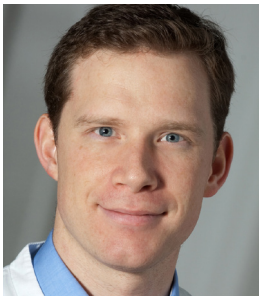
CELL & GENE THERAPY INSIGHTS

EDITORIAL



Challenges in gene therapy for desminopathies

Markus B Heckmann, Hugo A Katus and Oliver J Müller

"Although of monogenetic etiology, desminopathies pose significant challenges for gene therapeutic approaches owing to their inherent pathophysiologic differences."

Desminopathies are diseases linked to mutations in the desmin gene (*DES*). Clinical manifestations include skeletal muscle weakness, respiratory dysfunction, cardiomyopathy, cardiac conduction disease and, infrequently, changes in bowel habits [1,2]. Most patients exhibit a combined phenotype comprising neurological and cardiologic symptoms, while 22% show an isolated cardiac phenotype [3]. Cardiac involvement has been reported in 74% of patients and is the most life-limiting feature of desminopathy [4]. Variable phenotypes have been described for the same mutations and correlations between mutations and specific symptomatic patterns have been discussed controversially [3]. Disease onset has been described in the first decade for severe autosomal recessive forms and the second to fourth decade for autosomal dominant variants [1].

The first animal models for desminopathies were desmin knockout mice, followed by mice carrying a deletion and mice carrying a point mutation [5–7].

Although of monogenetic etiology, desminopathies pose significant challenges for gene therapeutic approaches owing to inherent pathophysiologic differences. *DES* codes for a type III intermediate filament protein featuring a typical structure allowing the formation of coiled-coil

dimers [8]. These dimers subsequently form filamentous networks that are mainly found in muscle cells. Its critical role in maintaining the cellular structural integrity is evident by its direct physical interactants, including desmoplakin, ankyrin, syncoilin, nebulin, lamin B, myotubularin, myospryn and plectin, linking its filamentous network to the nucleus, the cell membrane, the dystroglycan network, sarcomers, lysosomes and mitochondria (Figure 1).

Desmin's ability to form functional filamentous networks greatly depends on an undisturbed life cycle comprising protein expression, filament formation and protein degradation. *DES* mutations affect different steps of the protein's life cycle warranting distinct gene therapeutic approaches. Lack of desmin expression has been reported in humans and successfully treated with recombinant adeno-associated viral (AAV) therapy in mice [9,10]. Recessive mutations might also benefit from AAV-mediated wild-type desmin expression, although no research has been conducted yet, to prove this assumption.

The majority of known *DES* mutations, however, lead to protein misfolding and are inherited in an autosomal dominant manner [4]. Misfolded desmin exerts a dominant negative effect on wild-type desmin filament assembly and impairs the ubiquitin proteasome system, leading to the formation of desmin rich protein aggregates, which are less likely to be subjected to autophagy [2,11,12]. Although a critical ratio of wild-type to mutant desmin is necessary for mutant desmin to exhibit this effect, a simple increase in wild-type desmin expression might probably just increase protein aggregates [2].

Downregulating mutant desmin expression with selective siRNA might represent a more sophisticated approach. However, engineering siRNAs able to reliably distinguish mRNAs differing only in one base exceeds the method's potential. A less specific approach using siRNA-mediated downregulation of endogenous desmin expression combined with vector-mediated expression of wild-type desmin might shift the ratio of mutant to wild-type desmin to a sufficient extend in order to attenuate or prevent the dominant negative effect of mutant desmin [2]. This approach would require high levels of recombinant gene expression as endogenous desmin expression makes up 2% of the total protein mass in cardiac tissue [13].

Genome editing might offer a more elegant solution to this problem. An AAV-mediated CRISPR-Cas9 approach has been used in Duchenne muscular dystrophy (DMD) in postnatal and adult mice resulting in 37 and 67% of fibers expressing dystrophin in skeletal muscle, respectively [14–16]. With a relative expression level of 8%, dystrophin expression was still significantly lower in treated DMD animals than in wild-type controls [15]. As the pathophysiology of DMD resembles the type of desminopathy characterized by a lack of desmin expression, results from these studies might not easily transfer to desmin mutations exhibiting a dominant negative effect. However, with further optimization of efficiency, this method might become a promising tool targeting these mutations.

