Desmin in Muscle Formation and Maintenance: Knockouts and Consequences

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ABSTRACT. Desmin, the muscle-specific member of the intermediate filament (IF) family, is one of the earliest known myogenic markers in both skeletal muscle and heart. Its expression precedes that of all known muscle proteins including the members of the MyoD family of myogenic helix-loop-helix (mHLH) regulators with the exception of myf5. In mature striated muscle, desmin IFs surround the Z-discs, interlink them together and integrate the contractile apparatus with the sarcolemma and the nucleus. In vitro studies using both antisense RNA and homologous recombination techniques in embryonic stem (ES) cells demonstrated that desmin plays a crucial role during myogenesis, as inhibition of desmin expression blocked myoblast fusion and myotube formation. Both in C2C12 cells and differentiating embryoid bodies, the absence of desmin interferes with the normal myogenic program, as manifested by the inhibition of the mHLH transcription regulators. To investigate the function of desmin in all muscle types *in vivo*, we generated desmin null mice through homologous recombination. Surprisingly, a considerable number of these mice are viable and fertile, potentially due to compensation by vimentin, nestin or synemin. However, desmin null mice demonstrate a multisystem disorder involving cardiac, skeletal and smooth muscle, beginning early in their postnatal life. Histological and electron microscopic analysis in both heart and skeletal muscle tissues reveals severe disruption of muscle architecture and degeneration. Structural abnormalities include loss of lateral alignment of myofibrils, perturbation of myofibril anchorage to the sarcolemma, abnormal mitochondrial number and organization, and loss of nuclear shape and positioning. Loose cell adhesion and increased intercellular space are prominent defects. The consequences of these abnormalities are most severe in the heart, which exhibits progressive degeneration and necrosis of the myocardium accompanied by extensive calcification. Abnormalities of smooth muscle included hypoplasia and degeneration. There is a direct correlation between severity of damage and muscle usage, possibly due to increased susceptibility to normal mechanical damage and/or to repair deficiency in the absence of desmin. In conclusion, the studies so far have demonstrated that though desmin is absolutely necessary for muscle differentiation in vitro, muscle development can take place in vivo in the absence of this intermediate filament protein. However, desmin seems to play an essential role in the maintenance of myofibril, myofiber and whole muscle tissue structural and functional integrity.

Introduction

During muscle development an embryo must coordinate cell division, cell migration, cell recognition, cell adhesion, cell fusion and ultimately, formation of a properly functioning muscle. During these developmental processes signals by extracellular morphogens, extracellular matrix and direct cell-to-cell interactions must be sensed by the cells through different cell signaling molecules and transmitted to the nucleus where, in coordination with internal signals, induction of muscle specific gene expression takes place. A variety of independent observations have suggested that changes in cell shape due to interactions with either the extracellular matrix or neighboring cells lead to reorganization of the cytoskeleton that causes changes in gene expression, possibly by directly interacting with the nuclear matrix or by activating and facilitating the transport of regulatory

factors to the nucleus (for review see 7, 8, 9, 22, 54, 71, 73, 97, 109, 118, 121).

The cytoskeleton of the higher eukaryotic cell is composed of three different filamentous networks, microtubules, actin microfilaments and intermediate filaments (IF). The different members of the IF superfamily show very restricted tissue specific and developmentally regulated expression (for review see 35). This unique expression pattern distinguishes IF from the other two cytoskeletal networks and its biological significance has not been revealed yet (for review see 59, 117).

The IF cytoskeleton of mature muscle is composed predominantly of the muscle specific IF protein desmin (66) and lower amounts of synemin (46), which, as recent sequence analysis revealed, is also a member of the IF family (4). During early muscle development, in addition to vimentin, the specific IF protein nestin (56, 104) is also transiently coexpressed with desmin both in skeletal and cardiac muscle (56, 57). Desmin is encoded by a single copy gene (12, 13) which is expressed in all muscle

