

Muscular Dystrophy Meets the Gene Chip: New Insights into Disease Pathogenesis

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The human genome project reached a major milestone earlier this year, with the completion of a rough draft of the sequence (Pennisi, 2000). An accessible catalog of all human genes could greatly accelerate the pace of breakthroughs in medical research by allowing global analyses of gene expression changes in a variety of developmental and pathological states. However, it has never been clear whether these types of analyses could be efficiently performed, or whether significant sets of important data would emerge from the study of large sets of genes. Many diseases arise from single gene mutations in which the corresponding protein products are known. Would knowledge of global gene expression changes add to our existing understanding of these monogenic disorders or lead to the development of treatments? A paper in this issue provides a test of these questions and suggests that large scale gene expression profiling can contribute greatly to an understanding of monogenic disease mechanisms, may simplify diagnosis of genetic diseases, and could eventually provide important clues to assist with the development of novel molecular therapies.

Chen et al. (2000) report a survey of gene expression changes in two types of muscular dystrophy whose primary gene defect was known (Chen et al., 2000). The results demonstrate both the power and value of this type of analysis while providing a number of interesting insights into the underlying disease pathologies. This work also serves as a model for the application of this approach to other monogenic disorders. Chen et al. have carefully considered approaches to overcoming many of the inherent difficulties in analyzing gene expression changes from diseased tissues, and provide a preliminary check of some of the more intriguing observations of altered mRNA levels by immunolocalization studies of the corresponding proteins. Together, these observations have confirmed some previously known features of muscular dystrophies, have uncovered several novel alterations that may contribute to disease pathology, and have revealed interesting differences between two similar forms of dystrophy that may be as important to understanding the disease pathogenesis as is knowledge of the primary defects that initiate muscle cell degeneration.

The focus of the work by Chen et al. (2000) was on two related diseases, Duchenne and limb-girdle muscular dystrophies (DMD and LGMD)¹. DMD is the most common form of the disease and arises from defects in the dystrophin gene (Monaco et al., 1986). Dystrophin is an enormous cytoskeletal protein that links the muscle cytoskeleton to the extracellular matrix (Koenig et al., 1988; Ervasti and Campbell, 1993). This linkage facilitates force transduction from the contractile apparatus to the connective tissue surrounding muscle fibers, stabilizing the sarcolemma and minimizing contraction induced injuries (Petrof et al., 1993; Lynch et al., 2000). Dystrophin works by interacting with a large number of integral and peripheral membrane proteins known as the dystrophin glycoprotein complex (DGC), which includes four members encoded by the sarcoglycan gene family (Ibraghimov-Beskrovnaya et al., 1992; Ozawa et al., 1995). Mutations in any of the sarcoglycan genes lead to different types of LGMD, which are distinct, yet similar diseases to DMD (Bönnemann et al., 1995; Bushby, 1999).

Most models for the function of the DGC imply that mutation of either dystrophin or one of the sarcoglycans should render the DGC nonfunctional and lead to essentially identical phenotypes, yet a surprising number of differences have been observed. Some groups have speculated that the sarcoglycans and other DGC members such as the syntrophin and dystrobrevin family members could play auxiliary roles in maintaining normal muscle function by regulating or localizing signal transduction molecules. These signaling pathways would presumably help muscle cells adapt to the myriad forms of stress imposed by muscle activity. However, despite extensive analysis in transgenic mice and induced knockout models for the sarcoglycans, the nature and importance of these signals remains obscure (Hack et al., 1998; Grady et al., 1999; Crawford et al., 2000).

Understanding the precise function of the DGC may facilitate development of an efficient therapy for the muscular dystrophies. Despite knowledge of the primary genetic defects, the mechanisms that lead to muscle cell dysfunction

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¹Abbreviations used in this paper: DGC, dystrophin glycoprotein complex; DMD, Duchenne muscular dystrophy; LGMD, limb-girdle muscular dystrophy.

tion and death in the muscular dystrophies are poorly understood. For example, how does the absence of dystrophin lead to instability of the sarcolemma? Why do different disruptions of the DGC lead to different manifestations of the dystrophic process? The overall phenotypic manifestations of these diseases presumably result from a combination of muscle fiber necrosis, incomplete regeneration, infiltration of damaged muscle with immune effector cells, perturbed metabolic capacity, reduced blood supply to exercising muscle, activation of apoptotic pathways, and many other unknown processes. Developing a complete understanding of these multiple pathological events could help guide new types of therapies, as well as provide reference points for assessing the effectiveness of therapeutic intervention. For these reasons, the global gene expression analysis of Chen et al. (2000) plays an especially important role, by providing the first overall picture of the gene expression state of dystrophic muscle tissue.