Finally, as changes in protein folding, recycling and degradation are a well-studied pathophysiologic feature accompanying desmin's life cycle, facilitating the correct function

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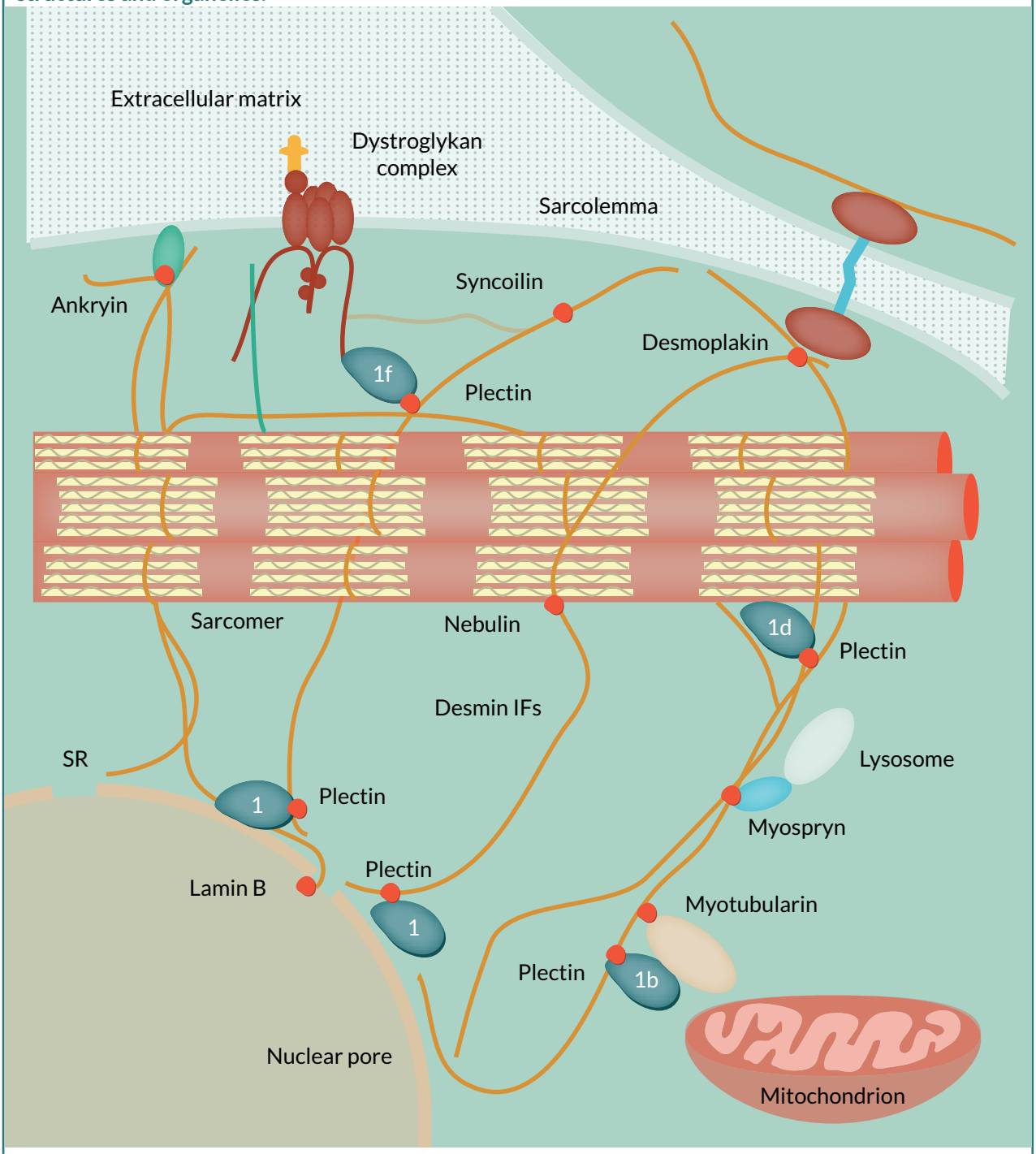
FIGURE 1

Desmin's ability to form filamentous networks is essential for maintaining structural integrity in muscle cells. Its critical role is evident in its interactions with other structural proteins connecting all major structures and organelles.



Physical interactions are marked with red dots. IF: intermediate filament; SR: sarcoplasmatic reticulum. Figure printed with permission and adapted from [19].

of these processes represents another therapeutic target. Mutations in alpha-B-crystallin, desmin's main chaperone, show similar cellular and clinical phenotypes as autosomal dominant *DES* mutations [17]. Overexpression of wild-type alpha-B-crystallin is an interesting

approach to decrease protein aggregates and promote proper filament formation. Aside from gene therapy, chemical chaperons, such as 4-phenylbutyrate, have also shown promising results [18].

In conclusion, desminopathies are challenging and very intriguing from a gene therapeutic point of view as pathophysiology varies with different mutations. Animal models mirroring their distinct pathophysiologic mechanisms are still scarce. Recent scientific advances have revealed promising new therapeutic strategies.

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