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tissues, cardiac, skeletal and smooth (for review see 65, 67). It is one of the earliest known myogenic markers both in heart and in somites (20, 50, 58, 61, 72, 101). During mouse development, desmin first appears at 8.25 days post coitum (dpc) in the neuroectoderm, where it is transiently expressed with vimentin, nestin and keratins. At 8.5 dpc it appears in the heart rudiment, and at day 9 it can be detected in the myotome (36, 101). Recent studies in transgenic mice have shown that the desmin gene is activated at least half to one day earlier than the time the protein can be detected (Fig. 1; 61). In contrast to most muscle specific genes, desmin is also expressed at low levels in satellite cells (2) and replicating myoblasts (58). During development desmin expression precedes all muscle specific structural genes and the myoD transcription factors myoD, myogenin and MRF4 (87, 123), with the exception of Myf-5 (for review see 69, 79, 80, 100), suggesting that it might play some modulating role in myogenic commitment and differentiation. Studies on the regulation of desmin gene expression have demonstrated that all four of the myogenic HLH transcription factors in cooperation with members of the MEF2 family of transcription regulators (for review see 89) control desmin expression during development (61, 69, 70). Furthermore, it has been shown that MEF2 factors are major regulators of desmin gene expression both in heart and skeletal muscle during mouse embryogenesis (61).

In mature striated muscle desmin IFs form a three dimensional scaffold which seems to extend across the entire diameter of the myofiber (for review see 67, 92). The IFs surround the Z-disks, extend from one Z-disk to the other and seem to traverse and potentially associate with the sarcoplasmic reticulum, the T-tubules, in the case of cardiac muscle, as well as mitochondria (90, 112, 113). Finally, IFs extend from the Z-disc to the plasma membrane, at the level of the costameres (27), and they project from the perinuclear myofibrils to the nuclear membrane (5, 31). A lower proportion of IFs also seem to form a longitudinal lattice which appear to form a cage around the myofibrils (113, 114). On the basis of these associations with the IF lattice it has been proposed that the desmin or desmin and vimentin IFs could mechanically integrate all contractile actions of a muscle fiber by linking individual myofibrils laterally at the periphery of the Z-disks and by linking Z-disks to the plasma membrane, nuclear membrane, and to other cytoplasmic membranous organelles (46). Costameres are periodic rib-like regions of membrane skeleton overlying the flanks of the Z-disks of subsarcolemmal myofibrils (27). It has been shown, mostly in cardiac and in several skeletal muscles, that IFs link the Z-disks to costameres, most possibly through spectrin and ankyrin (for review see 67, 86). The major components of costameres in all muscles are α - and β -spectrins, ankyrin, γ -ac-

tin, vinculin, and possibly talin and dystrophin (for review see 67, 93). The composition of costameres and the nature of the association of myofibrils with costameres depends not only on the muscle type but also on the location along the length of the muscle. The molecular basis of the association of the IF proteins with the costamere membrane skeleton is suggested by biochemical evidence of binding of IFs to spectrin (33, 63) and to ankyrin (38, 39, 41). The contribution of desmin to the assembly and stabilization of the structure and composition of costameres, as well as the nature of the association of myofibrils with costameres is unknown. Some muscle cells are interconnected in part by extracellular matrix filaments localized adjacent to the Z-disks (11, 16). There is also a strong correspondence between the presence of costameres with attached IFs and extracellular filaments enriched overlying the costameres (105). The linkage of the extracellular matrix and the costamere/cytoskeletal complex appears to involve a number of proteins including the integral membrane sialoglycoprotein Sgp130 (95) and several integrins, the receptor of a variety of extracellular matrix components including fibronectin, vitronectin, laminin and different collagen types (for review see 1). It seems that costameres might require this cell-EC matrix interaction to be organized. However, it is unknown if desmin IFs influence membrane-ECM organization. Collagen is a major ECM component in muscle. The collagen struts coursing between muscle cells could structurally integrate the cells across the diameter of the entire myocardium. This way the IF cytoskeleton/myofibrils as a unit could act to distribute force throughout a muscle cell, and then through their connection with ECM via membrane skeleton/transmembrane protein component would distribute force and coordinate movement in the transverse axis across the whole muscle tissue. The longitudinal force transduction, which is the most important element in muscle function, is mediated at the ends of striated cells by specialized junctions, the intercalated disks in cardiac muscle and myotendinous junctions in both skeletal and cardiac muscle. Though these are the attachment sites for actin filaments, intercalated disks are believed to be further strengthened by loops of intermediate filaments that connect desmosomes (for review see 47). Striated muscle has been one of the first cell types in which the attachment of the IFs to the nuclear membrane has been very clearly demonstrated (5, 31). Biochemical studies have further shown that vimentin and desmin bind to lamin B of the karyoskeleton through their carboxy-termini (38, 39, 40, 41). The existence of trans-cellular desmin-lamin B filament network has also been reported for cardiac myocytes (76). Consequently, the IFs form part of a network that connects the extracellular and the nuclear matrix and thus could participate in mechanochemical signaling to the nucleus both

