Review

Disease models of myositis: overview of cell culture and rodent systems

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ABSTRACT

Inflammatory myopathies (in short: myositis) display a heterogenic group of rare inflammatory diseases of the skeletal muscle and other organs such as lung, heart and skin. Patients typically display muscular weakness, wasting and a variable response to treatment. The pathogenesis involves invasion of muscle fibres by mononuclear cells and deposition of autoantibodies. In vitro and in vivo models are crucial to understand the so far unresolved complex network of pathomechanisms and how to design future treatment strategies. So far, no model can represent all features of the human disease, but each facilitates analysis of distinct mechanisms of the disease. A range of different in vitro and in vivo models have been developed in recent years to functionally study myositis pathology. This review provides an overview of muscle cell culture systems and transgenic as well as inducible animal models that each represent distinct features of myositis.

Introduction

Inflammatory myositis (in short: myositis) is a group of rare diseases that includes dermatomyositis, polymyositis, immune mediated necrotising myopathy, anti-synthetase syndrome, inclusion body myositis and myositis in overlap syndrome (1). Hallmark of these diseases is an inflammation of the skeletal muscle that leads to different degrees of muscle weakness and muscle atrophy (wasting). Apart from the skeletal muscle, several organs can be affected as well such as the lung, heart and skin. Treatment is often a challenge in severe cases and particularly organ involvement of the heart or lung are associated with a high mortality rate. The pathology of the myositis subtypes includes mechanisms of the innate and adaptive immune system such as various pro-inflammatory cytokines and chemokines, production of pathogenic auto-antibodies, lymphocyte infiltration of the muscle and attack of muscle fibres by auto-aggressive T-cells and macrophages (2, 3). Several non-inflammatory cascades such as protein metabolism, mitochondrial integrity and energy production, and cell stress pathways such as heat-shock and endoplasmic reticulum also play important role in tissue destruction or weakness (3). The design of more effective therapies is hampered by insufficient understanding of the pathomechanisms and how to best tackle them (4). The future development of treatments will depend on suitable models that reflect the most relevant pathways of the disease. We here review the models available for myositis, spanning from cell culture systems to animal models. We aim to demonstrate how the models can be used to achieve better understanding of the relevant pathomechanisms in myositis and which limitations exist.

Cell culture models of myositis

Cell culture models are essential to further understanding of pathomechanisms relevant to myositis including cellular inflammation of skeletal muscle, auto-antibody pathology, cell stress pathways, protein homeostasis, muscle regeneration, cytokine and chemokine signalling network. Moreover, *in vitro* models can help to pin-point novel treatment avenues. Therefore, disease specific, reproducible models are fundamental to tackle the complexity of the disease and complement or replace studies in animals. Several model systems for the *in vitro* study of myositis were developed during the last decades, based on 2D cell culture, 3D engineered skeletal muscle tissue and neuromuscular organoids (Table I) (5).

2D Monolayer cell cultures

Well established 2D monolayer monocultures of muscle cells using myoblast cultures derived by isolation of muscle stem cells from muscle biopsies (6), induced pluripotent stem cells (iPSC) (7, 8) or reprogrammed non-myogenic fibroblasts (9) are typically used but exhibit limitations regarding the organisation, maturation and interaction of different cell types (10). In monoculture assays, muscle cells were often stimulated by the addition of cytokines and chemokines to induce a phenotype comparable to different myositis subtypes. Especially for inclusion body myositis (IBM), fundamental insights could be achieved regarding immune cell attraction, β-amyloid accumulation and cytokine expression (IFN-γ, TNF-α, IL-1β) in myotubes (11-13). In addition to monocellular myoblast cell cultures and direct or indirect interaction in co-culture systems with immune cells, a recent paper showed a suitable *in vitro* model for inclusion body myositis using patient-derived fibroblasts (14). IBM-fibroblasts isolated from patients' biopsies were studied using transcriptome analysis and functional experiments. Inflammatory gene expression (*e.g.* HLA genes and IFN genes), cytokine secretion and impaired autophagy were comparable to findings in IBM-muscle tissue and could at least partially mimic the molecular mechanisms of IBM (14).

