Advanced microscopic and histochemical techniques: diagnostic tools in the molecular era of myology

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Over the past two centuries, myology (i.e. the basic and clinical science of muscle and muscle disease) has passed through 3 stages of development: the classical period, the modern stage and the molecular era.

The classical period spans the last part of nineteenth century and the earlier part of the twentieth century. During this time, several major muscle disease were clinically and pathologically characterized, including Duchenne muscular dystrophy (DMD), myotonic dystrophy (DM) and facioscapulohumeral dystrophy (FSHD).

The modern stage in the second half of the twentieth century is characterized by the adaptation of histo and cytochemical techniques to the study of muscle biopsies. These tools improved the diagnostic accuracy and made possible the identification of new changes and structures (Engel and Cunningham, 1963; Scarlato, 1975). Examples of this are the demonstration of nemaline rods in nemaline myopathy (Shy et al., 1963) and ragged red/blue fibers in mitochondrial diseases (Olson et al., 1972). The advent of modern cytochemical techniques permitted the identification of various enzyme defects/storage diseases such as Pompe’s disease (Meola et al., 1984) or to study the intracellular lipids and membrane network in human muscle cultures (Santilli et al., 1989). The histochemical techniques were also applied in de novo innervated human muscle cultures (Meola et al., 1994) in myogenic clones from adult human muscle cell cultures (Meola et al., 1991) to the show restoration and persistence of a cytoplasmic enzyme i.e. G6PDH in stable hybrid myotubes (Sansone et al., 1993).

The molecular era was made possible by the development of molecular biology and its application to muscle diseases. This permitted the identification of gene defects in many inherited neuromuscular diseases, leading to accurate and specific diagnosis.

The best example of this, is DMD and the discovery in last 1980s of the gene at locus Xp21 whose mutation causes the deficiency of an absolutely essential protein, dystrophin (Figure 1) in muscle fibers (Hoffman et al., 1987). Parallel with the spectacular development of genomic in relation to muscle disease, immunohistochemistry produced remarkable discoveries. A number of sarcolemmal proteins were identified whose deficiency causes different forms of limb girdle dystrophy, including dysferlin (Bashir et al., 1998); sarcoglycan (Duggan et al., 1997); calpain (Richard et al., 1995); and caveolin (Minetti et al., 1998; Royuela et al., 2003).

Diagnostic advances also occurred in immunopathology using in situ hybridization, immunohistochemistry, immunofluorescence and western blotting in the molecular diagnosis of non-genetic dysimmune muscle diseases (i.e. idiopathic inflammatory myopathies) (De Paepe et al., 2004).

In the pre-molecular era, the classification of muscle diseases was based on characteristic clinical and/or microscopic pathological feature. For example, a disease with an early onset, x-linked recessive progressive proximal muscle weakness, large calves, and dystrophic microscopic pathology, was justified for the category of muscular dystrophy. Furthermore, episodes of the profound hypotonic limbs muscle weakness along with reduced serum potassium level, would place such a disease in the category of periodic paralyses.

In the molecular era, the classification of above mentioned diseases would change in dystrophinopathy in former case and hereditary skeletal muscle channelopathies in latter case (Meola et al., 2003).

In the molecular era, the basis of classification has changed and is still evolving and includes: mutational characteristics, affected proteins, microscopic features, the nature of the abnormal cellular process(es), principal organelle involvement and distinctive clinical features. Three categories serve as basis for molecular classification:
Mutational profile plus organelle involvement

a. primary sarcolemmal diseases involving the plasma membrane or basal lamina: dystrophinopathies, sarcoglycanopathies, merosin-deficient disease, dystrophinopathies and caveolin-related diseases (Johnson, 2001);

b. diseases with primary myonuclear abnormalities: emerinopathies, lamin A/C-related diseases and myotubular related centronuclear myopathies (Maraldi et al., 2003);

c. diseases with a primary involvement of myofibrils or cellular cytoskeleton: actinopathies (Goebel et al., 1997), core diseases (McCarthy et al., 2000), nemaline myopathies (Wallgren-Pettersson i 1999), plectin (Smith et al., 1996) and telethonin related myopathies (Moreira et al., 2000) myosin heavy chain type 2 syndrome (Martinsson et al., 2000) and desminopathy (Karpati and Sinnreich, 2004);

d. diseases with ion channel or ion transporter defects: chloride/calcium/potassium sodium channelopathies (myotonic or other periodic paralyses) (Davies and Hanna, 2003), sarcoplasmic reticulum (SR) calcium release channel (ryanodine receptor) and SR ATPase-related myopathy (Brody’s disease) (Odermatt et al., 1996).