Fig. 1. Pattern of desmin gene expression during mouse development. Whole mount staining for lacZ activity of transgenic embryos, from 8.5–13.5 dpc, harboring the desmin-lacZ transgene. LacZ activity can be detected in the somites between 8–8.5 dpc (62).

from the outside of the cell as well as from the contractile apparatus in regards to its physiological state. The latter is obviously possible due to the mutual anchorage of the IF lattice and the contractile apparatus at the Z-disc.

The role of the IF cytoskeleton in muscle morphogenesis and function has only been speculated throughout the years (for earlier reviews see 65, 67). One of the main restrictions during the development of the different hypotheses was the fact that desmin is the product of a single gene expressed in all muscle types and throughout development. Consequently, the challenge was to either assume multiple functions in different muscle types and developmental stages or to look for a function common to all muscle tissues throughout development. An additional issue related to potential differences in the function of the different IF proteins in the same muscle cell. Is there a functional difference between desmin and vimentin or are they redundant for security purposes? Why do adult muscle cells need desmin and not vimentin? Why does growing muscle need more IFs than mature muscle and how does this correlate with their function in adult tissue?

Two hypotheses have been developed so far regarding the function of desmin. The older one was based on several of the morphological observations described above which suggested that in terminally differentiated myotubes a potential role for desmin is to form a trans-cytoplasmic integrating matrix, linking individual myofibrils laterally at their Z disks and to the sarcolemma thus being responsible for their lateral alignment, their striated appearance and the normal function of the contractile apparatus (for review see 45, 65, 67). According to the second hypothesis, desmin might mediate signal transduction and transport processes from the cell surface to the nucleus. This suggestion was based both on the unique developmental pattern of the expression of desmin, described above, as well as its position in the cell, where it interlinks the sarcolemma with the nuclear envelope by specifically binding to ankyrin, other components of the costameres and lamin B, respectively. The available data discussed in this article, obtained by *in vitro* studies, support the latter hypothesis, whereas *in vivo* knockout experiments are in favor of the older suggestions.

Desmin is very crucial for normal myogenesis *in vitro*

Studies using antisense RNA strategies (71) in differentiating C2C12 myoblasts have demonstrated that inhibition of desmin expression not only blocks myoblast fusion and myotube formation but also interferes with the normal induction of muscle specific gene expression including the myogenic HLH transcription regulators myoD and myogenin (71). These results suggested that the muscle-specific IF protein desmin, so far considered

merely a component of the supporting cell architecture, might be a central molecule regulating myogenesis through direct or indirect signaling that can modulate nuclear events. The possibility that desmin might be involved in signal transduction and transport processes between the cell surface and the nucleus is supported by several pieces of recent information, including its position in the cell where it interlinks the sarcolemma with the nuclear envelope by specifically binding to ankyrin, other components of the costameres and lamin B, respectively (for review see 38, 39, 41, 67, 76, 86, 93, 113, 114). Lamin B binds to the nuclear matrix associated regions (MARs) (77) which can activate gene transcription by binding to the nuclear matrix (108). Furthermore, it was very recently reported that lamin A and desmin can also specifically bind MARs *in vitro* (78). On the other hand, IFs serve both as sites of attachment as well as major substrates of several kinases that seem to be involved in signal transduction (for review see 29). Finally, recent data on mechanotransduction across the cell surface and through the cytoskeleton, including the intermediate filament system (30, 121; for review see 54) favors this hypothesis.

To confirm the intriguing results obtained with C2C12 cells and further investigate the role of desmin in all three types of muscle cells, we mutated both desmin alleles in embryonic stem (ES) cells. A differentiating system based on ES cell derived embryoid bodies (EBs) which recapitulates the *in vivo* cardiac, skeletal, and smooth muscle myogenesis of mouse embryos was developed and used (124). Wild type, heterozygous and homozygous cell lines with the mutated desmin allele were evaluated. Skeletal myogenesis was totally inhibited in desmin null mutant EBs, as manifested by the absence of myotube formation, contractility, and myoD, myogenin, myf5 and myosin heavy chain expression. Smooth muscle formation was also completely blocked in the absence of desmin. On the other hand, there were no obvious effects on cardiomyocyte differentiation in these desmin null mutant EBs (124). This data suggests that in contrast to early cardiocyte differentiation, desmin is indispensable for skeletal and smooth muscle formation *in vitro*.

