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Modifications in the myogenic program induced by in vivo and in vitro aging

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Abstract

In this study, we have used high density cDNA arrays to assess age-related changes in gene expression in the myogenic program of human satellite cells and to elucidate modifications in differentiation capacity that could occur throughout in vitro cellular aging. We have screened a collection of 2016 clones from a human skeletal muscle 3′-end cDNA library in order to investigate variations in the myogenic program of myotubes formed by the differentiation of myoblasts of individuals with different ages (5 days old, 52 years old and 79 years old) and induced to differentiate at different stages of their lifespan (early proliferation, presenescence and senescence). Although our analysis has not been able to underline specific changes in the expression of genes encoding proteins involved in muscle structure and/or function, we have demonstrated an age-related induction of genes involved in stress response and a down-regulation of genes involved both in mitochondrial electron transport/ATP synthase and in glycolysis/TCA cycle. From this global approach of post-mitotic cell aging, we have identified 2 potential new markers of presenescence for human myotubes, both strongly linked to carbohydrate metabolism, which could be useful in developing therapeutic strategies.

Keywords: Transcriptome; Skeletal muscle; Macroarrays

1. Introduction

Skeletal muscle is a post-mitotic tissue composed of multinucleated myofibres, elements that arise from the fusion of mononucleated myoblasts during development (Hawke and Garry, 2001). In the adult muscle, there is a population of mitotically quiescent cells, called satellite cells, sequestered between the basal lamina and the plasma membrane of the myofibres (Mauro, 1961). Following damage to the muscle fibres, these cells become rapidly activated, proliferate and then fuse either together to form new myofibres or to repair damage segments of existing muscle fibres. During regeneration some of these cells escape differentiation and restore the pool of quiescent satellite cells under of the basal lamina of the newly formed muscle fibres (Schmalbruch, 1991; Hawke and Garry, 2001).

Abbreviations: ALP, alpha-actinin-2-associated LIM protein; BLAST, basic local alignment search tool; BrdU, 5-bromo-2-deoxyuridine; cDNA, complementary DNA; COX, cytochrome oxidase; DNA, desoxyribonucleic acid; E, early proliferation state; EAP 30, EAP30 subunit of ELL complex; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine phosphoribosyltransferase; IGFBP4, insulin like-growth factor binding protein 4; mRNA, messenger RNA; MPD, mean population doubling; PCR, polymerase chain reaction; PS, presenescence; RNA, ribonucleic acid; S, senescence; SD, standard deviation; SDS, sodium dodecyl sulfate; SSC, sodium citrate saline; UV, ultraviolet; ZASP, Z-band alternatively spliced PDZ motif-containing protein.

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Human aging is characterized by a decline in muscle mass associated with a decreased muscle strength and an increased fatigability, commonly named sarcopenia (Doherty, 2003). These processes are the result of many molecular and cellular changes including progressive denervation (Luff, 1998), a loss of motor units (Larson et al., 1987), a reduction in the speed of contraction (Bilodeau et al., 2001), increased oxidative stress (Squier, 2001) and an altered equilibrium of growth factors and hormones involved in the maintenance of muscle function (Ferrannini et al., 1996). Skeletal muscle atrophy is associated with both a decrease in the cross-section area as well as a reduction in the number of fibres within the muscle (Lexell, 1995; Porter et al., 1995; Frontera et al., 2000). During aging, the number of satellite cells available for regeneration and maintenance of muscle mass also decreases in human skeletal muscle suggesting that regeneration may be compromised in older individuals (Renault et al., 2002).

During human aging, gene expression analysis of whole tissues is complex in particular due to the large inter-individual variability which may be related to either disease factors or different life styles. In addition, adult skeletal muscles exhibit a complex anatomic organization uniquely adapted to the physiological demands of each muscle (LaFramboise et al., 2003). Muscle regenerative capacity is influenced by autocrine growth factors intrinsic to the muscle itself, as well as extrinsic host factors including hormones and growth factors secreted in a paracrine manner by cells of immune, innervation and vascularisation systems (Hawke and Garry, 2001). To overcome the in vivo complexity of this tissue, we have investigated the intrinsic myogenic differentiation program using satellite cells in culture as a simple model to mimic myogenic differentiation. These cells can be isolated from skeletal muscle biopsies, cultured in vitro as myoblasts and be induced to differentiate into plurinucleated cells named myotubes, mimicking the first step of muscular regeneration (Decary et al., 1997). In addition, their proliferative lifespan is limited and decreases with increasing donor age (Renault et al., 2000). The use of myotubes derived from satellite cells of different aged donors at different stages of their proliferative lifespan provides us a good tool to study not only the age-related intrinsic myogenic program but also modifications in differentiation capacity that could occur throughout in vitro cellular aging.

In a recent study, we have used this biological model together with cDNA array technology to investigate the molecular mechanisms implicated in muscle aging using isolated human satellite cells (Bortoli et al., 2003). Gene expression profiling technology allows the analysis in parallel of the expression levels of thousands of genes and may lead to a better understanding of the molecular basis of age-related phenomena. We have shown that expression profiles of myoblasts vary with donor age, and depend upon the position of the cells within their proliferative lifespan. Even though some of the modifications are similar to those observed in other cell types, we have observed that many changes in gene expression are characteristic of the myoblasts, confirming the hypothesis that the program of replicative senescence is specific for each cell type (Bortoli et al., 2003).

In the present study, we have screened a collection of 2016 cDNA clones from a skeletal muscle library in order to evaluate variations in the genetic program of myotubes formed by the differentiation of myoblasts isolated from skeletal muscles of 5-day-old, 52-year-old and 79-year-old individuals at different stages of their lifespan.

2. Materials and methods

2.1. cDNA array

A collection of 2016 clones has been assembled from a sequenced human skeletal muscle 3′-end cDNA library (Lanfranchi et al., 1996). Information available for each clone were their 5′- and/or 3′-end sequences and a chromosomal assignment or more precise localization of the corresponding gene on radiation hybrid panels. The average size of the inserts was estimated to be between 0.3 and 0.6 kb.

Control DNA probes have been added at least in duplicate, including positive controls (GAPDH, actin, tubulin, c-jun, c-myc, HPRT) and negative controls (2 genes from *Arabidopsis thaliana*, plasmid DNA).

2.2. Sources of human skeletal muscle

Biopsies of skeletal muscle were obtained during surgery, in accordance with the French legislation on ethical rules. Normal muscle biopsies were obtained from a 52-year-old man and a 79-year-old woman during orthopedic surgery. The sample from a 5-day-old female infant was obtained upon autopsy. Histological analysis was performed indicating an absence of any obvious sign of neuromuscular disease.

2.3. Explant cultures

Satellite cell populations were isolated from each muscle biopsy by the explant culture method, as described previously (Decary et al., 1997). All cell populations were considered to be at 1 mean population doubling (MPD) at the time of cell isolation.

2.4. Cell culture conditions

Cell culture, plating conditions and myogenic purity were performed as previously described (Bortoli et al., 2003). All of the primary cultures of human cells made in our laboratory contain on the average 75–95% desmin positive cells. The myoblast population isolated from the