Improvements of the 2D cell cultures were achieved by co-culture systems using two or more cell populations in the same *in vitro* setup (Table I). 2D co-culture experiments of muscle cells and relevant surrounding cell types, *e.g.* immune cells, neural cells or fibroblasts, appeared superior to 2D monocultures regarding differentiation, maturation and autoimmune phenomena (15, 16). Of particular interest are co-cultures of immune cells and muscle cells with a focus on the impact of myositis patients' derived T cells (17-19) to mimic the *in vivo* relevance of infiltrating immune cells in autoimmune myositis. Another co-culture used dendritic cells (DCs) and myotubes revealed a stimulation of HLA-ABC and cytokine secretion, promoted by activated DCs (20). In this system, an increased muscle proliferation and migration, but reduced muscle differentiation was observed, indicating a crucial interaction between muscle cells and DCs in myositis.

Since muscle fibres in disease are typically surrounded by fat and fibrosis, the impact of adipogenic cells and fibroblasts was studied in several ways. Enhanced myoblast migration and differentiation was observed in co-culture assays using murine fibroblasts and muscle cells in spatially separated, paracrine systems (21, 22). Muscle cell apoptosis was reduced in co-culture experiments with chicken myoblasts and fibroblasts. These data indicated the requirement of the β1 integrin-PI3K/Akt pathway activation for myoblast protection from apoptosis during differentiation (23). Apart from paracrine effects of fibroblasts on myoblasts or *vice versa*, cell-to-cell-mediated effects were studied by co-culture of C2C12 myoblasts and 3T3 fibroblasts (both of murine origin) (24), revealing that direct cell-cell-contacts promoted fibroblast-dependent myoblast alignment. To overcome the restrictions of *in vitro* co-culture systems with two, often physically separated, cell populations, an *in vitro* triple co-culture method was designed (25). This method allowed the co-culture of at least three cell populations with some degree of direct cell contacts and revealed positive effects on myoblast proliferation and migration of either macrophages or fibroblasts. By contrast, triple co-culture of myoblasts, macrophages and fibroblasts resulted in a macrophage-dependent inhibition of fibroblast-driven myoblast migration. Yet, at the same time, macrophages continued to promote myoblast proliferation, independent of fibroblast presence or absence (25).

Beyond interactions between myoblasts and fibroblasts, the cross talk of muscle cells and neurons is an important area of research on neuromuscular diseases. In a co-culture of myoblasts and neural cells, the muscle cell viability and myotube formation was enhanced compared to monocellular systems (26-28).

3D engineered muscle and organoids

Limitations of 2D mono- or co-culture approaches occur with respect to mimicking central neuromuscular disease hallmarks including loss of muscle strength and contraction forces. To overcome these limitations *in vitro*, 3D muscle engineering appears as a versatile tool to study *in vitro* muscle function comparable to primary muscle biopsies from patients (29, 30). A central composition of 3D engineered muscle is the scaffold which defines the mechanical support of muscle cells to enable cell proliferation, organisation, differentiation and final maturation (31). Different scaffold types can be distinguished, based on the origin of their components. Synthetic-derived scaffolds comprise the advantage of a precise and customised composition that mainly includes polyurethane (PU), polyethylene glycol (PEG), polyvinyl alcohol (PVA), poly-L-lactic-acid (PLLA) and polylactic-co-glycolic-acid (PLGA) and can easily be supplemented with growth factors or other bioactive molecules (32, 33). Natural-derived scaffolds were obtained from decellularised tissues and organs and result in tissue-specific and well-preserved extracellular matrix components (34, 35). Frequently, these scaffolds were applied as hydrogel formulations in combination with collagen, matrigel or fibrin to achieve *in vitro* a proper simulation of *in vivo* conditions (36, 37). Generally, 3D models were self-organised using myoblasts and a scaffold of varying composition and formulation (38-40). Thereby, cells were embedded in a hydrogel mixture and 3D organisation occurred due to the presence of attaching points (41-45).