At the present, we can only speculate that the loss of desmin either directly blocks some specific step during early myogenic commitment and differentiation or alternatively it interferes with the maintenance of the myogenic phenotype. How could desmin be involved in these processes? Myogenesis can be divided into several phases, including cell commitment, exit from the cell cycle, initiation of differentiation, cell recognition and alignment, cell adhesion and cell fusion (for review see 122). During most of these processes extensive signal transduction from the cell surface to the nucleus takes place. Desmin could actively participate in one or more

of these processes. If desmin somehow modulates signals that lead to a post mitotic state, this could explain the absence of an obvious effect during cardiac cell differentiation, since these cells, in contrast to skeletal myoblasts, can both proliferate and differentiate simultaneously. If desmin is involved in cell recognition or attachment processes rather than fusion itself, this might explain why smooth muscle development is also severely inhibited in desmin null mutant EBs, since these cells do not fuse. Desmin, through its interaction with the membrane skeleton (for review see 41, 67, 86) and particularly components of the costameres (93, 113, 114), might be involved in integrating and stabilizing several of their potential functions, including cell recognition, adhesion and signal transduction. Adhesion molecules and receptors have already been reported to play important role(s) in myogenesis (52, 83, 96). Finally, desmin could be directly involved in myoblast fusion. This possibility could be favored by several reported observations. Ectopic expression of desmin in the lenses of transgenic mice causes, among other abnormalities, partial plasma membrane fusion (60). Furthermore, our antisense studies (71) as well as those from other investigators (20, 72) have suggested that fusion can take place only between desmin positive cells. Finally, the correct quantity of desmin seems to be necessary for maintaining the phenotype in newly formed myotubes, because reduced levels of desmin protein in heterozygous EBs, caused a collapse of most myotubes.

As with the antisense RNA studies, the EBs studies also demonstrated that in the absence of desmin the expression of the mHLH regulators is suppressed. Therefore, it could be speculated that desmin regulates myogenesis by influencing nuclear events, possibly through its potential involvement in myogenic signal transduction and transport processes between the cell surface and the nucleus. This could be a way by which this part of the cytoskeleton can participate in the establishment and maintenance of the myogenic phenotype, in the initiation of differentiation, fusion and maintenance of the terminally differentiated state. As pointed out above, desmin could be responsible for one or more of these processes. However, the observation that the absence of desmin causes also suppression of myf5, the only member of the myoD family to be expressed in the mouse embryo as early as desmin and prior to muscle cell differentiation (107), suggests that desmin must not participate mainly in terminal differentiation of skeletal muscle but rather acts upstream in processes crucial for the establishment and maintenance of the myogenic phenotype. The available data do not address directly the mechanism of the mHLH factor suppression. The desmin antisense studies showed that the inhibition of at least myoD and myogenin was at the RNA level (71). It is possible that desmin might be involved in the nuclear

transport or stabilization of the RNA or protein of these factors and when this process is blocked, even partially, these proteins cannot efficiently transactivate their own genes and their downstream partners and are quickly degraded. Analysis of myf5 expression in the desmin null spindle shape cells of the EBs showed that a small fraction of them do seem to express low levels of this factor. If stabilization is a level at which desmin might function, this low signal might be due to residual myf5 protein. Alternatively, it might be possible that desmin directly influences nuclear events, including the activation of the mHLH factors, through its connection to the nuclear matrix associated lamins (38, 39), which in turn specifically bind to MARS (77, 78). Thus, one could envision that the connection of the cytoplasmic IF with the nuclear lamins could influence nuclear events that might be modulated through lamins-MARS association. Studies on mechanotransduction across the cell surface and through the cytoskeleton, including the IF system (121; for review see 54), favors and complements the above considerations. In addition, recent data in cardiomyocytes have also suggested the potential involvement of desmin IFs in stretched induced changes in chromatin (10). Furthermore, a variety of independent observations in different cell types have suggested that changes in cell shape due to interactions with either the extracellular matrix or neighboring cells cause changes in gene expression (7, 8, 21, 71, 73, 97, 109, 118). Though the exact mechanism of this signal transduction process has not been revealed, it has been speculated that the cytoskeleton is responsible for converting changes in the cytoarchitecture to effects on gene transcription (9, 32, 54, 121). The antisense studies (71) and the desmin knockout experiments in differentiating EBs (124) provide the first direct evidence of gene regulation by the cytoskeleton and specifically the IF system during myogenesis. The significance of the previously reported sequence homology between desmin and the mHLH regulators (71) on the presently speculated mechanisms of desmin function remains elusive. Relationship and association of other members of the IF with several transcription factors and their potential involvement in the modulation of nuclear events has previously been reported (14, 81; for review see 117). It was recently demonstrated that disruption of IFs interferes with normal neuron development in *Xenopus laevis* (74). Finally, it could be speculated that IFs and the cytoskeleton generally could modulate gene activity possibly by directly interacting with the nuclear matrix and/or by participating in the control of transport of regulatory factors to the nucleus. The latter possibility has been suggested from recent studies which showed that depolymerization of microtubules controls gene expression by activating NF- κ B (97). The exact mechanism of the above suggested possibilities remains elusive.

