Minireview

Muscular expressions: profiling genes in complex tissues

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Abstract

Gene-expression profiling has yielded important information about simple systems, but complex tissues have not yet been widely profiled. Four recent studies of mammalian skeletal muscles have added to the catalogs of their gene expression differences, but have yet to lead to better understanding of the molecular processes underlying their physiological differences.

Skeletal muscle is a good complex tissue to study using expression profiling, because muscles are biochemically and functionally similar and yet have clear anatomical and physiological differences, the molecular bases for which are as yet mysterious. Major features that distinguish between muscles are their distinct metabolic and contractile properties, and during the past century many differences in protein and cognate mRNA levels were characterized that underlie these distinctions (reviewed in [1]). These studies provided reagents with which to dissect muscle development and function but, in general, other types of work were required to find underlying control mechanisms. A hope with expression profiling is that, by obtaining a complete mRNA picture, quantitatively minor components with important control functions may be elucidated and can then be analyzed. Alternatively, correlated changes in several mRNAs may highlight the involvement of a previously unsuspected regulatory 'system'. How far do the four recent reports [2-5] on gene-expression profiling on mammalian skeletal muscles take us?

In one analysis on mice [2], the traditional classification of muscles into 'red' and 'white', composed of slower- and faster-contracting fibers, respectively, was confirmed at the level of RNA profile. Around 20% of the approximately 6,000 genes on the Affymetrix Mu6500 oligonucleotide microarray were significantly expressed in the tissues examined, and around 12% of expressed genes (or about 150 genes in total) were found to be differentially expressed between the two tissue types. A number of these are genes already known to be differentially expressed between red and white muscle (such as myosin isoforms), but some (such as the gene for the homeodomain transcription factor LIM1) were not. In their discussion [2], the authors speculate on several expression-level differences that they find striking. Perhaps the most interesting is the observation that the calcium-activated protein phosphatase calcineurin is more highly expressed in white muscle, the tissue in which calcineurin is thought to be less active [6,7].

A second study [3] found about 36% of the around 10,000 arrayed murine genes on Affymetrix MG-U74A chip to be expressed in muscle, and then used stringent difference criteria to compare extraocular muscles (which attach the eyeball to its socket, permitting and controlling eye movement) with leg and jaw muscles. Extraocular muscle is known to be distinct from other muscle types by virtue of its function and the combinations of myosin motor proteins that are expressed in single fibers. Of the expressed genes, 2.6% (or 1% of the arrayed genes) differed between extraocular and both jaw and leg muscles. The authors couch their conclusions in terms of distinguishing muscle 'allotypes' [3], and the results clearly confirm that extraocular muscle is more different from the other two samples than the latter are from each other. Whether or not this validates the allotype concept is unclear. The authors go on to highlight differences they find interesting but, as in the other recent

studies, the significance for the muscle biologist is hard to assess at this stage. Further analysis of the data together with future studies will hopefully determine the functional importance of the differences observed.

The third paper [4] compares mRNA levels in the muscle of muscular dystrophy sufferers (people with Duchenne and limb-girdle muscular dystrophies) to levels in healthy muscle using the Affymetrix HuGene FL microarray. Numerically, the results are comparable to the previously mentioned two studies [2,3]: around 34% of genes are expressed and 10% of these (around 3% of arrayed genes) are differentially expressed between normal and diseased muscle. Encouragingly, changes in some genes are confirmed at the protein level in this study. For example, on the basis of upregulation of the expression of genes of the immune system, evidence is provided for the invasion of diseased muscle by dermal dendritic cells. Further results from this study have been reviewed elsewhere [8].

An earlier muscle-profiling article [5] characterized differences triggered in murine muscle by muscle aging and by an environmental change, caloric restriction, which, it was confirmed using Affymetrix oligonucleotide arrays, partially reverses the aging-related changes. As in the more recent studies [2-4], the importance of the data for increasing our understanding of developmentally, genetically or environmentally driven changes in muscle has yet to become clear. Nevertheless, all four studies will prove valuable to muscle researchers, simply by adding to the large catalog of genes known to be expressed and/or differentially expressed in muscle [9-11].

Beautiful studies have been described in yeast and mammalian cells that prove the potential of expression profiling (for example, see [12,13]). These have generally entailed analysis of the consequences of changing a single variable in a single cell type. But in applying the approach to a complex tissue such as muscle, new potential pitfalls arise. Firstly, tissue heterogeneity is a problem. The experimenters in all four of the reports discussed here employ methods to prevent sample-to-sample variability from yielding false positives. But just as northern blotting and reverse-transcriptase-coupled-PCR analyses of tissues generally need to be followed up with *in situ* mRNA hybridization to confirm and interpret the findings, so profiling a tissue requires knowing which cell type is responsible for which changes in profile. This issue may hamper the search for coordinated gene expression changes that are a 'signature' of particular control pathways. Perhaps, when this becomes technically feasible, expression changes in a single cell or in individual cell types isolated from the tissue should first be examined; then comparison with changes in the tissue as a whole would be more informative. A second issue that arises is that differences in gene expression could reflect either altered cell content within the tissue or distinct cell-cell interactions, or both. A general method to distinguish between these alternatives is needed. Thirdly, all the studies consider a twofold change in mRNA levels significant. This begs the question of how often a twofold change in mRNA level leads to a physiologically significant change in protein activity. The frequent lack of phenotype in mice heterozygous for null alleles raises the specter that a twofold change may have little biological effect. Methods are needed to sort out which changes in gene expression level are in fact important. Finally, once gene expression changes that robustly correlate with tissue differences have been discovered, how are we going to pursue their roles in causing tissue differences? In contrast to yeast, where generating specific mutants in genes of interest is quick, specific mutants and genetic crosses are less cost-effectively made in mice. With the advent of large-scale reverse-genetic screens this may change, but simpler, cheaper 'model' organisms may provide the best route for those wanting to get to mechanisms from tissue-level genomic and proteomic studies.

In summary, in the field of muscle biology, expression profiling has already provided valuable data sets, but the new research avenues the data suggest have yet to be productive. The four recent reports [2-5] fail to reach the current gold standard for what can be learnt from systematic profiling, set by, for example, Lee Hood and colleagues [12], who unveiled an entire control network for the raffinose metabolic pathway in yeast. Is it realistic to expect such detailed insights from the use of this approach in complex tissues? We believe so, if the experiments are carefully targeted and the analytical tools are developed at the pace and in the directions predicted [14]. It is frustrating to realize, however, when observing from beyond the fray and considering the costs involved in such experiments, that the analyses described here will probably need to be repeated when complete genome arrays are available. These reports are the first and necessary steps on a long road. An encouraging milepost to pass would be a spectacular mechanistic discovery (for example, a disease etiology) that derived directly from genes or systems whose involvement had been identified solely by profiling. We are not there yet, but as Lao Tsu said, a journey of a thousand miles starts with a single step.

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