Bioprinting of biomaterial can overcome some of the limitations of manually produced, self-organised 3D engineered muscle. This technique allows construction of complex 3D structures through accurate positioning of cells, extracellular matrix (ECM) components and bio-reactive factors using bio-inks (46, 47). 3D bioprinting approaches have been successfully used to form *de novo* myofibres upon *in vivo* implantation (48, 49).

Co-cultures of 3D muscle cells with immune cells, neural cells or endothelial cells has been used to study molecular mechanisms of myositis. Neuronal in-

Table I. Selected *in vitro* models of myositis.

Method	Involved cell types	Disease model	Ref.
Cell Monolayer	Human primary skeletal myocytes	Inclusion body myositis	(11, 12)
Cell Monolayer	Patient-derived fibroblasts	Inclusion body myositis	(14)
2D co-culture	T cells and skeletal myocytes	Polymyositis	(17-19)
2D co-culture	DC and myocytes	Myositis	(20)
2D co-culture	Fibroblasts and myocytes	Myositis	(21-23)
2D co-culture	Fibroblasts, macrophages and mycocytes	Myositis; Muscle Regeneration	(25)
3D Culture	Immortalised myoblasts	Duchenne	(38)

DC: dendritic cell.

nervation in 3D engineered muscle co-cultures was found to be much faster and more efficient compared to 2D culture models (50). Vascularisation of 3D muscle engineering can help to avoid necrosis of the tissue core (51) and facilitate studying the pathophysiology of trans-vessel immune cell-mediated neuromuscular diseases and muscle regeneration. The supplementation of 3D engineered muscle with endothelial cells and pericytes was found to present a suitable *in vitro* model for muscle vascularisation (52, 53).

The development of complex organoids, representing self-organising, functional multi-cellular tissues represent the most recent addition to the growing repertoire of *in vitro* models for myositis. Neuromuscular organoids are a simplified mimic of the complex tissue structure and can be used to study functional approaches that cannot be addressed with other *in vitro* models, including evaluation of disease related functions of specific cell types (54). So far, only few studies could successfully employ an *in vitro* neuromuscular system using human pluripotent stem cells in organoids (40, 55, 56). These organoids were shown to be useful for studying muscular functionality including development, regeneration and contraction.

Take home messages

- 2D cultures are instrumental in addressing key pathomechanisms that are relevant to one or several myositis subsets
- 3D cultures offer the opportunity to track interactions between lymphocytes and myotubes in a more complex environment compared to 2D
- 3D cultures allow measurement of muscle contraction and strength and how that is affected by inflammatory mechanisms

Inducible animal models of myositis

Over the last decades, several inducible animal model systems have been developed to study myositis. Using different modes of induction, selective aspects of the myositis pathology or approach to design novel treatments could be studied (Table II).

Experimental autoimmune myositis (EAM)

Efforts to induce autoimmune myositis by immunising animals with muscle homogenates began over 60 years ago (57). Initially, muscle homogenates were injected along with complete Freund's adjuvant (CFA) in rats or guinea pigs. Following multiple refinements, EAM can be induced in SJL/J mice by subcutaneously injecting muscle homogenates or "partially purified" myosin B (MB) along with CFA for 3-4 times at one-week intervals (58, 59). EAM is histologically characterised by the predominant infiltration of $CD4^+$ T cells and macrophages. Autoantibodies against muscle antigens including MB have been detected in the serum of EAM mice. The transfer of T cells or IgG from EAM mice to naïve mice was capable of inducing myositis, which was attenuated in complement deficient mice, highlighting the crucial role of humoral immunity in this model (60). Studies utilising EAM have revealed the efficacy of various treatments, including $CD4^+CD25^+CD62^+$ regulatory T cell infusion (61), anti-CX3CL1 antibodies (62), a synthetic retinoid (63), and rapamycin (64). However, it is important to note that SJL/J mice have a defect in *dysferlin*, leading to spontaneous muscle fibre necrosis and inflammatory cell infiltration (65). Although the EAM induced in the young SJL/J mice should be independent of the defect in *dysferlin*, it is possible that this defect might have some impact on EAM development. In fact, only a few groups have successfully induced EAM in other strains, such as C57BL/6 (64, 66). Additionally, EAM in SJL/J mice displayed mild symptoms, and clinical manifestations such as muscle weakness were rarely observed (67). Methods for inducing EAM have continued to evolve: in Lewis rats, a more severe myositis was induced with reduced muscle strength by immunisation with purified MB with CFA, in combination with intraperitoneal injection of pertussis toxin (PT) (68). C protein isolated from purified MB derived from human skeletal muscle was used for immunisation with C protein and CFA, leading to severe myositis, suggesting C protein as a potential primary antigen in EAM (69). A successful induction of EAM was established in BALB/c mice by administrating increased dosage of rat MB, along with intraperitoneal PT injection. This EAM model exhibited reduced muscle strength, confirmed by the inverted screen test (70), and has been used to highlight the benefits of physical training in preserving muscle strength (71, 72).

