Mapping Aging Biomarkers and Pathways Using Proteomics

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Abstract: Aging is the leading factor in many diseases. During aging, a lot of proteins and pathways are dramatically changed, which may serve as potential aging biomarkers and targets for anti-aging drug development. In this review, we summarize the current progress in aging-related biomarkers and pathways that have been identified by proteomics. We find that many proteins in metabolism pathways are involved in aging process, which have a positive- or a negative-correlation. Changes in many have strong relationship with aging, including mTOR pathway, pro-inflammatory pathway, insulin/insulin-like signaling pathway, mitochondrial function pathway.

Keywords: Aging, Protein, Proteomics, Biomarker

1. Introduction

As the world's population grows, the elderly persons are also rapidly increasing in number. Ageing affects all organ systems by changing molecular, cellular and physiological characteristics, and contributes to cognitive decline, heart disease, neurodegeneration, cancer and many other diseases [1]. Therefore, it is important to study the complex mechanisms behind aging process [2]. Scientists have considered aging as a shared cause of diseases, and developed numerous hallmarks using transcriptome, epigenetic, proteome, microbiome, metabolome, and even imaging data [3]. These hallmarks contain impaired DNA repair, genomic instability, epigenetic alterations, toxicants accumulation, mitochondrial dysfunction, dysregulated metabolism, stem cell exhaustion and telomere attrition [4].

Protein is the basic material of life activities and undertakes most of the work in organs. For example, telomeres are ends of chromosomes that get shorter after each replication. Its length is associated with age and life span [5]. Since proteomics provides of higher coverage and more in-depth analyze of proteins, proteomics is a powerful tool for the identification of aging factors. Currently, it is certain that aging involves proteins and pathways in many parts of the body working together. These biomarkers are important for revealing the underlying mechanisms of aging and development of aging interventions. Indeed, several critical factors and pathways have been identified by proteomics.

Therefore, in this review, we specifically focus on aging-related factors and pathways identified by proteomics and analyze their potential mechanisms of action. Biomarkers of metabolism and immune system in brain, kidney and skin are summarized from published studies, which can monitor the proteins that are related with the aging process and predict trends of aging [5]. Biomarkers are downregulated or upregulated due to aging, with some exceptions of dysregulation. We find that proteins involved in metabolism are significantly affected by aging, as well as metabolism pathways. Activation or inhibition of cell pathways result in changes in cellular products and cell properties. Here, pathways related to pro-inflammatory, insulin signaling, and mitochondrial functions are discussed.

2. Aging-Related Biomarkers

Numerous proteins have been found to be associated with aging, which may serve as biomarkers for this process. As age growing, proteins in different organs of the body have different responses, some are upregulated or downregulated, while others remain constant (Table 1).

Nerve cells of the central nervous system have little ability to regenerate, neurons are long-lived cells that live as long as human. Therefore, neurons experience a long age-related decline [6], and protein

alterations in the brain are crucial potential markers to the aging process. Age associated disease such as Alzheimer's disease (AD) shows change in protein structure and abundance. As brain ages, IgG (Ighg2c), B2m, inflammatory proteases such as lysozyme 2 (Lyz2) and cathepsin D (Ctsd) and protease inhibitor cystatin 3 (Cst3) showed changes in relationships. CD5 antigen-like protein (Cd5l), potentially harmful to autoimmunity, is suggested to be upregulated as aging. Most importantly, 14-3-3 proteins YWHAZ and YWHAB-demonstrated a strong association with brain aging, memory, and such features-are abundant in AD patients [7].

Table 1: Biomarkers indicating aging process summarized from previous studies.

