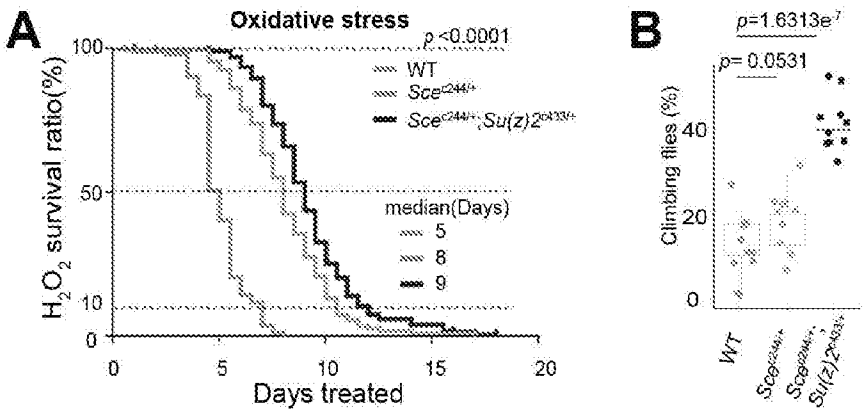
A
Oxidative stress
p <0.0001
WT
median(Days)
5
8
9
100
50
10
0
0
5
10
15
20
Days treated
B
p=1.6313e-7
p= 0.0531
Climbing flies (%)
40
20
0
WT

Fig.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2018/090791

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; C12Q 1/68(2018.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS,CPRSABS,SIPOABS,DWPI,CNTXT,WOTXT,EPTXT,USTXT,CNKI,BAIDU XUESHU,WEB OF SCIENCE, PubMed: Histone 2A Protein, UBIQUITYLATION, ubiquitin, aging, Alzheimer Genbank,EMBL: sequence search on SEQ ID NOs: 1-11

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015117145 A1 (UNIV CHICAGO) 06 August 2015 (2015-08-06) See Description, paragraph 93 and Example 4	1-25
Y	WO 2015117145 A1 (UNIV CHICAGO) 06 August 2015 (2015-08-06) See Description, paragraph 93 and Example 4	26-44
Y	WO 2017049192 A1 (UNIV MASSACHUSETTS) 23 March 2017 (2017-03-23) see claims 1-101	26-44

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 March 2019	**25 April 2019**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **ZHAO,Yanhao** Telephone No. **62411043**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2018/090791

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **42**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Although claim 42 is directed to a method of treatment of the human/animal body by therapy (PCT Rules 39.1 (iv)), the search has been carried out and based on the alleged use in the manufacture of the pharmaceutical composition for decreasing ubiquitylation of Histone 2A protein.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2018/090791

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2015117145 A1	06 August 2015	EP 3102721 A1	14 December 2016
		US 2016341743 A1	24 November 2016
		EP 3102721 A4	01 November 2017
		JP 2017506073 A	02 March 2017
		CN 107002289 A	01 August 2017
WO 2017049192 A1	23 March 2017	EP 3349749 A1	25 July 2018
		CA 2998745 A1	23 March 2017
		AU 2016324144 A1	22 March 2018
		US 2018256749 A1	13 September 2018
		JP 2018528968 A	04 October 2018