In vivo prenatal muscle development can take place in the absence of desmin

To investigate the function of desmin in all muscle types *in vivo*, we generated desmin null mice through homologous recombination. Surprisingly, a considerable number of these mice were viable, demonstrating that prenatal muscle development can take place in the absence of desmin. The desmin null mice display no obvious anatomical defects when compared to wild type and heterozygous littermates, except for slightly reduced size in a very small percentage of individuals. Both male and female desmin null mice are fertile, although null females produce slightly smaller litter sizes when compared to wild type females.

Obviously, the *in vivo* results do not support the *in vitro* data. However, the difference could be explained by the potential use of vimentin or nestin *in vivo* to compensate for the absence of desmin during development. Such compensatory mechanisms seem to be very efficient *in vivo* not only for members of the IF family (23, 43, 75, 91) but also for several other genes, particularly in muscle (88). In embryoid bodies, however, these compensatory mechanisms seem to take place effectively only in cardiac and not in skeletal and smooth muscle development (124). In addition to vimentin and desmin, nestin is also expressed in both skeletal and cardiac early muscle development (56, 57). Generation of a double null mutation of desmin and vimentin and/or nestin will directly address whether or not either of these two IF proteins can compensate for the absence of desmin during development *in vivo*. However, an additional complication is the recognition that there is now a fourth IF protein, synemin, expressed in muscle tissue (4, 46). Like desmin, synemin also shows similar localization at the Z-line in striated muscle (46, 85). Compensation by non IF cytoskeletal proteins is also possible.

The absence of desmin does not interfere with the down regulation of vimentin and nestin in mature muscle

During embryonic development of skeletal muscle, levels of desmin expression increase as terminal differentiation proceeds, and desmin filaments reorganize at the periphery of the Z-discs (37, 44). Vimentin expression is initially high in replicating myoblasts, but gradually decreases as terminal differentiation occurs (6, 37). Like desmin, vimentin can be detected at the Z-discs at low levels, but it gradually disappears and is at very low levels or totally absent from adult skeletal muscle (115). Nestin has also been shown to be present in replicating myoblasts and early myotubes, but like vimentin, it is also absent in adult skeletal muscle (56, 104). Recent knockouts of keratins 14 and 10 have suggested that in the absence of these IF subunits, expression of other keratin IFs are able to at least partially compensate for the loss of these subunits (75, 91). Analysis of the desmin

null mice revealed that neither vimentin nor nestin are expressed in tongue and thigh skeletal muscle of adult mice. The same is true for cardiac muscle. (84) Thus, we concluded that in the absence of desmin, neither vimentin nor nestin expression is maintained in adult striated muscle. We do not know at the present the pattern of synemin expression or potential induction of any other IF protein.