Nature of the relevant cellular processes

a. muscle metabolism: catabolic metabolism, including lysosomal disorders (lamp-2 calpainopathy and proteosomal disorders); carnitine and fatty acid metabolism (Di Donato and Taroni, 2002); glycolytic pathways (Moxley et al., 2001) and mitochondrial oxidative phosphorylation defects (Taylor et al., 2004);

b. neuromuscular transmission: congenital myasthenic syndromes and autoimmune myasthenia gravis (McConville and Vincent, 2002);

c. glycosylation: inclusion body myopathy with GNE deficiency (Muntoni et al., 2002), muscle-eye-brain syndrome, and Fukuyama’s congenital muscular dystrophy (Michele et al., 2002).

Special complex molecular mechanisms

a. trinucleotide (CTG) repeat expansion: in DMPK (myotonin) gene in myotonic dystrophy type 1 (DM1) (Brook et al., 1992);

b. trinucleotide (GCG) repeat expansion: in PABPN1 gene (oculopharyngeal muscular dystrophy) (Meola et al., 1997);

c. tetranucleotide (CCTG) repeat expansion: in the gene encoding zinc-finger protein (ZNF9) in myotonic dystrophy type 2 (DM2) (Liquori et al., 2001; Cardani et al., In press) (Figure 2);

d. large telomeric deletion: on chromosome 4 in the D4Z4 repeat zone (facioscapulohumeral muscular dystrophy) (Wijmenga et al., 1992).

In the molecular era the diagnostic process of genetic or other myopathies must still start with obtaining a detailed history (including ascertainment of symptoms, pedigree, etc.) and performing a careful physical examination that is streamlined.
for characteristic signs of muscle diseases. The next steps are electrodiagnostic studies and microscopic study of muscle biopsies, using advanced histochemistry, immunohistochemistry and immunoblotting analysis. However, it should be emphasized that molecular testing is necessary at present. This includes mutational analysis and immunohistochemistry and immunoblotting on muscle biopsies.

For mutational analysis, one must focus on a highly suspected culprit gene. In many cases of genetic myopathies where a certain type of mutation is predominant, conventional technique with polymerase chain reaction (PCR) is the method of choice. In other instances, sequence analysis is necessary, which can be time consuming and expensive.

Some investigators give first preference to non-invasive molecular analysis versus microscopic study of an invasive muscle biopsy (Muntoni, 2001), for example in case in which clinical and genetic history is highly suggestive for DMD, but confirmation is necessary for differential diagnosis from Becker or other forms of muscular dystrophy.

Others investigators advocate the demonstration of dystrophin deficiency by histochemistry/immunoblot on muscle biopsy. Another approach is to first perform mutational analysis by multiplex PCR of the dystrophin gene’s coding sequence and to perform muscle biopsy if the former approach is not diagnostic (Flanigan et al., 2003).

Another example for the absolute need for mutational analysis is carrier detection or prenatal diagnosis in DMD.

Despite the molecular discoveries pertaining the diagnosis of many myopathies, much remains to be explored.

In many genetic and non-genetic muscle diseases, the culprit gene and its product remains unknown.

In some important diseases, where the genetic defect has been clarified, we still lack any understanding of the pathogenesis of muscle fibre damage. An example of this is FSHD.

Some basic pathogenetic mechanisms that are possibly operating in myopathies or have potential therapeutic usefulness need to be explored (Chaubourt et al., 1999).

These include the role of signalling systems, apoptosis, oxygen radical-induced damage (Rando, 2001), muscle cell development and differentiation as well as related molecules, post translational processing of proteins, the interaction of nuclearly coded and mitochondrially-coded molecules, and perfecting gene therapeutic methods.
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