Chen et al. (2000) provide a significant amount of clarity to the overall pattern of gene expression in dystrophic muscle because of their elaborate and well controlled approach. A direct comparison between a dystrophic and a normal muscle sample is difficult to interpret due to phenotypic variations within an individual muscle biopsy, as well as patient-specific differences arising from genetic variability. In addition, comparison of samples only from DMD patients and normal controls might reveal both specific and nonspecific alterations, making it difficult to identify the most important differences. Chen et al. (2000) solved some of these dilemmas by analyzing pooled samples from multiple controls in parallel with pooled samples from multiple patients that had either DMD, or LGMD resulting from mutations in the alpha-sarcoglycan gene. To reduce the problems that might arise from variations within a given biopsy and provide independent confirmation of the data sets, each muscle specimen was divided in half before preparing pooled sets for parallel processing. Pooled probes made from the sample sets were analyzed for gene expression by hybridization to the Affymetrix HuGeneFL microarray that contains sequences from ~6,000 human genes. Sophisticated data analysis tools were then used to compare each result and identify trends. The authors focused on several types of changes. Initially, genes whose expression was consistently elevated or reduced in either type of dystrophy were identified. Next, genes whose expression was consistently altered only in one of the two types of dystrophy were identified. As a result, the authors derived a list of genes whose products were likely to be altered in any general dystrophic process and those that might contribute to specific features of a single form of muscular dystrophy. Finally, a select subset of the more interesting and accessible alterations was confirmed by immunologic analysis of muscle biopsies. While this enormous data set will require a great deal of further analysis, a variety of interesting and novel trends have already emerged.

An initial observation from the data sets is that many secondary features of the disease, which had been hinted at in earlier studies were confirmed and extended, such as a generalized metabolic crisis manifested by a significant reduction in the expression of many nuclear-encoded mitochondrial genes. Upregulated gene expression was also observed for many cell surface and extracellular proteins, perhaps reflecting the widespread fibrosis and mononu-

clear cell infiltrates seen in dystrophies. In addition, a number of calcium-regulated signaling molecules were downregulated, potentially providing new insights into pathways affected by the altered calcium homeostasis that results from ruptures in the sarcolemma membrane. Perhaps more importantly, a number of novel observations were apparent that should inspire increased efforts to examine the consequence of these changes on the dystrophies. A few examples of the many novel observations from the gene expression analysis include reduced levels of the protein kinase ERK6 and the protein phosphatase pTPH1 in DMD, but not in LGMD. The expression of numerous genes involved in cell growth and signaling was also upregulated. Intriguingly, several markers for activated dendritic cells were elevated in dystrophic samples, supporting a role for immune effector cells altering the microenvironment of dystrophic muscle.

One of the more striking observations made by Chen et al. (2000) is that dystrophic muscle may exist in a generalized state of incomplete differentiation. Widespread elevated levels of embryonic muscle isoforms for both myosin heavy chain and alpha-actin support the idea of a generalized state of incomplete differentiation, and the observed changes can not be accounted for solely by the number of actively regenerating fibers. Reduced mRNA levels for the alpha subunit of the acetylcholine receptor could contribute to neuromuscular junction structural abnormalities seen in DMD and may inhibit complete muscle maturation by contributing to a functional denervation of the muscle (Rafael et al., 2000). The authors also provide evidence for a process by which cells in dystrophic muscle tissue might be undergoing a dedifferentiation into alternate lineages such as bone and connective tissue precursors.

Despite the enormous wealth of information produced by Chen et al. (2000), it is clear that many additional observations remain to be mined from these data sets. Further interpretation of the emerging trends will also be aided by additional studies on biopsies obtained at different stages of disease progression and from additional forms of muscular dystrophy. However, the present data have already provided numerous new insights that will immediately aid studies of the disease pathogenesis. These data should also lead to a variety of new targets to treat secondary pathological features of the diseases, and may well provide a generalized method to monitor the efficacy of such interventions, which might be used either singly or in combination with cell and gene based therapies (Hartigan-O'Connor and Chamberlain, 2000). Finally, as Chen et al. (2000) note, these approaches may also serve as a basis for differential diagnosis of the many types of muscular dystrophy by enabling test of specific molecule signatures associated with each individual primary genetic defect. This convergence of the human genome project and molecular medicine may well be a critical factor in finding a cure for muscular dystrophy.

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