Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

A review of Genetic Etiology and Emerging Molecular Therapies for FSHD in Preclinical Studies

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ARTICLE INFO

Article type
Review article

Article history
Received: 15 Feb 2021
Revised: 10 March 2021
Accepted: 20 Apr 2021

Keywords
Facioscapulohumeral muscular dystrophy
Genetic therapy
Small interfering RNA

ABSTRACT

Facioscapulohumeral muscular dystrophy is one of the most common musculoskeletal diseases with a considerable burden. Most of the affected individuals experience muscle weakness as the common muscular symptom. Despite the underlying genetic mechanism which is extensively studied, curative treatment is not available for patients with facioscapulohumeral muscular dystrophy, and only supportive care is considered as the treatment of choice. Recently, several studies addressed the treatment of facioscapulohumeral muscular dystrophy by genetic engineering strategies, most of which indicate the effectiveness of different types of small interfering ribonucleic acids. However, these studies are still in the preclinical phase and it seems that there is a long way ahead of curing facioscapulohumeral muscular dystrophy despite recent advances in the field of genetic engineering. This study aimed to review the underlying genetic mechanism of Facioscapulohumeral muscular dystrophy alongside providing the latest preclinical studies related to the treatment of this disease.

Please cite this paper as:
Seyyed Taghia MR, Jafarzadeh Esfehani R, Boostani R, Shariati M, Sadr Nabavi A. A Review of Genetic Etiology and Emerging Molecular Therapies for FSHD in Preclinical Studies. Rev Clin Med. 2021;8(1):35-40.

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common musculoskeletal disorder after dystrophinopathy and myotonic dystrophy (1,2). Before the widespread use of genetic diagnostic techniques, the prevalence of FSHD was reported to be as high as 5 cases per 100,000 individuals, which is now reached up to 7 cases per 100,000 people (3). FSHD is now considered a genetic disorder with variable symptoms and a complex etiology (4).Among these variable clinical symptoms, weakness of the facial muscles leading to the loss of the ability to express emotions is a common clinical finding making it difficult to drink or pronounce certain words (5,6). Muscle weakness can also affect the eye, making it difficult to close eyes properly, resulting in conditions including dry eye and keratopathy (7,8). The weakness of the scapulae muscles is also present. The weakness of the scapula muscles leads to the winging and overriding

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scapula or poly-hill sign (8). Muscle weakness can affect the abdominal muscles, known as beevor's sign, impairing breathing in some patients (8).

Moreover, lumbar muscle weakness in some patients may cause abnormal posture and hyperlordosis (5, 8). Regardless of our current knowledge about the clinical manifestations, the genetic basis and emerging molecular treatment options for treating FSHD is still a challenging issue which is the main focus of the present review.

Literature review

Base on the genetic basis of the disease, FSHD is divided into two groups: FSHD1 and FSHD2. FSHD1 accounts for 95% of patients, while FSHD2 is less common accounting for 5% of patients. The dux4 (completely double homeobox4) gene is responsible for the clinical symptoms. The gene is located at the end of the long arm of chromosome 4 at position 4q35, within the D4Z4 satellite iterations (9)(8). The gene is expressed in healthy individuals only during the fetal period and remains un-expressed after birth due to the condensation of the gene's area. The density of this region depends on the number of D4Z4 repeats. Healthy individuals usually have 10 to 100 repetitions while people with different types of FSHD have less than 10 repetitions. The reduced number of replicates has a less inhibitory effect on gene expression and consequently, a greater amount of DUX4 protein will produce in cells (10) (Figure 1).

Moreover, the inhibitory effect of telomere has also been demonstrated in this area. Telomere shortening with age along with the accumulation of harmful effects of DUX4 protein justifies the cause of worsening of symptoms as affected individuals ages and therefore the progressive nature of the disease occurs (11). An interesting