C protein-induced myositis (CIM)

Recognising that skeletal muscle C protein is a primary immunogen of EAM

Table II. Inducible animal models of myositis.

Model	Induction method	Major mouse strain	Histological findings	Autoantibody	Clinical features	Pathogenesis
Experimental autoimmune myositis (EAM) (59, 60)	Repeated immunisation of muscle homogenates or myosin B	SJL/J	Muscle fibre necrosis, inflammatory infiltrates ($CD4^+$ T cell and macrophage dominant)	Anti-myosin B, anti-troponin, anti-actin antibodies (varies by immunogen)	Myositis	$CD4^+$ T cells, antibody, complement activation
C protein-induced myositis (CIM) (73, 77, 79)	Single immunisation of skeletal C protein fragment	C57BL/6	Muscle fibre necrosis, inflammatory infiltrates ($CD8^+$ T cell and macrophage dominant) surrounding/invading muscle fibres, upregulation of MHC class I molecules in muscle fibres.	Anti-C protein, anti-nuclear antibody	Myositis	$CD8^+$ T cells, innate immunity
Jo-1 mediated myositis (86, 87)	Single immunisation of Jo-1 fragment	C57BL/6	Sporadic inflammation, muscle fibre degeneration	Anti-Jo-1 antibody	Myositis, pneumonitis	Innate immunity
TIF1γ-induced myositis (TIM) (88)	Repeated immunisation with TIF1γ	C57BL/6	Muscle fibre necrosis, inflammatory infiltrates ($CD8^+$ T cell dominant), upregulation of MHC class I molecules in muscle fibres	Anti-TIF1γ antibody	Myositis	$CD8^+$ T cells
Humanised mouse model of IMNM (89)	Repetitive transfer of IgG derived from anti-HMGCR or anti-SRP antibody-positive IMNM patients	C57BL/6	Scattered necrotic muscle fibres, infiltration of macrophages, complement deposition	Anti-HMGCR or anti-SRP antibody	Myositis	Antibody, complement activation

TIF1γ: transcription intermediary factor 1γ; IMNM: immune-mediated necrotising myopathy; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase; SRP: signal recognition particle.

