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Review

Proteome analysis in the assessment of ageing

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ABSTRACT

Based on demographic trends, the societies in many developed countries are facing an increasing number and proportion of people over the age of 65. The raise in elderly populations along with improved health-care will be concomitant with an increased prevalence of ageing-associated chronic conditions like cardiovascular, renal, and respiratory diseases, arthritis, dementia, and diabetes mellitus. This is expected to pose unprecedented challenges both for individuals and societies and their health care systems. An ultimate goal of ageing research is therefore the understanding of physiological ageing and the achievement of 'healthy' ageing by decreasing age-related pathologies. However, on a molecular level, ageing is a complex multi-mechanistic process whose contributing factors may vary individually, partly overlap with pathological alterations, and are often poorly understood. Proteome analysis potentially allows modelling of these multifactorial processes. This review summarises recent proteomic research on age-related changes identified in animal models and human studies. We combined this information with pathway analysis to identify molecular mechanisms associated with ageing. We identified some molecular pathways that are affected in most or even all organs and others that are organ-specific. However, appropriately powered studies are needed to confirm these findings based in in silico evaluation.

Contents

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1. Introduction

Ageing is an inevitable passage of living organisms. In humans, ageing is superficially characterised by the appearance of grey hair, declining in vision and hearing, wrinkles in the skin and a decline in physical strength of muscles and bones. While the progression of ageing can easily be observed in individuals over the years, it appears to be one of the most complex biological events. The turning point on ageing research was the remarkable discovery made that life span could be genetically controlled by mutating specific genes in the nematode *Caenorhabditis elegans* (Johnson, 1990; Kenyon et al., 1993; Klass, 1983). Since then, a plethora of research activities were carried out to shed more light on the mechanisms that underlie the process of ageing.

The major complication of normal "healthy" ageing is the increasing risk for age-related diseases like cardiovascular diseases (North and Sinclair, 2012), diabetes mellitus (Sue et al., 2012), and dementia (Corrada et al., 2010) that can adversely affect quality of life in general, increase the risk of co-morbidities, and increase mortality. The burden caused by ageing-associated pathologies is therefore obvious.

On a molecular level ageing can be defined as a progressive deterioration of physiological functions ultimately leading to systemic dysfunction and death (Campisi, 2013). This might include the accumulation of senescent cells (Lopez-Otin et al., 2013) thereby limiting regenerative abilities (Collado et al., 2007; Onyema et al., 2012).

Ageing is a complex systemic process and the major gap in ageing research remains the insufficient knowledge about pathways derailing normal "healthy" ageing to disease-associated pathological ageing. Therefore, global "omics" approaches may help to study cellular and even molecular mechanisms and obtain detailed insights into ageing-associated processes. The proteome, being more close to the phenotype than transcriptome and more stable than the metabolome (Schanstra and Mischak, 2014), might be the most promising "omics" field in ageing research.

To date, most studies on ageing have been conducted within the context of chronic pathologies and it appears challenging to clearly separate "healthy ageing" from pathological ageing in most of the proteomic studies published. In this review on the use of proteomics in studying ageing processes, we present a condensed overview of the different proteomic technologies and ageing studies using proteomics. Finally, we used pathway analysis to integrate the currently available proteomics data in ageing and identify additional candidate proteins. This analysis suggested that ageing processes differ between organs.

2. Proteomics approaches in ageing studies

Although ageing enhances the risk for developing a host of human ailments and thus comprises an underlying cause for disease, ageing in itself is not a disorder but instead a normal physiological process. As such, attempting to identify ageing-related proteomic alterations might not be of any direct clinical applicability but might rather enable the assessment of preventive interventions. Regardless of clinical applicability, however, identifying ageing-related proteomic changes will play an important role for understanding ageing at a molecular level with ramifications for investigating root causes for age-related diseases.

2.1. Technical aspects

The complexity of tissue and body fluid proteomes calls for a separation step ahead of mass spectrometric (MS) analysis. Depending on the composition of the proteome, several well studied separation techniques are available including two-dimensional gel electrophoresis (2D-DE), liquid chromatography (LC) and capillary electrophoresis (CE). For a brief overview of the strengths and limitations of these major proteomic techniques see Table 1. With respect to the separation and mass spectrometric technique selected, proteins may have to be fractionated in a controlled manner into peptides through enzymatic digestion using e.g. trypsin up or downstream of the separation step (Chait, 2006).

2.1.1. Two dimensional gel electrophoresis

The principle of two-dimensional gel electrophoresis (2-DE) coupled to MS is based on the separation of complex protein mixtures via a two-step protocol (Natale et al., 2012). Classically, proteins are first separated according to their isoelectric points (Ip) in a pH gradient gel strip and second according to their molecular weight (MW) using SDS-PAGE. Both physicochemical properties of a protein, Ip and MW, are independent and can be altered by post-translational modifications (PTMs) such as phosphorylation, glycosylation and oxidation. 2-DE can therefore not only be utilised to analyse differential protein expression but also to detect aberrations of PTMs. For in-gel protein detection, various staining methods exist (Steinberg, 2009) which also include some PTM-specific dyes (Miller et al., 2006). Staining-based relative quantification to compare the abundance of proteins in different samples can be compromised by limitations in the linear dynamic range of dyes. Further complications of a 2-DE protein separation approach include labour-intensiveness, limited separation of hydrophobic and highly acidic or basic proteins (Magdeldin et al., 2014), and gel-to-gel variability. The latter can be partly overcome by two-dimensional fluorescence differential gel electrophoresis (2-D DIGE) where two samples can be compared in one gel (Qin and Ling, 2012). Proteins of interest can be identified, typically by tryptic digestion of the selected protein spots in the gel and subsequent identification by mass spectrometry.