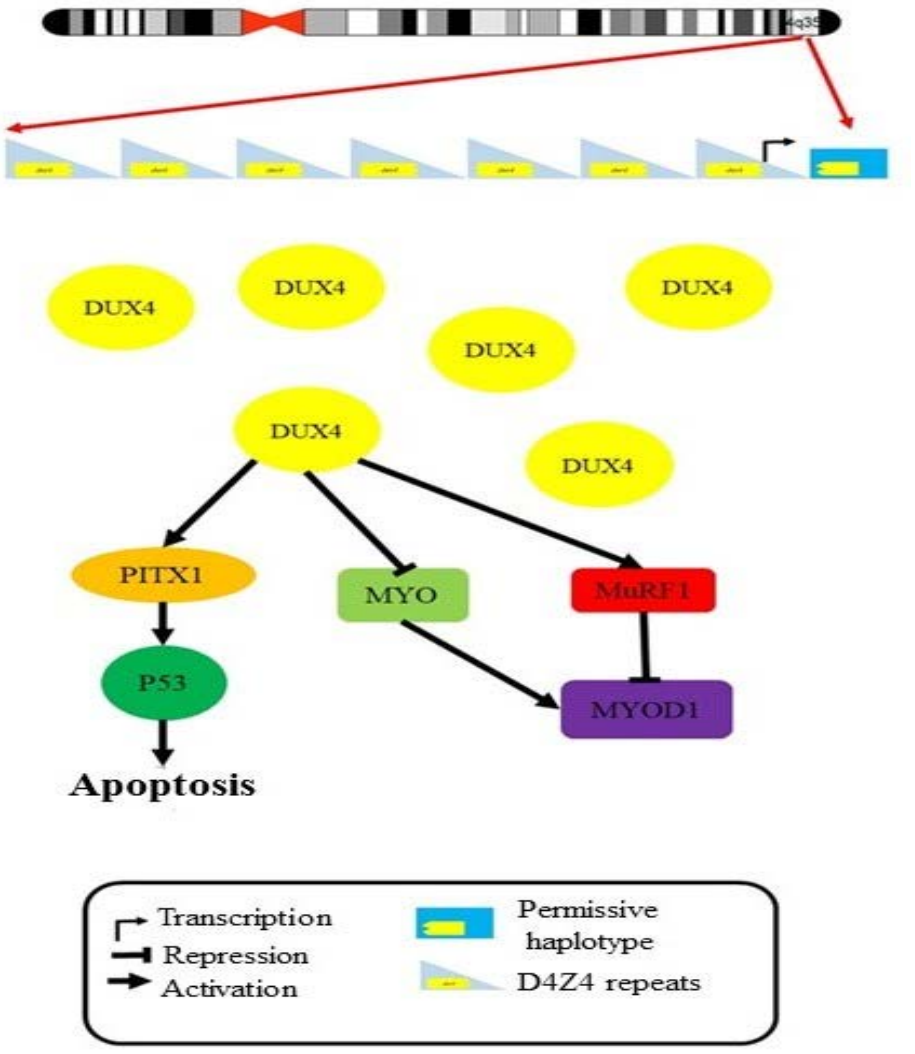

Figure 1. Reduction of the number of D4Z4 repeats within the pathogenic range (1-10 repeats) and presence of an authorized sequence bearing a polyadenylation signal leads to expression of DUX4 protein (yellow circle). DUX4 protein induces disease phe

fact about FSHD is that all D4Z4 repeats have a sequence encoding the DUX4 protein, but the

expression of this gene occurs only from the last repeat (closest to the telomere). The reason behind this phenomenon is the presence of a permissive haplotype or pLAM sequence in affected individuals (12). This haplotype has an ATTAAA sequence known as a polyadenylation signal. The presence of the polyadenylation signal leads to complete transcription of the gene and the production of a functional product. Other alternative transcripts of this gene are not functional because of the lack of major domains of this protein (13). Therefore, euchromatinization of this region alongside the presence of permissible haplotypes is necessary to cause the disease. So that, FSHD is considered to follow type 1 of the Digenic inheritance (14).

