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Extracellular vesicles and extracellular RNA in aging and age-related disease

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Abstract

Circulating factors are well known to influence aging and age-related disease. As part of the Aging Science Talks: Science for the Community series, data was presented on two types of circulating functional biomarkers: extracellular RNA (exRNA) and extracellular vesicles (EVs). EVs in the context of type 2 diabetes mellitus was also discussed, as this is an area of interest due to the growing global epidemic of this age-related disease.

Keywords

EV; exRNA; microRNA; Exosome; Microvesicle; Microparticle; Type 2 diabetes mellitus; Biomarker

1. Extracellular RNA changes with human age

Aging is inevitable, but how we age is influenced by numerous biological, environmental, epigenetic and genetic factors. It is well documented that individuals age at different rates and that certain demographic groups age more rapidly than others.

Research in our laboratory aims to identify circulating biomarkers that indicate an individual's chronological versus biological age, which may give clues as to why individuals age at different rates. In a sub-cohort of young (~30 yrs) and old (~64 yrs) participants in the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study [1], we reported that human aging decreased the levels of microRNAs (miRNAs) in peripheral blood mononuclear cells (PBMCs) and in biofluids such as serum [1–3]. miRNAs are a family of small noncoding RNAs that post-transcriptionally regulate gene expression by binding to target genes. Circulating miRNAs are remarkably stable and have been the focus of intense research since they regulate a plethora of gene targets, can easily be isolated from serum and are altered with many different disease processes and during aging [4, 5]. The levels of many circulating miRNAs declined with age. Several of these miRNAs were found to reduce the expression of inflammatory markers, indicating that lower levels of these

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Declaration of competing interest

None.

circulating miRNAs may exacerbate inflammation in the elderly [3]. miRNAs may play important roles in driving aging and also be regulated as part of the aging process as suggested by data from *Caenorhabditis elegans* and cells undergoing cellular senescence [6]. Future studies employing mammalian models will aid in defining the roles of miRNAs in aging.

In addition to examining circulating miRNAs with human age, we developed a sequencing pipeline to identify both small and long RNAs in one sequencing reaction with the goal to establish a catalog of extracellular RNA (exRNA) in human aging [7]. As many previous studies have focused on exRNA profiles with a specific disease process, we believe it is important to identify age-dependent differences, which may help guide the development of references for investigating age-related disease. RNA is classified into categories or biotypes by the Ensembl database. The distribution of most RNA biotypes was similar between young and old, highlighting the validity of our sequencing approach and a relative consistency amid exRNA profiles. However, biotypes including mitochondrial tRNAs (Mt_tRNA), mitochondrial ribosomal RNAs (Mt_rRNA), and RNA transcribed from unprocessed pseudogenes were significantly higher in older individuals (Figure 1).

Pathway analysis revealed that exRNAs from older individuals included more mitochondrial RNAs and more RNAs implicated in chromatin remodeling and in the response to oxidative stress. This finding is consistent with data in the literature showing that mitochondrial dysfunction is a hallmark of aging [8]. Further validation of age-specific changes in a larger cohort was performed, highlighting changes in snoRNAs, miRNAs and circular RNAs (circRNAs).

2. Extracellular vesicles (EVs) as functional biomarkers of aging

exRNAs are thought to be stable in the circulation through their interaction with RNA-binding proteins, high-density lipoproteins and extracellular vesicles (EVs) [4,9]. EVs is a general term encompassing vesicles derived from different mechanisms, including exosomes, microvesicles and apoptotic bodies [10,11]. Recent interest in these nanosized vesicles has been spurred by the potential diagnostic and therapeutic use of EVs in age-related diseases [11,12]. However, for EVs to be utilized as biomarkers it is imperative to characterize them in human populations and to decipher if there are age-dependent differences in EVs.