in Lewis rats, an improved murine autoimmune myositis model was induced in C57BL/6 mice, referred to as CIM (73). CIM is induced by a single intradermal injection of CFA-conjugated recombinant human C protein fragment, combined with an intraperitoneal injection of PT (73). CIM causes muscle dysfunction and weakness substantiated by the rotarod test and the grip strength test (73, 74). Histologically, CIM is characterised by $CD8^+$ T cell-dominated muscle tissue inflammation (75). CIM recapitulates the histological features characteristic of polymyositis (PM), including the infiltration of $CD8^+$ T cells in the endomysium surrounding and invading non-necrotic muscle fibres, and upregulation of MHC class I in muscle fibres (73). CIM exhibits a peak in inflammation around 2–3 weeks after the immunisation, followed by spontaneous resolution (76). While anti-nuclear antibodies as well as anti C protein antibodies were present, known myositis-specific antibodies (MSA) have not been detected in the serum of CIM (73). Muscle inflammation was attenuated in mice lacking β2-microglobulin or perforin, and adoptive transfer of $CD8^+$ T cells from CIM mice into naïve mice resulted in more severe muscle inflammation compared to that of $CD4^+$ T cells, indicating the role of auto-aggressive $CD8^+$ T cells in its development (77). Additionally, transfer of the bone marrow-derived dendritic cells presenting a CD8 epitope peptide was capable of inducing myositis in the recipient mice (78). Furthermore, activation of innate immunity in muscles with CFA is essential for the development of CIM (79). CIM, as an autoimmune myositis model involving both innate and acquired immunity, has uncovered potential therapeutic targets for myositis, including IL-6, IL-1, IL-15, IL-23, CXCL10, L-selectin, and CD80/86 (73, 75, 76, 80-83). Additionally, injured muscle fibres are capable of activating local innate immunity, promoting the muscle inflammation of CIM (84). A recent study on CIM has shown that injured muscle fibres undergo necroptosis, a form of lytic regulated cell death accompanied by the release of pro-inflammatory mediators, including HMGB1. The treatment of CIM with a necroptosis inhibitor or anti-HMGB1 antibodies suppressed muscle inflammation and recovered muscle strength (74). Furthermore, a GLP-1R agonist was identified to ameliorate CIM through suppressing muscle fibre necroptosis (85).

Jo-1 mediated myositis

While MSA have been utilised for diagnosing and predicting prognosis of myositis, their role in the pathophysiology remains elusive. To explore the potential involvement of MSA in the pathology of myositis, a few immunological murine models have been developed. A Jo-1 mediated myositis was developed by immunising mice with a fusion protein consisting of the amino terminal fragment of murine histidyl-tRNA synthetase (HisRS, Jo-1) and maltose binding protein (MA/MBP), along with CFA (86). A single subcutaneous injection of MA/MBP in B6.G7 mice re-

sulted in muscle and lung inflammation after 8 weeks. Histologically, sporadic inflammatory infiltrates were observed in muscles. In lungs, perivascular and peribronchiolar dominant lymphocytic infiltrates were observed (86). Intramuscular MA/MBP inoculation without an exogenous adjuvant could induce muscle inflammation in multiple congenic strains, including C57BL/6 and B6.G7 (87). In both models, anti-Jo-1 antibodies were detected in the serum, and a $CD4^+$ T cell response specific to HisRS was confirmed (86, 87). Notably, the mice lacking *Rag2,* with impaired antigen-specific T and B cell responses, still developed myositis (87). *Tlr4*-mutant mice also developed myositis, with lower serum anti-Jo-1 antibody levels compared to wild type mice (87). These studies implied a potential role of the innate immune response rather than HisRS-specific autoimmunity.

Transcription intermediary factor 1γ (TIF1-γ)-induced myositis (TIM)

TIM is an experimental myositis model induced by the immunisation of the molecule targeted by MSA. TIM was induced in C57BL/6 mice by intradermal injection of recombinant human TIF1γ-conjugated with CFA weekly for 4 times, along with an intraperitoneal injection of PT (88). The mice developed muscle inflammation 2 weeks after the last immunisation, with the presence of TIF1γ-specific T cells and anti-TIF1γ antibodies. Histologically, the majority of muscle-infiltrating inflammatory cells were $CD8^+$ T cells and MHC class I molecules were upregulated in muscle fibres. Experiments utilising knock out mice, antibody-mediated depletion, and adoptive transfers have highlighted the crucial role of autoreactive $CD8^+$ cells in TIM, with less involvement of B cells, $CD4^+$ T cells, and autoantibodies. MX1, an IFN-induced molecule, was upregulated in the affected muscle fibres in TIM. Furthermore, TIM development was partially suppressed in mice deficient of IFN-α/β receptors, underscoring the involvement of type I IFNs in this model. The treatment with a Janus kinase (JAK) inhibitor attenuated the muscle inflammation in TIM (88).