Unlike FSHD1, the FSHD2 patients have D4Z4 repeats within the normal range. However, this area is euchromatin in and dux4 expression occurs (15). This process is mostly due to a disruption in the pathway involved in creating density in this area. To induce chromatin condensation, a set of histone methyltransferases, polycomb, and lncRNA proteins (DBE-T in this case, which together with the ASH1L protein induces tritorax complex)

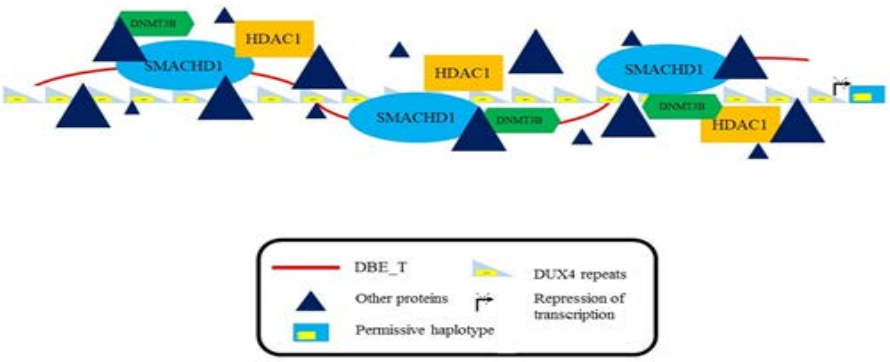

Figure 2. if the number of D4Z4 repeats are is within the normal range of 10-100 repeats, and a set of proteins alongside with lncRNA (DBE_T) will result ininduce condensation in this region. In this case, it inhibits gene expression becomes inhibit.

SMACHD1 and DNMT3B proteins are among the most important proteins involved in this process. Other proteins in this pathway are probably intolerable to damaging mutations as their generality in the chromatin compression mechanism (18, 19). Inactivated homozygous or compound heterozygous mutations in any of these genes, along with the permitted haplotype, leads to FSHD type 2 disease. This type of disease follows autosomal recessive inheritance (12,20).

dux4 acts as a transcription activator for another protein called PITX1 (paired-like homeodomain transcription factor 1). The PITX1 induces p53 apoptotic protein expression. Muscle cell death causes muscular weakness and disease-related symptoms (22). On the other hand, dux4 protein leads to the expression of MuRF1 protein. The MuRF1 is an E3 ubiquitin ligase specific for MYOD1 protein. The MYOD1 promotes the breakdown of muscle cells in the proteasome. Inhibition of transcription of the MYO gene activates the MYOD1 gene, inhibited by dux4 expression. These two processes lead to disruption of the myogenesis process (Figure 1) (23,24).

While considering the heredity of FSHD in families with a positive family history of the disease is important in diagnosing the affected individuals with even mild symptoms, however, it is important to note that about 1.3 patients may deal with new mutations (reduced pathogenic D4Z4 replications) misleading as a lineage similar to autosomal recessive inheritance (2,26,27). The standard diagnostic method for molecular diagnosis of FSHD type 1 is Southern blotting (28). For this purpose, DNA extracted from the patient's blood is treated with the MseI restriction enzyme, cleaving both sides of the D4Z4 repeat. In addition to chromosome 4, duplicates of the D4Z4 repeats have been observed on chromosome 10 having a high homology without pathogenic effects, challenging the interpretation of southern blotting for FSHD patients. To solve this problem, the restriction enzyme Bln1 is used. The enzyme only cut D4Z4 repeats on chromosome 10 (29).

In contrast, the Xap1 enzyme cuts only D4Z4 repeats on chromosome 4. Enzyme treatment and transfer on nitrocellulose paper using the Kpnl band probe related to repeats can also be used (30, 31). The number of repeats can be measured from the band position. A reduction in the number of repeats (less than 10) confirms FSHD type 1. In case of a normal number of repeats, FSHD type 2 should be considered as the possible diagnosis. Eighty percent of FSHD type 2 cases are caused by mutations in the SMACHD1 gene (12).

Different types of mutations observed in this

gene result in the development of the disease by haploid insufficiency and predominantly negative mechanisms (12,32). The other gene considered in the diagnosis of FSHD is SMACHD1. If sequencing of SMACHD1 becomes unremarkable, DNMT3B gene and its common mutations (p.Pro691Leu and p.Cys-527Arg) came into consideration (19). If there is no mutation in these genes, other myopathies including Limb Girdle type 2A, maltase deficiency disease, and mitochondrial diseases should be considered as differential diagnoses (33-37).