To address this need, we analyzed circulating EVs in a cross-sectional and longitudinal study in humans with age [13]. A sub-cohort of African Americans and whites who were young (30–35 years; n = 30), middle-aged (40–55 years; n = 30), and old (55–64 years; n = 14) were chosen from the HANDLS study. These participants contributed plasma samples at two different time points approximately 5 years apart. We found a significant decline in EV concentration with age (Fig. 1). EV concentration is a balance of both the cellular release and internalization of EVs. To test whether the decrease in EV concentration with age is due to an increase in internalization, we developed a FACS-based assay where fluorescent-labeled EVs were incubated with freshly isolated human PBMCs, since lymphocytes and monocytes are known to interact with EVs in the circulation [14]. Among them, we found

that EVs were internalized primarily by monocytes and moderately by B cells, but not by T cells. Interestingly, we found that B cells internalized more actively EVs from older individuals than EVs from younger individuals, whereas EV donor age did not affect internalization by monocytes [13]. EVs from older individuals activated monocytes as measured through increased cell surface expression of MHC-II on monocytes. The age of the EV donor did not alter B cell activation. These data indicate an age-dependent decline in EV concentration, which may in part be due to an increase in EV internalization with advancing age (Fig. 1).

EVs contain molecular bioactive cargo that can be delivered to recipient cells. To examine whether EV proteins are altered with human age, we analyzed the protein content of EVs in our cohort. We found that the levels of several apoptotic proteins decreased, and cancer-associated proteins increased in EVs with age.

We further examined the proteomic profile of EVs in each study participant over time. There was a strong degree of similarity in the EV protein content between visits, as the levels of 39 out of 46 proteins were significantly correlated between visit 1 and visit 2, which were ~5 years apart. Although there was a significant decrease in EV concentration between visit 1 and visit 2, each individual's EV concentration was highly correlated between visits. These data suggest that for each individual their own EV concentration and protein profile is consistent over time, which is important to note since only a few studies have examined EVs from the same person over a longer time interval. This finding also highlights the fact that if EV cargo or concentration are to be utilized as biomarkers of disease progression, it may be important to establish an EV baseline reference value to accurately monitor disease or treatment progression.

3. Type 2 diabetes mellitus and extracellular vesicles

Recent data suggest that EVs may play an important role in type 2 diabetes mellitus (T2DM) [15], a chronic metabolic disease that rises in incidence and prevalence with age and is a risk factor for age-associated co-morbidities [16,17]. T2DM is a major health problem worldwide, as over 400 million people have this disease [16,17] and are at particular risk for cardiovascular disease and damage to nerves, kidneys, and eyes among other complications. We thus sought to study how EVs might contribute to T2DM and its complications.

In cross-sectional cohort studies, we found that individuals with diabetes had elevated circulating EV levels compared to euglycemic controls (Fig. 2) [18]. Furthermore in a longitudinal cohort, individuals that developed diabetes over a 5-year time period on average had a higher concentration of circulating EVs than euglycemic controls [18]. Using cell-specific surface markers, we found that EVs derived from erythrocytes ($CD235a^+$) were significantly more abundant in the circulation of diabetic individuals. In humans, homeostatic model assessment (HOMA) quantitatively measures insulin resistance (HOMA-IR) and β-cell resistance (HOMA-B) and this mathematical model can provide another measurement of diabetes severity [19]. HOMA-IR and HOMA-B were significantly positively associated with EV concentration and negatively associated with several insulin signaling proteins in EVs. To test whether insulin resistance leads to higher EV

concentration, we established a model of insulin resistance *in vitro* using primary cortical neurons. Insulin resistance significantly increased EV concentration [18]. Therefore, these data suggest that higher circulating levels of EVs in individuals with diabetes may in part be due to insulin resistance driving EV secretion.

