Multifarious microarray-based gene expression patterns in response to exercise

MULTIPLE GENE EXPRESSION responses of leukocytes to physical exercise are well known. They are dependent on type, intensity, and duration of exercise; training status of athletes; and environmental conditions. Nevertheless, we are far away from having a complete list of changed genes and from the complete understanding of the regulatory mechanisms. Microarrays are widely used tools for the comprehensive analysis of gene expression and may be applied to investigate this issue in a systematic way. They enable the analysis of hundreds to thousands of genes simultaneously. Specific patterns of gene expression, so-called gene expression fingerprints, and/or new candidate genes in a certain situation can be found. Whole genome arrays may even facilitate the analysis of genes that were until that time not associated with exercise. These data may help to characterize and define the complex stress response to acute and chronic exercise on the molecular level.

In humans, the easiest accessible source to perform measurements of stress parameters on the cellular and molecular level is peripheral blood. The microarray analysis of leukocytes after exercise allows the genetic profiling of immune-competent cells in response to exercise to gain more insight into mechanisms through which exercise changes immune function. Moreover, changes of certain genes in leukocytes may serve as surrogate markers for systemic or local exercise-induced modifications (7) and will potentially obviate the need of muscle biopsies.

The study of Büttner et al. (1) in this issue of the Journal of Applied Physiology presents a very well-designed study using the microarray technology. They analyzed the gene expression of leukocytes in response to both moderate and exhaustive treadmill runs in the same individuals. Notable interindividual similarities specify characteristic and intensity-dependent gene expression fingerprints. Some hitherto unknown exercise-responsive genes were revealed. Furthermore, the study confirms some results of earlier reports.

There are only few previous studies that investigated leukocyte gene expression after physical exercise on a large scale using microarrays (2–6, 8, 9). They revealed several interesting candidate genes and component parts that might be important in the exercise response. Inflammatory and heat shock response genes were mainly affected [heat shock proteins (1, 2, 8), IL-1 receptor antagonist (2, 8), interferon-induced sequences (5), ubiquitin C (5), dual-specific phosphatase-1 (2, 5), inflammatory protein-1 (2), RANTES (regulated upon activation, normal T-cell expressed, and secreted) (2)] which indicates that exercise-induced hyperthermia and inflammation might account for at least some of the observed changes in leukocyte gene expression. However, consistently in all array studies, IL-6 does not appear to be one of them, in agreement with the hypothesis that mainly muscle cells produce circulating IL-6 during exercise. Furthermore, genes grouped to cellular communication [CD11c, CD81 (8)], signal transduction [mitogen-activated protein kinase activating protein kinase 2 (8)], cellular protection [thioredoxin (8)], tumor suppression [glutathione S-transferase M (8)], growth and repair [epiregulin (2), PDGF (2), hypoxia-inducible factor-1 (2, 8)], bronchoconstriction, and asthma [arachidonate 5-lipoxygenase (ALOX5); ALOX5-activating-protein (2)] were significantly affected. New findings in Büttner et al. (1) were regulations of matrix metalloproteinase-9, potassium channel-associated-proteins, S100P, YES-1 oncogene, and natural killer cell receptor CD160. For a number of the significant genes, they suggest a nice interaction model. These results have the potential to provide novel insights into the molecular mechanisms of exercise.

Despite these interesting results, there are some methodological influencing conditions (different microarray platforms, RNA and array preparation methods, sampling points, cell populations) that make the comparison of the real exercise-related responses difficult. Cross-platform comparisons identify only a small fraction of genes similarly affected by exercise. Matching Büttner et al. (1) (whole gene array) with Zicker et al. (8) (homemade cDNA array) revealed only seven similarly affected genes. A list of just 11 concordant genes was the result comparing Büttner with Hilberg et al. (4) (custom-made oligo array), and only three genes in Connolly et al. (2) (whole gene array) vs. Zicker et al. (8).

Using the same microarray platform makes comparisons easier. Connolly et al. (2) vs. Büttner et al. (1) results in more coincident expression changes (53 significant genes) despite different exercise protocols, subjects, sampling points, cell populations, and RNA preparation methods. It is interesting to note that only a rather limited number of genes of the 14,500 sequences on the whole gene chip were significantly altered by exercise. This indicates the existence of particular exercise-responsive genes. All concordant genes may be accounted for as exercise specific and used to design a special “exercise stress chip.” Customized arrays will allow one to quickly apply experimental results obtained with one big array to subsequent, specified experiments. Such an application-specific stress chip may be used to monitor the physiological and pathophysiological exercise response (8).

However, more interesting are the unequal expression profiles that may be associated with the different exercise protocols. One of the aims of microarray analysis is to differentiate samples according to their pattern of regulated genes. At this point, the procedure of Büttner et al. (1) becomes eminently important. Gene expression in response to two exercise protocols exclusively differing in maximal $\text{O}_2$ uptake (60 vs. 80 %) but otherwise identical conditions was investigated. This design specifically allows one to analyze the influence of exercise intensity. The magnitude of the changes of a pattern of genes could be related to the strength of exercise. Furthermore, the reproducibility of the technology was illustrated by comparable basal gene expression before both runs.

Despite differences in methodology and findings, the exercise-microarray studies demonstrate acute bouts of exercise produced time- and intensity-dependent patterns of gene expression that are easily detectable in circulating peripheral blood cells, which can be easily obtained. Significant new exercise-specific candidate genes may give hints to regulatory pathways that were activated by exercise stress. The analysis of
exercise-related gene expression profiles or surrogate marker genes in leukocytes may replace, e.g., muscle biopsies. Especially the study by Büttner et al. (1) verifies that microarray analysis of leukocytes is a valuable tool to monitor different training protocols.

Nevertheless, there are also some disadvantages of the microarray technology: analyzing whole leukocytes is an important limitation in that they represent a heterogeneous cell population. Measured changes of expression can result from either actual changes in transcript level within individual cells or from exercise-induced shifts of subpopulations (8).

Cut-off problems are produced by stringent statistics used to avoid false-positive results. Out of hundreds to thousands of genes only the most significant will become relevant. This problem becomes more obvious as the number of genes on the chip grows. Last, but not least, gene expression analysis is only one cornerstone in the exercise response. Additional analysis of corresponding proteins, functions, and pathways is necessary to yield further information about functional relevance in exercise.

Conclusively, microarray analysis is applicable to discriminate exercise intensity-dependent gene expression profiles in human leukocytes. Using a whole genome chip gives novel insights into the molecular mechanisms of exercise and defines a group of exercise-specific genes. A subsequently designed particular "exercise stress chip" will cover individual gene responses to exercise stress, minimizing cut-off problems and reducing costs. An exercise-related gene expression fingerprint may become helpful to characterize the immune response to different types of exercise and may mirror in part the whole body response. A better understanding of the pathways associated with normal responses to exercise will provide the basis for diagnosis and treatment of diseases such as overtraining, chronic fatigue syndrome (6), asthma (4), and exercise-induced immune suppression or for evaluating individual training regimes. Potentially, microarray analysis may also be helpful in doping analysis, which is actually tested in some research projects (http://www.wada-ama.org/).

In the future, microarray researchers in exercise physiology should identify compact problems for practice. Meta-analyses may assist to define clusters of informative genes to make specialized problem-directed chips. Performing longitudinal and multicenter studies with corresponding specialized chips may prove and improve microarrays as a valuable diagnostic tool. Adjusting protocols and compiling common databanks will make results more readily comparable. The organization of cooperations or workshops of interested research groups will help to achieve those goals.

REFERENCES


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