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Cellular and Functional Mechanisms Underlying Muscle Aging and Associated Diseases

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Abstract

The loss of skeletal muscle mass and function during the aging process (sarcopenia) has a major impact on muscle function and is a key component of frailty. A clear understanding of the mechanisms of sarcopenia through the identification of selective biomarkers, and thus of potential therapeutic targets, is of paramount importance in ensuring quality of life in old age. This presentation will provide an overview of the studies associating immunohistology and omics investigations during sarcopenia and associated pathologies in humans.

Introduction

In the developed world, life expectancy increases at a rate of two years per decade. However, health span is not keeping pace with increasing life span. For example, in a 20-year period (from 1990 to 2010), male life expectancy increased by 4.2 years in the U.S., but healthy life expectancy lagged behind at 2.7 years.¹ Understanding the factors influencing health in old age and developing and validating interventions to combat the negative aspects of aging is therefore a major issue.

Aging affects most tissues and many physiological functions. However, one of the most dramatic effects of increasing age is the atrophy of skeletal muscle, referred to as sarcopenia, which is predictive of all-cause mortality in the elderly.² Sarcopenia is a universal, age-related loss of muscle mass associated with a loss of strength and function resulting

Glossary of Abbreviations

ECM: Extracellular Matrix

MALDI: Matrix-Associated Laser Desorption/Ionization

MSMI: Mass Spectrometry Imaging

in muscle weakness. Sarcopenia is a prevalent condition, as it can be detected in 13 to 24% of adults over 60 years of age and in 50% in individuals older than 80.³ Estimates of the rate of muscle loss are 1 to 2% per year after the age of 50 years,⁴ and sarcopenia can result in a loss of about 30 to 50%

of the muscle mass by the age of 80 years.⁵ Interindividual differences in the prevalence of sarcopenia depend not only on genetic factors but also on food habits, activity patterns and general lifestyle.

Healthy skeletal muscles are central not only for coordinated movements and postural control but also for general well-being. Hence, age-related loss in skeletal muscle contractile strength increases the risk of impaired mobility, poor balance, falls, and loss of autonomy. Skeletal muscle, which is the most abundant tissue in the adult body, also plays a central role as a reserve for energy and amino acids and is a major site of fatty-acid oxidation, carbohydrate metabolism and maintenance of heat homeostasis.⁶ Hence, age-related loss of muscle mass also triggers severe metabolic side effects, including metabolic syndrome and frailty in the elderly.

Metabolic syndrome is a cluster of interrelated risk factors for cardiovascular diseases and type-2 diabetes. Metabolic syndrome occurrence strongly increases with aging, and among its components, hypertension is the most prevalent.⁷ When associated with weight loss, poor physical reserves, weakness, reduced balance, and physical inactivity, sarco-

penia can further result in frailty,⁸ which is accompanied by loss of independence, institutionalization and increased mortality. A clear understanding of the mechanisms of muscle aging through the identification of selective biomarkers, and thus of potential therapeutic targets, is of paramount importance in ensuring quality of life in old age.

Numerous theories have been proposed to explain sarcopenia. Obviously, muscle aging is a multifactorial phenomenon that implicates intrinsic factors such as perturbations in the endocrine system (somatopause, menopause, andropause, adrenopause), an increase in proinflammatory cytokines (IL6, TNF α) with attendant chronic inflammation (referred to as inflamm-aging),⁹ motor units denervation/reinnervation,¹⁰ decreased muscle regeneration capacity,^{11,12} and increased mitochondrial reactive oxygen species produced during energy metabolism,⁶ Undoubtedly, extrinsic factors such as diet and exercise, and probably other unknown mechanisms,¹³ further play important roles.

To better understand the mechanisms of aging of the human skeletal muscle, we have undertaken top-down differential proteomic approaches and combined immunohistology, proteomics, transcriptomics, and mass spectrometry molecular imaging (MSI) to investigate “healthy” aging and two common age-related pathologies: metabolic syndrome and hypertension. Using these methodologies, we identified fiber-type specific alterations and several potential biomarkers of aging.

Muscle Fiber Morphometry and Chronological Aging in Men

Human skeletal muscles are of mixed fiber-type composition, as they comprise slow-oxidative (type-I) fibers, fast-oxidative glycolytic (type-IIA) fibers, fast-glycolytic (type-IIX) fibers, together with hybrid fibers.¹⁴ Aging at the cellular level involves decline in both number (hypoplasia)¹⁵ and size (cross-sectional atrophy) of muscle contractile cells (also named (myo)fibers). Immunohistochemical studies not only revealed the importance of aging but also age-related pathologies, such as metabolic-syndrome, for fiber-type specific characteristics.¹⁶ Notably, metabolic syndrome is sufficient to strongly modify the characteristics (size, mitochondrial oxidative activity, lipid droplets) of muscle fibers.