We utilized our FACS-based EV internalization assay to assess whether PBMCs differentially internalize EVs from euglycemic or diabetic individuals. Both B cells and monocytes internalized EVs more readily from individuals with diabetes compared to EVs from euglycemics (Fig. 2) [18]. Strikingly, gene expression pathways including those related to oxidative stress, apoptosis and immune function were altered in monocytes treated with EVs from diabetic individuals. In addition, these monocytes secreted inflammatory markers in EVs and in the soluble fraction (Fig. 2). These data suggest that EVs from individuals with diabetes induce inflammatory pathways and alter gene expression in monocytes.

4. Conclusions

We have explored circulating markers of aging and an important age-related disease, type 2 diabetes mellitus. Our discovery of differences in exRNAs and EVs contributes to establishing a baseline reference for how these circulating molecules change with human age. As risk for most chronic diseases increases with age, it is important to consider aging when examining these factors for a specific disease. We propose that the circulating biomarkers we have begun to catalog in normal aging will help identify individuals with faster rates of biological aging that may result in diminished health span and life span. Further characterization of exRNAs and EVs in diverse cohorts will expand the value of circulating biomarkers as diagnostic and prognostic markers of disease.

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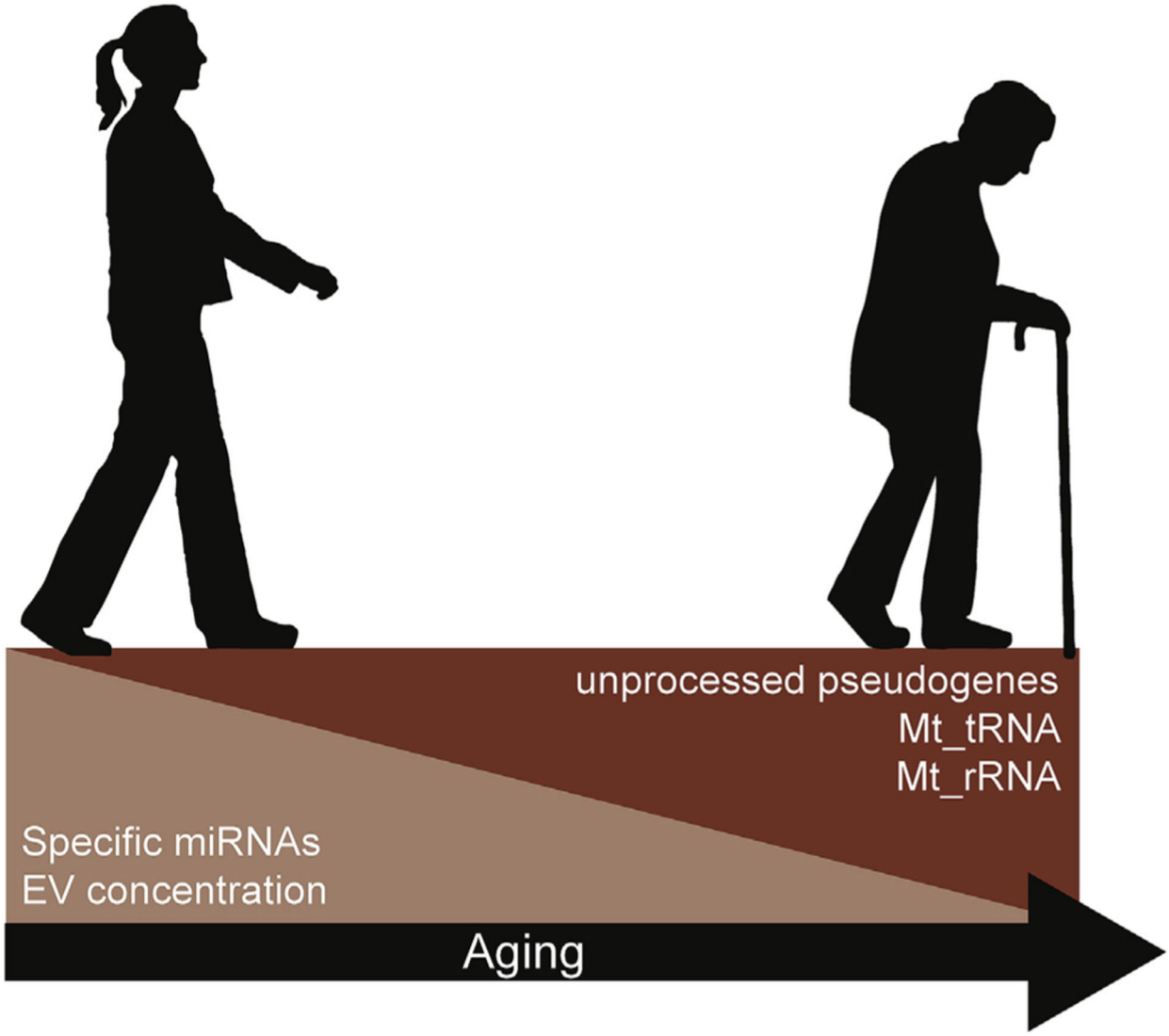