Humanised mouse model of immune-mediated necrotising myopathy (IMNM)

Anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and anti-signal recognition particle (SRP) antibodies are frequently detected in patients with IMNM. It was demonstrated that transferring IgG derived from patients with anti-HMGCR or anti-SRP antibody-positive IMNM into C57BL/6 mice could induce muscle weakness (89). Histologically, this model exhibited scattered necrotic muscle fibres with infiltration of macrophages and C5b-9 deposition, recapitulating the histological findings of IMNM. The muscle weakness in this model was mitigated in mice lacking C3 (89), emphasising the pathogenic impact of these MSA and complement activation in the pathophysiology of IMNM. The research team also showed that transferring the patient-derived IgG along with human complement further exacerbated muscle weakness (89), labelling this model as a humanised mouse model of IMNM. They identified that the treatment with a C5 inhibitor (90) or the human IgG1 Fc fragment (91) restored the muscle functions and histological findings in this model.

Take home messages

- Inducible animal models in mice or rats harbour acute inflammatory mechanisms of myositis including endomysial T-cell infiltration
- Antibody-mediated muscle inflammation in mice such as from anti-Jo1 resembles inflammatory mechanisms relevant for the respective myositis subset

Transgenic mouse models of myositis

There are significant challenges to developing pre-clinical animal models that recapitulate the autoimmune features seen in sporadic diseases such as myositis. To circumvent this, several animal models have been developed that manifest with myodegenerative features but not the inflammatory components observed in human myositis. These models have particularly emphasised disease models that develop protein aggregates and vacuoles similar to that found in muscle tissue from inclusion body myositis (IBM) patients.

One of the pathologic features seen in IBM patient muscle is the presence of proteinaceous inclusions often associated with "rimmed vacuoles (92)." These inclusions are stained with congo red and exert green birefringence under polarised light supporting that these inclusions are amyloidogenic (93). This finding led to further speculation that the myodegeneration seen in IBM was similar to that seen in neurodegenerative disorders that also accumulate amyloid inclusions such as Alzheimer's disease (AD). The principal component of amyloid plaques in AD brains is a proteolytic fragment from the amyloid precursor protein (APP) or Aβ (94). Dominant mutations in APP lead to an early onset AD phenotype with prominent amyloid plaques (95). Both APP and β-amyloid are present in IBM patient muscle although mutations in APP have not been found to be associated with muscle disease (96, 97).

Nonetheless, the overexpression human APP harbouring the "double Swedish mutation" associated with early onset AD in skeletal muscle recapitulated some of the pathologic features and progressive weakness seen in IBM patients (98). Specifically, MCK-βAPP mice develop age-dependent weakness, myopathy, Aβ-positive inclusions and inflammation (98). Augmenting Aβ levels in skeletal muscle by co-expression of APP and mutant presenilin-1 hastened weakness and myopathology (99).

These data supported that increasing the burden of an amyloidogenic protein may lead to myopathology similar to IBM. This was further demonstrated by expression of a non-IBM associated amyloid, Gelsolin. Dominant mutations in gelsolin lead to systemic amyloidosis with ophthalmological, neurological and dermatological symptoms (100). Restricted overexpression of gelsolin in mouse skeletal muscle resulted in muscle weakness, myopathologic features, and intracellular and extracellular amyloid accumulation (101). Surprisingly, these mice devel-

Table III. Genetic animal models of myositis.

Genetic model	Overexpression or knockin	Histological findings	Inflammation	Protein inclusions	References
Amyloid precursor protein (APP)	Muscle specific overexpression	Myopathy with central nuclei	Mononuclear cells	Intracellular Aβ	(98)
APP+presenilin-1(PS1)	Muscle overexpression (APP) and PS1 knockin	Myopathy with central nuclei	CD8+ T cells	Intracellular Aβ	(99)
Gelsolin	Muscle specific overexpression	Myofibre atrophy, rimmed vacuoles, basophilic inclusions	none	Congo red amyloid intra/extracellular Aβ, ubiquitin	(101)
Valosin containing protein (VCP)	Muscle specific overexpression (R155H)	Fibre size variability	none	Congo red, ubiquitin, TDP-43	(105, 111)
VCP	Systemic overexpression (R155H and A232E)	Internal nuclei; myofibre necrosis, rimmed vacuoles	none	Ubiquitin, TDP-43	(103, 106)
VCP	Knockin R155H	Central nucle; rimmed vacuoles		Ubiquitin; TDP-43	(104)
TDP-43	Muscle specific overexpression	Tubular aggregates		TDP-43; NT5C1A	(110)

