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Novel ageing-biomarker discovery using data-intensive technologies

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ABSTRACT

Ageing is accompanied by many visible characteristics. Other biological and physiological markers are also well-described e.g. loss of circulating sex hormones and increased inflammatory cytokines. Biomarkers for healthy ageing studies are presently predicated on existing knowledge of ageing traits. The increasing availability of data-intensive methods enables deep-analysis of biological samples for novel biomarkers. We have adopted two discrete approaches in MARK-AGE Work Package 7 for biomarker discovery; (1) microarray analyses and/or proteomics in cell systems e.g. endothelial progenitor cells or T cell ageing including a stress model; and (2) investigation of cellular material and plasma directly from tightly-defined proband subsets of different ages using proteomic, transcriptomic and miR array. The first approach provided longitudinal insight into endothelial progenitor and T cell ageing.

This review describes the strategy and use of hypothesis-free, data-intensive approaches to explore cellular proteins, miR, mRNA and plasma proteins as healthy ageing biomarkers, using ageing models and directly within samples from adults of different ages. It considers the challenges associated with integrating multiple models and pilot studies as rational biomarkers for a large cohort study. From this approach, a number of high-throughput methods were developed to evaluate novel, putative biomarkers of ageing in the MARK-AGE cohort.

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1. Introduction

1.1. Biomarkers of ageing discovery

We have been aware of the normal age-associated changes in human physiology, for example loss of arterial elasticity, since the days of the ancient Greeks; these changes may in some individuals predispose to cardiovascular events for which clinical intervention is essential (Lee and Oh, 2010). This sort of epidemiological evidence, of age-associated characteristics and diseases, has provided the foundation for creating hypotheses about the ageing process (Jacob et al., 2013). For example, protein oxidation has been implicated in increased crosslinking of the extracellular matrix with age and which contributes to skin wrinkling and poor vascular tone, neither of which normally demand an intervention for survival of the ageing organism (Baraibar et al., 2013; Ratnayake et al., 2013). Increased oxidative DNA damage in short-lived animals provides further support for a sub-category of the "damage" or "error theory of ageing" (Sastre et al., 2000; Barja, 2013). Other observations in the physiology of ageing include an increase in the appearance of senescent cells; this observation is one of several used to inform the "programmed theory of ageing" (Kirkwood and Melov, 2011; van Deursen, 2014). From such theories, a number of ageing-biomarkers have been proposed and investigated, many within the MARK-AGE project. Importantly, ageing is a normal process of development and is not inherently a disease requiring treatment. Therefore, by understanding and being able to define normal ageing better, we may also be better placed to predict those people whose health is deviating from normal age-associated change and who are therefore are at increased risk for age-associated disease. Hypothesis-driven approaches have been commonplace for scientists over centuries, however, the advent of high data intensity approaches has fuelled the opportunity to identify novel biomarkers and possibly also develop new hypotheses about ageing.

2. Rationale for adopting high data-intensity, hypothesis-generating methods

Biomedicine, as with all areas of biology, has undergone a rapid change in the last 15 years through the development of technologically advanced methods providing large datasets. We can now question gene expression patterns simultaneously, the profile of regulatory microRNA expression, mRNA and the study of protein forms, their frequency and location or interactions at a particular moment in time, offering unprecedented insight into many aspects of human biology. Developing large scale datasets, e.g. of biological ageing in the case of MARK-AGE, provides opportunities for discovering hitherto unrecognised biomarkers and can also offer increased understanding of the ageing process, generating new hypotheses *in silico*. These biomarkers can then be tested through the associated development of high throughput screening methods such as qPCR, ELISA or flow cytometry for proteins. Here, we describe the approaches, challenges and validation of methods that have been applied in the discovery of novel putative biomarkers of ageing. The data arising from the discovery process has been introduced into the larger MARK-AGE dataset for statistical evaluation and will be published elsewhere. All tissues are used here in biomarker discovery (T cells, circulating endothelial cells, plasma, peripheral blood mononuclear cells) are available from a normally collected human blood sample. It is not anticipated that any new biomarkers of healthy ageing which are discovered would be consistent between different tissues and indeed the differences may provide further insight into healthy ageing.

3. Data-intensive methods in ageing biomarker discovery

The sensitivity of mRNA and miR analysis afforded by their relatively simple amplification together with the immense capability for data mining, has contributed to their popularity in biomarker discovery. Microarrays are typically used to identify new targets in a low or medium throughput scale. After identification of specific mRNA or miRNAs as biomarkers, real-time PCR is used for validation and high throughput application. Microarray platforms enable the comparison of thousands of genes expressed in a cell at any given time which are simultaneously monitored; they have been applied to study the effects of development and ageing on gene expression. However, the variation in gene expression changes with age, probably due to declining transcriptional efficiency during ageing remains a problem (de Magalhaes et al., 2009).

Microarray has been used to study healthy ageing-related changes in gene expression in a several studies since the concept of MARK-AGE was developed (ElSharawy et al., 2012; Nakamura et al., 2012; Lazuardi et al., 2009; Uehara et al., 2006). These studies have largely been based on cross-sectional cohorts. Varying approaches have been adopted previously for the use of microarray in biomarker discovery including a pooling approach, where samples from patients are pooled and compared to controls. This offers advantages in terms of normalizing outliers but correspondingly restricts the data that can be generated from the system (Rudolf et al., 2013). The comparative nature of the analyses also presents a problem when samples are blinded as was the case during MARK-AGE; to overcome this we adopted two different approaches. First, single-blinded analysis was undertaken after the sample codes were broken by the biobank, so enabling leukocytes to be pooled for miR analysis from multiple donors within a given decile of years of age. Second, a subset of donors was recruited to serve as a source of larger cell numbers of known ages for comparative profiling at rest and under hyperoxic stress which is known to induce senescence. This approach allows us to follow longitudinal changes in CD4+ T cells that have been exposed to controlled "ageing".

A range of proteomic approaches are available to investigate the expressed proteome. These include one-dimensional and two-dimensional polyacrylamide gel electrophoresis with options for specific labelling using 2D-DIGE to enable simultaneous sample separation; gel-free high approaches are also adopted such as isotope-coded affinity tag ICAT; SILAC; isobaric tagging for relative and absolute quantitation (iTRAQ); and shotgun proteomics which is proving increasingly popular in recent times owing to the high resolving power for complex samples through innovations in mass spectrometry hardware and methods (Nikolov et al., 2012). MARK-AGE began in 2008 and use of 2DE with LC–MS was commonplace at that time and so was adopted here. The article by Capri et al. (Capri et al., 2015) in this edition, has reviewed the few proteomic ageing biomarker studies to date. These include a differential plasma protein pattern in subjects that associates with amyloid deposition in the brain; a differential expression of ApoE and antioxidant proteins was observed in the plasma of 10 japanese supercentenarians; and our own work has shown age-related changes to the transferrin glycoform (Dunston et al., 2012; Thambisetty et al., 2010; Miura et al., 2011).

Two different proteomic strategies were explored as sources of putative ageing biomarkers in the MARK-AGE project; (1) comparing the proteomes of a small healthy population of younger and older adults, which was carefully controlled for health status; (2) exploring endothelial markers identified during PROTEOMAGE by fishing with antibodies. The workflow is illustrated in Fig. 1 and the approaches used are described in detail in Section 3 below.