Myofibrillogenesis can take place in the absence of desmin

As myofibrils are assembled in desmin null mice, it is obvious that the presence of desmin is not necessary for the assembly and initial lateral alignment of myofibrils, as was suggested originally by Hill and colleagues (51). Disruption of IFs in cultured muscle cells using truncated desmin dominant mutants in transient transfection experiments (103) agree with the present data in that desmin is not absolutely necessary for myofibril assembly. These studies, however, could not address issues regarding the role of IFs in the maintenance and proper integrity of these structures in the tissue. On the other hand, disruption of IFs by microinjection of DNA coding for truncated vimentin or desmin in fertilized *Xenopus* eggs also did not seem to influence myofibril assembly but destabilized the intersomite junction in myotomal muscle (15). Analysis of regions of muscle from desmin null mice which had not yet undergone any degeneration (see below) has shown that the absence of desmin does not influence the localization or level of expression of some individual myofibrillar proteins (84). In striated muscle, sarcomeric α -actinin is a major component of the Z-disc. We were unable to detect any discernible difference in localization or intensity of α -actinin staining in wild type or desmin null cardiac or skeletal muscle. Tropomyosin is not localized along the Z-lines itself but appears on either side of the Z-line, thus displaying a wider band. No obvious differences between wild type and desmin null muscle could be seen, indicating that the absence of desmin does not influence the localization or expression of this protein. Similar results were obtained for nebulin and myosin heavy chain. Thus we conclude that the absence of desmin does not influence the localization and levels of expression of these proteins of the contractile apparatus and the cytoskeleton.

Desmin is essential for the maintenance of integration of muscle structure and function

It has been clearly demonstrated that desmin null mice have defects in all three muscle types (84). Common features in all cases include overt cell and tissue damage with signs of instability and degeneration. While most desmin null mice are anatomically identical to wild type and heterozygous mice, by the second week

after birth they are somewhat more lethargic than their wild type and heterozygous littermates. Dissection of desmin null mice revealed the presence of extensive calcium deposits in the myocardium. Histological and electron microscopic analysis revealed the appearance of a general loss of cardiomyofiber tension, with areas of considerable disruption and disorganization of the cardiac myofibers and myofibrils. Extensive interstitial fibrosis and necrosis are hallmarks of the observed defects. Degeneration of the myocardium was more prominent throughout the right ventricle, and the lesions appeared to progress from the exterior of the myocardium to the interior, suggesting that over time, the degeneration of cardiac tissue works from the exterior of the myocardium inward.

Occasionally, the extent of muscle loss was very severe and this was easily manifested upon dissection by the transparency of areas of the myocardium (84). While analysis of cardiac sections from neonates shows little degeneration and no calcification, these defects are visible as early as one week after birth in some individuals and gradually increase with age. Additionally, in cases of severe lesions the calcium deposits disappear together with the degenerating tissue. Electron microscopy of sections from desmin null ventricular muscle revealed that while there are some apparently normal fibers, several myofibers demonstrate severely disrupted architecture (Figs. 2 and 3). In contrast to wild type fibers, which display laterally aligned myofibrils and nicely packed strands of mitochondria, these myofibers have lost the normal lateral alignment of myofibrils and often show disrupted myofibrils, particularly at the positions of the intercalated discs which often look abnormal (Fig. 4). Loss of myofibril anchorage to the plasma membrane is one of the hallmarks of the observed defects (Figs. 2 and 3). Additionally, the mitochondria have lost their organization and shape (Figs. 2 and 3) and they often appear to be swollen and disintegrating (Figs. 2, 3 and 4).

Histological examination of skeletal muscle of the tongue, leg and diaphragm revealed similar defects in the integrity and organization of the myofibers, although to a lesser extent than that seen in cardiac muscle (84). Abnormalities included loss of myofiber tension, ragged and disorganized myofibers with fewer, if any, discernible striations, some loss of stable nuclear positioning and indications of degeneration (84). The extent of damage depends on the origin of the muscle examined. In the tongue, a majority of the fibers display the ragged, disintegrating morphology, while in muscle from the back, relatively few fibers show this morphology. Cross sections of skeletal muscle from desmin null and wild type mice also demonstrate that in mice lacking desmin, many fibers are ragged in appearance, of smaller diameter and frequently not closely packed

Fig. 2. Disruption of muscle architecture in mice lacking desmin. Transmission electron microscopy of cardiac muscle from wild type (WT) and mice lacking desmin ($-/-$). Wild type cardiac muscle shows aligned arrays of myofibrils terminating and inserting at intercalated discs. Myofibrils are interrupted occasionally by packed strands of mitochondria. Disorganization and signs of damage are the hallmarks of desmin null myocardium. The myofibrils are disorganized, separated from the sarcolemma, hypercontracted and damaged. Loss of cell adhesion and generation of abnormally enlarged intracellular space is obvious. The mitochondria are also abnormal and highly disorganized.

