Supplemental data

 # methods

Study design

Each of the 8 biological samples was compared to a common reference sample consisting of a pool of equal quantities of mRNA from 7 other biological samples. This complex mRNA pool was used as a standard or a common point of measurement that enabled a comparison between the individual mRNA samples.

Control of technical and biological noises inherent to microarray experiments was incorporated in the study design. To account for the technical fluctuation of the expression measurements, eight technical replicate values were obtained for each muscle sample. To determine the fluctuation due to tissue sampling, two biological replicate samples were obtained from each biopsy.

"Myochips": Microarray production

The 3588 genes represented on the microarray were selected for involvement in skeletal muscle. Selection was based on (i) subtractive hybridization experiments using a rat model of muscular atrophy and *mdx* mice [1,2], (ii) genome-wide microarray hybridizations in two muscular pathologies: Duchenne Muscular Dystrophy and Myotonic Dystrophy type 1 (unpublished data). Other genes involved in muscular disease were added after literature database searches. The supplemental table 3 shows the 3588 genes spotted on the Myochips. Microarrays were prepared in-house using 50-mer oligonucleotides probes (MWG Biotech). The probes were spotted onto epoxy-silane coated glass slides using the Lucidea Array Spotter (AMERSHAM, Uppsala, Sweden). Each gene was spotted in quadruplicate and therefore spatially distributed on the slide. For more information see: http://cardioserve.nantes.inserm.fr/ptf-puce/myochips_en.php

Target preparation

Normal and patient total RNA was purified using Trizol Reagent (Life technologies). A small aliquot of RNA was then used for quantification and quality control using the RNA 6000 labchip kit and Agilent Bioanalyser (Agilent Technologies, Palo Alto, United States). Poly (A)+ RNA was purified from total RNA using oligo-dt linked oligotex resin (QIAGEN). Cy3- and Cy5-labeled cDNA was prepared using the CyScribe cDNA post labeling Kit (Amersham Pharmacia Biotech). Samples from DMD and AGING patients were each labeled individually with Cy5. Reference patients were pooled and then labeled with Cy3.

Hybridization

Microarrays were prehybridized for 1 hour at 42° C (in 10% BSA, 20XSSC, 10% SDS). 0.5 µg of labeled sample cDNA was mixed with 0.5 µg of labeled reference cDNA in 40 µl of hybridization mix (50X denhardt, 20XSSC, 10 µg/µl PolyA RNA, 10 µg/µl Yeast tRNA, 10% SDS, 50% formamide) and hybridized to the microarrays. Microarray hybridization was carried out overnight at 42° C in a humidified chamber. Finally, slides were washed, dried by centrifugation and read by a scanner. Two independent hybridizations were performed for each DMD and AGING patient sample.

Image acquisition and data consolidation

Array scanning was carried out using a GSI lumonics LITE dual confocal laser scanner with ScanArray Microarray Analysis Software. Raw scanner images were analyzed with Genepix Software (AXON LABORATORY). Analysis of the expression level was performed using MADSCAN (MicroArray Data Suites of Computed Analysis) developed in our laboratory [3]. MADSCAN can be applied to one microarray or a batch of replicated microarrays and consists of:

- *Physical validation,* aiming to flag flawed spots and to extract information from borderline features in raw data matrices.

- *Normalization*, in order to minimize experimental systematic biases so that the observed variation arises from biological differences rather than from defects in the microarray technology. Invariant genes were selected using the rank invariant method [4]. A non-linear regression method (lowess fitness) was applied to the invariant genes to calculate the normalization correction, which was then used to normalize all spots on the array [5].

- *filtering* to remove genes of which all signal intensities were below the background level.

- *Scaling* to bring the internal variance within or between slides within the same range.

- *Outliers:* the filtered and normalized quantification data matrices must be compared to statistically validate the replicates and to remove outliers. An outlier is a suspect value in terms of its relative distance to the median value. We labeled outliers with the MAD test (Median absolute deviation test), which is a modified z-test where the median absolute deviation is used instead of the standard deviation in the z score calculation.

 # Inflammation and AGING

Acute inflammation is thought to play an important role in muscle repair [6] but there is a growing body of evidence favouring the fact that chronic inflammation may also be associated with the loss of muscle mass (sarcopenia) and muscle strength with aging [7]. In this situation, sarcopenia is though to be a smoldering inflammatory state driven by cytokines and oxidative stress [8].

In the aging skeletal muscle from rhesus monkeys [9], while down-regulated genes mainly involved proteins of the energy metabolism, up-regulated genes encoded proteins linked to an inflammatory/immune response along with stress response genes. The genes from the inflammation signature represented the largest class of transcripts that display a large (2-fold

or more) change in expression pattern with aging. Higher levels of pro-inflammatory cytokines (interleukin-6 (IL6), tumor necrosis factor-α (TNF)) and C-reactive protein (CRP) increase the risk of muscle strength loss [10] and are correlated with lower muscle mass in healthy older persons [11], even if the increase is moderate. Increased expression of IL6 has implicated cytokine-mediated events in reduced skeletal muscle function with age [12,13] although IL6 may also possess anti-inflammatory properties within this context [14].

Aging results in a rise in basal inflammation that is thought to contribute to sarcopenia by causing a decline of physical functioning through the catabolic effects of inflammatory markers on muscle [15,16]. Such stimulation of protein catabolism, probably through the increased activity of the Ubiquitin/Proteasome proteolytic pathway, has been shown for instance in rat were administration of IL6 or TNF causes muscle protein breakdown [17,18].

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