Tissue/cell type	Factors	Changes with aging	Species
Human skin	Tubulin Beta-3	Upregulated	Human
Individual level	Insulin/Insulin-like Growth Factor 1 (IGF-1)	Downregulated	Mice
Ovarian Surface Epithelium	Mechanistic Target of Rapamycin (mTOR)	Upregulated	Human
Ovary	Mechanistic Target of Rapamycin (mTOR)	Upregulated	Mice
Skeletal muscle	5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK)	Upregulated	Mice
Dermal fibroblasts, Ovary	Sirtuins (SIRT1)	Downregulated	Mice
Peripheral blood mononuclear cells	Sirtuins (SIRT2)	Downregulated	Human
Ovary	Sirtuins (SIRT3)	Downregulated	Mice
Dermal fibroblasts, Ovary	Sirtuins (SIRT6)	Downregulated	Mice
Kidney	Aldehyde Dehydrogenase 1 (Aldh1a1)	Upregulated	Mice
Kidney	Ceramidase (Asah1)	Upregulated	Mice
Kidney	Glutaredoxin-1(Glrx1)	Downregulated	Mice
Individual level (Mitochondria)	Thioredoxin-dependent peroxide reductase/Peroxiredoxin 3 (PrxIII)	Upregulated	Human
Plasma	GFAP	Upregulated	Mice
Kidney, Skeletal muscle	Phosphoenolpyruvate Carboxykinase (Pck1)	Downregulated	Mice
Kidney	Aldehyde dehydrogenase 1 (Aldh1a1)	Upregulated	Mice
Kidney	IgGs	Upregulated	Mice
Kidney	Glutaredoxin-1 (Glrx1)	Downregulated	Mice
Kidney	Glutathione peroxidase-3 (Gpx3)	Upregulated	Mice
White blood cell	Interleukin-1β	Upregulated	Mice

The kidney is another organ that is widely studied for aging. Aldehyde dehydrogenase 1 (Aldh1a1) is the most upregulated biomarker that is found in the loop of Henle of aged mouse kidneys [2]. The mechanism behind its high expression in the kidney is still unclear. In aging kidney, ceramidase (Asah1) is highly expressed, which hydrolyzes ceramide into sphingosine. In line with this, ceramide C16 significantly decreased [2]. Moreover, peroxisome-associated proteins which contain enzyme that catalyze reactions are downregulated. However, studies showed that nicotinamide mononucleotide (NMN) treatment could increase peroxisomal proteins, as well as Glutaredoxin-1 (Glrx1), an enzyme that repairs oxidation [2].

Metabolism related proteins are major factors that affect aging, many of which have been discovered by proteomics. For example, insulin/insulin-like growth factor 1 (IGF-1) participates in metabolism regulations and senses glucose [5]. Decreasing IGF-1 levels is likely to increase lifespan of mice [8]. Mammalian target of Rapamycin (mTOR), which senses amino acids and other nutrients, increases in human ovarian surface epithelium with age [5]. In mice, mTOR is also upregulated in aged ovaries, and inhibition of mTOR promotes longevity in animal, while the mechanism still remains unclear [9]. One possible theory is that the accumulation of cytosolic proteins that occurs during aging may be caused by mTOR1 activity. 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) regulates metabolism and other physiological processes [10]. In the skeletal muscles, it is upregulated with age. Sirtuins link metabolism with protein activation and aging. The most common types of Sirtuins in humans, SIRT1 and SIRT3, are both downregulated during aging. Mouse SIRT1, SRT3 and SIRT6 levels are also decreased in aged ovaries [5].

As age increases, levels of protein within the immune system are also affected. Interleukin-1β, a type of inflammatory cytokine, is upregulated with aging. Antimicrobial peptides and lectins are major histocompatibility complex (MHC) proteins, which are all upregulated as age increases [11].

Lastly, protein markers in human skin cells can also indicate aging. A recent study indicates that

Tubulin Beta-3 chain, a component of microtubules, may be a reliable biomarker for human skin aging. As human skin ages, the amount of Tubulin Beta-3 increases, showing a positive correlation [12]. Moreover, mutations in the Tubulin Beta-3 chain may result in neurological disorders. Thioredoxin-dependent peroxide reductase (PrxIII), and peroxiredoxins 1 and 2 are also upregulated. However, 6-phosphofructokinase, platelet type, and cornifin-B are dysregulated during aging [12]. Another research has identified eight potential biomarkers for human skin aging using peptide location fingerprinting, which are biglycan, collagen VI alpha-3, fibulin-1, and galectin-7 from the dermis and keratins (K)-2 and −10, desmoplakin and heat shock protein 70 (HSP70) from the epidermis. These potential candidates of biomarkers may impact homeostasis, functionality, and damage mechanism of the skin [13].