Atrophy of type-II fibers is one of the most consistent observation for chronological aging.^{16,17} Altered fiber shape and/or fiber-type grouping represent the first signs of fiber disuse, cell death, denervation^{18,19} or reorganization of motor units in the old skeletal muscle.^{20, 21} Another critical morphological alteration is centralization of myofiber nuclei (myonuclei).²² Centralized myonuclei are recognized markers of regenerating fibers,²³ and in the old muscle, centralized myonuclei also could result from fiber denervation and branching,²⁴ or alterations in the microtubule network.²⁵

Intramyocellular Lipid Droplets and Chronological Aging in Men

Skeletal muscle is a major site of fatty-acid oxidation and insulin-mediated glucose disposal, and dysregulations of lipid metabolism with accumulation or delocalization of intramyocellular lipid droplets occur in the old muscle.^{16,26,27} Metabolic syndrome further alters intramyocellular lipid content and composition, and this occurs at a fiber-type specific level. Chronological aging and particularly metabolic syndrome are thus associated with an accumulation of intramyocellular lipid droplets, especially in type-I fibers. Matrix-assisted laser desorption/ionization (MALDI)-MSI was developed to characterize intramyocellular lipid profiles at the fiber-type level. Ionic maps of lipids highlighted several m/z distinctions among young men, healthy old men and old men with metabolic syndrome, which indicated that chronological aging and metabolic syndrome are associated with altered lipid composition in the human skeletal muscle.¹⁶

Extracellular Matrix and Chronological Aging in Men

The extracellular matrix (ECM) embedding contractile fibers is critical to maintain muscle structures and for the transfer of force from the muscle fiber out to the tendon and subsequent bone.^{28,29} ECM also provides an environment in which the contractile fibers can function.^{28,29} ECM further contains different types of stromal cells, such as fibroblasts, immune cells, adipocytes, and capillaries, which reciprocally are involved in the regulation of myofiber metabolism and of muscle stem (satellite) cells.^{22,30}

In men we showed that healthy aging is associated with more perimysium.³¹ The perimysium (surrounding bundles of myofibers) coordinates shape change during muscle contraction,³² and more perimysium might be important to preserve muscle shape despite age-related fiber atrophy. Importantly, we also demonstrated that hypertension in old men is associated with increased endomysium (surrounding each myofiber). A greater endomysium area in hypertensive elderly subjects could contribute to alter ECM hydration and interstitial fluid pressure, which might be harmful for transcapillary exchange.³¹

Microvascularization and Chronological Aging in Men

We further investigated microvascularization because it is representative of the potential for exchange of respiratory gases, fuel and metabolites, and is thereby an important determinant of muscle functionality. Although few differences were observed in lean healthy men during chronological aging, the microvascularization was significantly altered in old men with metabolic syndrome. We identified

hypertension (a major component of metabolic syndrome) as central for this regulation of microvascularization in the old muscle.³¹ Specifically, we reported less capillary surrounding types I and II fibers, smaller length of contact between capillaries and each fiber, and reduced tortuosity of capillaries in skeletal muscle of elderly men with hypertension or metabolic syndrome. Such structural changes, together with functional changes in capillary hemodynamics,³³ could have detrimental effects on oxygen/metabolites diffusive capacity, and thereby contribute to alter muscle functionality.

Omics of Chronological Aging in Men

The overall functional, structural and biochemical alterations in muscle have been studied for chronological aging, but the detailed molecular mechanisms implicated remain to be specified. At the molecular level, the differential expression profiles of mRNAs (transcriptomes) were previously described in rodents.³⁴ In humans, whole-genome expression profiling^{35,36} together with meta-analysis of microarray experiments³⁷ have been used to identify genes that change expression with chronological age in the skeletal muscle. The differential expression profiles of mRNAs constitute a first essential level of information, but analyses of the expression profile of proteins (the proteome) in aging also are required to understand the molecular mechanisms important for the muscle aging process.

In fact, unlike the genome, the proteome varies in response to many physiological or pathological factors. In addition, the proteome orders a magnitude more complex than the transcriptome due to post-translational modifications, protein oxidation or limited protein degradation.⁶ Several proteomic studies, including ours, have been conducted in rodent muscle, and profiling of chronological aging has demonstrated substantial alterations in muscle proteins involved in key metabolic pathways, myofibrillar remodelling, cytoskeleton organization and mechanisms of cytoprotection and cytodetoxification.^{38,39} However, few proteomic studies have been conducted about the chronological aging of the human muscle,^{40,41} and the results were contradictory.

Differential 2D-proteomic and transcriptomic approaches were thus performed to characterize young versus healthy old men (manuscript in preparation). These investigations identified 42 proteins and 484 ARNm's as potential biomarkers. In women we assessed 2,285 spots/proteins and identified 98 potential markers for chronological aging of the skeletal muscle.⁴²⁻⁴⁴ Most of the candidates partly accounted for the immunohistochemical and physiological modifications that we found associated with chronological aging and/or metabolic syndrome in men. Thus, chronological aging was associated with a decrease in glycolytic metabolism, a fast-to-slow transition and an upregulation of several proteins

involved in cytoprotection/cytodetoxification and membrane repair. In elderly men, metabolic syndrome was linked with perturbations of lipid metabolism and increases in components important for proteostasis.

Conclusions

With the continuous increase in the average life expectancy, the real societal challenge is to bridge the gap between life expectancy per se and healthy life expectancy. Sarcopenia is a main component of this burden, since it triggers frailty and is responsible for a decreased or loss in mobility and independence. Important information was obtained about fiber types morphology, oxidative metabolism, lipid droplets, apoptosis, microvascularization, satellite cells, and fibrosis of the extracellular matrix. Together with state-of-the-art transcriptomic and proteomic analyses, these data are required to improve our understanding of the factors influencing sarcopenia. Potentially they might both help identify new regulatory pathways and provide potential therapeutical targets.

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