when compared to wild type muscle. Additionally, there appear to be more nuclei present in cross sections from null mice, including some fibers which appear to have internal clusters of multiple nuclei. Electron microscopy of skeletal muscle from the diaphragm clearly revealed that, as in cardiac muscle, some myofibers from desmin null animals appear relatively normal, while others show disrupted intracellular architecture, including abnormally registered sarcomeres with miss-

Fig. 3. Extensive myocardium damage and degeneration in the absence of desmin. Transmission electron microscopy of cardiac muscle from desmin null mice at lower magnification ($\times 1,500$) reveals a variety of severe structural abnormalities. Complete loss of coordination and alignment between neighboring myocytes and extensive degeneration are the main obvious defects in the present section. The origin of the huge empty spaces is unknown; degenerated mitochondria might be a potential source. In most cases, the empty spaces are surrounded by membranous structures. Myofibril, mitochondria and nuclear structural abnormalities and loss of positioning are obvious in all cells. In extreme cases, as in the upper left myocyte (*), myofibrils are replaced by mitochondria.

Fig. 4. Changes in the architecture of intercalated discs in desmin null cardiomyocytes. Transmission electron microscopy of cardiac muscle from wild type (WT) and desmin null ($-/-$) mice. Desmosomes (arrowheads) can not be easily seen in the desmin null myocytes. Adherens junctions (arrows) defects include occasionally fragmentation and increased intraplaque space. Myofibril disruption and mitochondria degeneration is obvious here as well. (Original magnification: $\times 20,000$).

ing or disoriented Z-discs and an absence of typically striated A and I bands. Similarly, an additional severe effect was the disorganization of mitochondria. While the mitochondria are found in pairs at the Z-disc in wild

type diaphragm, this organization is lost in a number of fibers in desmin null muscle. Additionally, a number of mitochondria also show the swollen, disintegrating morphology similar to those seen in some desmin null cardiac myofibers. Indeed, association of mitochondria with intermediate filament networks has been reported previously (113, 114). Among the different skeletal muscle types studied, tongue demonstrated the most severe myofiber disruption and disintegration both by histological and electron microscopic analysis (84). The diaphragm was also severely effected, while skeletal muscle from the thigh is effected to a somewhat lesser degree. The muscles of the back show the least disruption and degeneration. These results suggest a possible correlation between the severity of the damage and the extent of muscle usage.

Histological analysis also revealed parallel defects in smooth muscle (84). Abnormalities of the smooth muscle of the aorta from desmin null mice include loose organization of smooth muscle cells, loss of the elongated nuclear shape a preponderance of empty space around the myofiber nuclei, hypoplasia and a low degree of degeneration at both the outtrack and the thoracic levels. The clearing of cytoplasm around the nuclei can also be observed at the electron microscopic level. The effect of the absence of desmin was also examined in gastric smooth muscle. The defects observed here include partial loss of the outer longitudinal smooth muscle layer and some hypoplasia of the circular layer muscle of the intestine. Loose cellular organization was also very pronounced.

The effects of the absence of desmin are most severe in cardiac muscle, with smooth muscle seemingly least affected and skeletal muscle exhibiting damage that is intermediate in severity. The defects we see are most possibly due to either increased fragility of the myofibers, or to impairment of normal myofibril and myofiber repair and regeneration (48). Additionally, these results seem to suggest that the more active the muscle tissue, the greater damage and degeneration it exhibits in the absence of desmin. This is supported by the fact that degeneration of cardiac muscle, which is constantly contracting, is the most severe that we observe, and that there seems to be a range of severity of degeneration seen in skeletal muscle. Very active muscles such as the diaphragm and tongue show a higher amount of degeneration than muscles which are less active, such as those of the back. However, only a fraction of the cell population in a given muscle tissue is defective. This indicates that desmin is not absolutely necessary for the formation and assembly of myofibrils, but rather it suggests that it is required for maintenance of their integrity and possibly for their repair or regeneration after mechanical damage. These data are consistent with the hypothesis that IFs form a transcytoplasmic integrating matrix

that might contribute to the integrity and strength of both myofibrils and myofibers (64, 67).

The role of IFs in cell integrity and strength has been suggested from numerous studies with keratins (18, 19, 25, 26, 62, 98, 94, 116, 120; for review see 35, 82). Furthermore, transgenic mouse models have provided evidence directly linking neurofilament abnormalities to neurodegenerative processes (22, 24, 125; for review 68). Thus, muscle that is deficient in desmin could be more fragile than wild type muscle and as such would be more easily damaged during contractile activity. This would lead one to expect that more actively contracting muscle would incur more damage and thus undergo more extensive degeneration. Our data from the desmin null mice support this idea.