oped rimmed vacuoles and a secondary accumulation of Aβ and ubiquitin inclusions (101). These data support that an increase in protein aggregate burden may lead to a collapse of myofibre proteostasis resulting in protein aggregation and vacuolation.

One form of hereditary myopathy with inclusions is due to mutations in the multifunctional ubiquitin adaptor protein valosin containing protein (VCP) (102). VCP is critical to maintain protein quality control and cellular homeostasis by facilitating the degradation and clearance of ubiquitinated proteins via the proteosome and autophagy. Dominant mutations in VCP lead to a variably penetrant multisystem degenerative syndrome that includes both frontotemporal dementia, amyotrophic lateral sclerosis and a myopathy with inclusions (102). Several mouse models of VCP associated disease have been generated including VCP mutant muscle restricted expression, global expression and knockin mouse models (103-105). These mice display the myodegenerative features of VCP-disease with weakness, myopathy, protein inclusions and in some cases rimmed vacuoles albeit at a late age. In fact, the VCP-A232E global transgenic mouse line has been utilised as a preclinical model for investigational drugs (103). Specifically, 10-month treatment of VCP-A232E mice with the heat shock inducer, arimoclomol, improved maximal tetanic force in the extensor digitalis longus muscle and reduced both ubiquitin and TDP-43 inclusions via immunohistochemistry (106).

TDP-43 is an RNA binding protein with an aggregate prone domain that is mutated in amyotrophic lateral sclerosis, frontotemporal dementia and more recently distal myopathies (107, 108). TDP-43 is present as ubiquitinated inclusions in ALS/FTD motor and cortical neurons and IBM patient muscle suggesting that its accumulation is primary driver of myodegeneration (109). Whether over expression of TDP-43 in skeletal can recapitulate IBM-like pathology remains to be seen. The overexpression of wild-type TDP-43 in mouse skeletal muscle generated prominent TDP-43 inclusions with a mild myopathy with tubular aggregates at 18 months of age (110).

Take home messages

- Genetic mouse models of myositis particularly resemble chronic, degenerative mechanisms in skeletal muscle with a strong relevance to IBM.
- Molecules that are used to induce protein accumulation in skeletal muscle as mechanism of myositis include APP, β-amyloid, TDP-43, and VCP.

Conclusion

In summary, a broad range of *in vitro* and *in vivo* models of myositis have been developed over the last decades (Tables I-III). Each model can contribute to the research on myositis pathophysiology and how to best design effective treatment modalities. The different approaches of 2D cell cultures, 3D cell cultures, organoid models, induced or genetic rodent models are complementary in that, as detailed above, each of the models has its advantages and disadvantages. Simple 2D cell cultures can help to quickly pin-point the timing and subcellular localisation of a certain inflammatory molecule. More complex models such as 3 D (co-)cultures, organoids, and inducible animal models will be superior in delineating the interactions between cell types and testing of therapeutic molecules. Genetically modified animal models can be instructive in understanding muscle function in conjunction with selected disease-relevant molecules or treatment approaches.

Some of the models uniquely recapitulate the histopathological findings characteristic of selective subtypes of myositis. However, most of the models display an acute onset followed by spontaneous resolution, which deviates from the chronic disease course of myositis. In addition, with few exceptions, the models fail to demonstrate extramuscular manifestations of myositis. Nevertheless, as exemplified by the involvement of both, acquired and innate immunity in the development of myositis, these models have unveiled the intricate interplay among different cell types in the pathophysiology and offer promise for identifying potential therapeutic agents in the future.

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