Fig. 1. Age-related changes in circulating exRNA and EVs.

With advancing age, the levels of specific circulating miRNAs and circulating EVs decline (represented by the light orange), while the levels of unprocessed pseudogene RNAs, mitochondrial tRNAs (mt-tRNAs), and mitochondrial ribosomal RNAs (mt-rRNAs) increase (represented with the dark orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

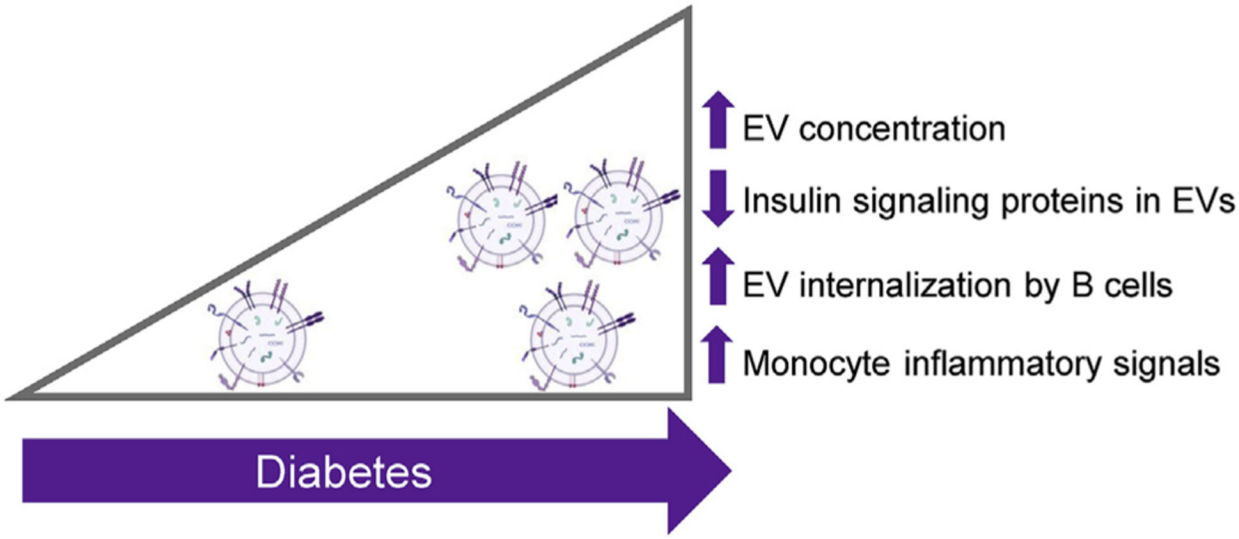

Fig. 2. Changes in extracellular vesicles in type 2 diabetes mellitus.

Higher extracellular vesicle (EV) concentration was observed in individuals with diabetes mellitus compared to euglycemics. This is accompanied by decreased levels of insulin signaling proteins in EVs. EVs from diabetics are more readily internalized by B cells and induce inflammatory signals in monocytes.