After making a definite diagnosis, many patients seek a cure for their disease. Unfortunately, there is no cure for the disease and the treatments are mainly based on reducing the symptoms and improve the patient's clinical condition (38). Mostly, aerobic exercises are recommended for these patients with a restricted intensity not reaching themaximum lactic acid threshold (39,40). Exercise, in addition to strengthening patients' muscles, helps to improve the immune system relieving oxidative stress (40,41). Correction of lordosis in patients with orthosis help improving patients' breathing. In more severe cases, surgery would be the treatment of choice (41). Among the available pharmaceutical treatments, the use of antioxidants and non-steroidal anti-inflammatory drugs is considered a palliative therapy in most patients. ATYR1940 (Resolaris), regulating the immune system and reducing inflammation, is currently used alongside ACE-083, increasing the muscle volume and strength by inhibiting TGFβ protein (42,43). Resolaris has completed phase one and two clinical trials, and ACE-083 is in phase two clinical trials (43,44). However, as mentioned earlier, none of these interventions cure the disease and will only slow down the progression of the disease. patients become wheelchair-bound. In addition to searching for a drug, gene therapy has also become an important field of research. So far, various The disease gets worsen by aging and some researches have been done on gene therapy strategies for this disease. We narratively reviewed studies in PubMed databases addressing novel therapeutic approaches by using molecular techniques. However, all of these studies are in the preclinical stages and have not entered the clinical trials phase and almost all of these strategies include interfering RNA (siRNA, miRNA, antisense oligonucleotide).

In 2013, a study by Miller et al., used the siRNA technique for activating Wnt / β-catenin signaling pathway in muscle cells, thus inhibiting DUX4-induced apoptosis. Two years later, a study by tapscott SJ et al. showed that targeting the upstream regions of the dux4 gene's transcription start site using exogenic siRNA inhibits the expression andincreases histone 3 methylation at the lysine 9 site (which Leads to condensation in this area).

This research group has previously shown that endogenous siRNA and miRNA, expressed from the same D4Z4 repeats, reduce dux4 expression. Also in 2017, Belayew et al. used the antisense oligonucleotide targeting the dux4 mRNA reporting that the atrophic phenotype of FSHD mutations could be suppressed but the irregular phenotype of FSHD mutations did not improve. This could be due to the over-expression of DUX4c. For this reason, this study has identified DUX4c as a therapeutic target for future researches. Other preclinical studies that examined different types of interventions in this disease are summarized in Table 1.

Table 1. Summary of intervention studies related to the treatment of FSHD

Fist author	Publication date	Type of intervention	Sample used	result	Clinical stage
Wallace LM et al. (45)	2017	Using miRNA (mi405) transcript into DUX4s	HEK293 cells	Effective	Preclinical
Dib C et al. (46)	2016	Fusing patients myoblast by pre-myoblast of healthy individuals	Muscle biopsy	Forming a hybrid myoblast consisted of 60% normal cells	Preclinical
Lim JW et al. (47)	2105	epigenetically repression of D4Z4 repeats by siRNA	Pre-myoblast from patients and controls	Potential therapeutic effects	Preclinical
Giesige CR et al. (48)	2018	Transfusion of follistatin (antagonist of myostatin)	Mice model	Recovery in volume and strength of muscle induced by dux4	Preclinical
Lindsay m Wallace et al. (49)	2011	Use of miRNA against FRG1 (miFGR1.984)	HEK293 cells and mice	Improve power and histology of muscles	Preclinical
Himeda CL et al. (50)	2016	Using sgRNAs (CRISPR/dCAS9) for exon1 and promotor of DUX4	Patients muscle biopsy	Repressed transcription of dux4-fl	Preclinical
Sergia Bortolanza et al. (51)	2011	Designing a shRNA and systemically injections in mice	Mice and C2C12 cells	Effective	Preclinical

Conclusion

FSHD is among the important neuromuscular diseases and patients with this disease face many challenges during their daily lives. Unfortunately, although the pathogenesis of this disease has been well studied, however, there is not any curative treatment and most treatments rely on supportive care. Regarding the recent advances in diagnostic genetic testing. Regarding the recent advances in using new genetic engineering techniques including the use of interfering RNA and their promising results in the preclinical phases, we may hope for effective treatment of this disease shortly.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgments

There is no source of funding to acknowledge.

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