An alternative explanation for the severity of the degeneration seen in cardiac muscle is that a vascular abnormality resulting from the absence of desmin in smooth muscle results in reduced blood supply to the myocardium, leading to progressive degeneration. This possibility is supported by the observed effect the absence of desmin has on the smooth muscle of the aorta. On the other hand, the possibility that the primary cause of myocardial degeneration is fragility of the myofibers is supported by the observed effects on skeletal muscle. However, these possibilities are not mutually exclusive, and could both contribute to the observed myocardial degeneration. Overall, the phenotype observed in smooth muscle was less dramatic than that seen in cardiac and skeletal muscle. As with striated muscle, the abnormalities observed in smooth muscle could be due to tissue instability as a consequence of the absence of desmin and/or inability to repair mechanical damage resulting from normal activity of the muscle. The fact that organs containing smooth muscle are less affected may be due to the sustained expression of vimentin which, in contrast to striated muscle, is maintained after differentiation (102). Very preliminary examination of uterine smooth muscle did not demonstrate extreme differences between non-pregnant wild type and desmin null tissue. However, uteri from pregnant desmin null females show marked hypoplasia of smooth muscle compared to pregnant wild type uterine tissue.

As in dystrophin deficiencies that lead to muscle degeneration (55), the causes of the observed muscle degeneration in the absence of desmin are not clear at this point. They could be the consequence of tissue damage due to muscle weakness in the absence of desmin, and/or an inability of the desmin null mice to efficiently repair damage caused by normal mechanical stress. Such defects in damage repair can be correlated with delayed wound healing found in BPAG1, or keratin bundling protein, knockout mice (49), possibly due to abnormal keratin filament behavior. The latter case could be due to defects in signaling which might be important for

both myofibril and myofiber repair and regeneration. This possibility is supported by recent reports from GFAP null mice, which have suggested involvement of the glial cell specific IF in communication between Bergmann glial and Purkinje cells (106).

Desmin and Muscle Disease

There are several reported myopathies and cardiomyopathies with abnormally accumulated granular and filamentous aggregates of desmin (for review see 42). There is no case, however, where desmin is completely absent. Similarly, there is no evidence, so far, demonstrating that these abnormalities in desmin distribution have a causal effect on the disease. Muscle damage and degeneration is the hallmark of most common myopathies, cardiomyopathies and muscular dystrophies (48). In addition, several of the lesions developed in all three muscle types of the desmin null mice are reminiscent of those reported in some familial desminopathies (3, 53, 119; for review see 42). These include dilation of the ventricle chamber, hypoplasia of the aorta, thinned intestinal walls and distended and fragmented Z-lines (3). The data with the desmin null mice support the idea that the observed abnormalities in desmin distribution in several reported myopathies might have a causal effect. This hypothesis is favored by the extensive similarity of the desmin abnormal aggregates to those of keratin filament aggregates which relate directly to different skin diseases (18, 19, 25, 26, 62, 94, 98, 116, 120; for review see 35, 82). Furthermore, natural human mutations in k14 (17, 99) and the knock out of the k14 gene (75) provided further support for the notion that cell fragility is a consequence of the absence of the keratin filament network rather than the presence of insoluble aggregates. Consequently, it could be suggested that the desmin null mouse can provide an animal model for several skeletal and cardiac myopathies which closely resemble those with abnormalities in desmin distribution (3, 42, 53, 110, 111, 119).

CONCLUSIONS

The studies summarized here indicate that the IF cytoskeleton is essential for maintaining integration of structure and function in muscle. This could be achieved by generating a continuous scaffold together with extracellular and nuclear matrix through the membrane cytoskeleton that extends throughout the muscle fibers. This cytoskeletal network could serve in coordination of contraction and relaxation, in transmission of mechanical forces and mechanochemical signals throughout the myofiber, to the nucleus, other cellular organelles and the entire muscle in a coordinated fashion, as well as in strengthening the muscle. Some of these functions could be common to embryonic and adult muscle. Dur-

ing muscle morphogenesis such functions are of great importance and might be guaranteed by redundancy and flexibility in the use of other members of the IF family or even other cytoskeletal proteins. The double or even triple knock outs of desmin, vimentin, nestin and synemin will address this issue.

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