3. Aging-Related Pathways

Several above proteins belong to known biological pathways, such as mTOR signaling, pro-inflammatory pathway, insulin/insulin-like signaling and mitochondrial function related pathways. The specific relationship can be identified using Kyoto Encyclopedia of Genes and Genomes (KEGG) or Gene ontology (GO) pathway enrichment analyses.

3.1. mTOR Pathway

mTOR signaling can be activated by protein kinase B (PKB, also known as Akt) signaling pathway [14]. Increased mTOR activity causes pathological change [15]. Growing evidence indicates that Rapamycin, the mTOR inhibitor, has the function of prolonging lifespan in various organisms including yeast, worms, flies and mammals [16-20]. The mechanism is speculated to be decreased mRNA translation, increased mitochondrial function, stem cell function enhancement and immune regulation [21].

3.2. Pathways Related to Pro-Inflammatory

Upregulation of pro-inflammatory pathways is one of the hallmarks of aging. The upregulation of pro-inflammatory are shown in the aging of mice lungs and the plasma of human [22, 23]. IL-1β signaling pathway plays an important role of regulating the pro-inflammatory. The signature of IL-1β signaling pathway appeared in capillary endothelial cells, mesothelial and smooth muscle cells, but not other mesenchymal cell types [22]. Inflammatory pathways are evolutionarily conserved and so do the most biological pathways changing with age [20, 24]. Leukocyte activation which releases the inflammatory molecule is detected to be overrepresented in kidney aging [25]. The main protein of blood coagulation which related with aging-- fibrinogen and fibronectin are also shown to have strong connection with pro-inflammatory state [20, 26].

3.3. Insulin/IGF-I pathway

Insulin/IGF-1 signaling plays a key role in lifespan regulation [27]. It takes part in the aging of human and the growth, proliferation, maturation of cells [28, 29]. AKT2 is a key protein in insulin signaling pathway, which decreases with aging [25]. Insulin or IGF-1 stimulation induces AKT-dependent phosphorylation of FOXO proteins, which is important in regulating aging [30]. It has been found that aging has influence on the aggregation and the toxicity of polyglutamine (polyQ) [31-33]. Insulin-like signaling can reduce the aggregation and toxicity of polyQ through DAF-16 and HSF1. Therefore, the inhibition of HSF1 can both enhance the aggregation of polyQ and suppress the insulin-like signaling pathway for longevity [34].

3.4. Mitophagy Pathway

Mitochondria maintains cellular homeostasis by removing damaged mitochondria that accumulates in aged individuals through 'mitochondria'. Mitochondrial dysfunction is associated with many human diseases, especially age-related diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), metabolic diseases, certain cancer and age-related cardiovascular disease (CVD) [35]. Moreover, Basisty et al. has concluded that when interventions are performed to slow aging, mitophagy pathway is the most affectable pathway [20].

3.5. Other Pathways

NAD+ is reduced with aging and can cause the decreasing of the muscle mass [36, 37]. And the Preiss-Handler pathways can produce the NAD+ [20, 38]. TNF receptor is also involved in a series of pathways that are important in aging. For instance, TNF receptor 1 (TNFRSF1A) and 2 (TNFRSF1B) are related with the function and disease of aging [39-41]. Therefore, the TNF/TNFR pathway and its downstream pathways, such as IKK/NF-κB pathway and caspase cascade apoptosis pathway may play important roles in the aging process [23].

4. Challenges and Future Perspectives

Proteins are the ultimate effectors of physiological activity and are viable candidates for biomarkers and anti-aging treatment. However, the major obstacle in protein study using proteome is the insufficient technology and method of identification, as well as the detection of low-abundant proteins. As technology continues to advance, mass spectrometry-based proteomics, proximity extension assay (PEA) and aptamer-based proteomics (SOMAscan) assay have achieved great success. We can learn more about biomarkers, pathways, and interventions for aging in organisms, which allows for the identification of novel clinical biomarkers and new clues to the mechanisms of aging. More importantly, how to use these findings we have obtained to delay aging and avoid age-related diseases remains to be further studied.

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