

Control of DUX4 Expression in Facioscapulohumeral Muscular Dystrophy and Cancer

Anna Karpukhina, Eugenia Tiukacheva, Carla Dib, Yegor S Vassetzky, Svetlana Dokudovskaya

▶ **To cite this version:**

Anna Karpukhina, Eugenia Tiukacheva, Carla Dib, Yegor S Vassetzky, Svetlana Dokudovskaya. Control of DUX4 Expression in Facioscapulohumeral Muscular Dystrophy and Cancer. Trends in Molecular Medicine, 2021, 27 (6), pp.588 - 601. 10.1016/j.molmed.2021.03.008 . hal-03375995

HAL Id: hal-03375995
https://hal.science/hal-03375995

Submitted on 16 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Highlights

- *DUX4* is a transcription factor expressed in early embryos and repressed in most adult tissues *via* multiple genetic and epigenetic mechanisms;
- *DUX4* aberrant expression caused by genetic abnormalities is linked to facioscapulohumeral dystrophy (FSHD);
- Recent data point to chromosomal rearrangements involving *DUX4* as driver in leukaemias and sarcomas;
- *DUX4* is reactivated in solid cancers;
- *DUX4* expression is regulated by several feedback loops;
- Several approaches targeting DUX4 in FSHD and cancer are currently being developed

Manuscript Click here to access/download;Manuscript;DUX4 regulation R1.docx

Control of *DUX4* expression in FSHD and cancer: from feedback loops to chromatin loops

Anna Karpukhina[1-3], Eugenia Tiukacheva[1], Carla Dib[1,4], Svetlana Dokudovskaya[1], Yegor S. Vassetzky[1,2,5]

[1]UMR 9018, CNRS, Université Paris Saclay, Institut Gustave Roussy, F-94805 Villejuif, France

[2]Koltzov Institute of Developmental Biology, Moscow 117334, Russia

[3]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119991 Moscow, Russia

[4]Stanford University School of Medicine, Stanford, CA 94305-510, USA

[5]To whom correspondence should be addressed:

Abstract

DUX4, a gene encoding a transcription factor involved in early embryogenesis, is heavily repressed in most somatic tissues while its aberrant expression is linked to facioscapulohumeral muscular dystrophy (FSHD). Recently, the deregulation of *DUX4* expression was found in many cancers. While DUX4 role in FSHD is well studied, its relevance in cancer is less explored. Here we discuss multiple levels of control of *DUX4* expression, including enhancer-promoter interactions, epigenetic modifications, non-coding RNAs and telomere positioning effect. We also connect disparate data on intrachromosomal contacts involving *DUX4* and emphasize feedback loops in *DUX4* regulation. Finally, we bridge data on *DUX4* in FSHD and cancer and discuss prospective approaches for future FSHD therapies and the potential outcomes of *DUX4* inhibition in cancer.

Keywords: FSHD; leukemia; Ewing-like sarcoma; rhabdomyosarcoma; DUX4; gene regulation; transcription; epigenetics

DUX4 expression in development and pathologies

DUX4 is a transcription factor containing two homeobox DNA binding domains and a C-terminal transactivation domain. The *DUX4* open reading frame (ORF) is present in multiple copies throughout the human genome. Most of the copies are located within 3.3kb **D4Z4** repeats, organized into long polymorphic macrosatellite arrays in the subtelomeric regions of chromosomes 4q35 and 10q26; many D4Z4-related sequences are also dispersed elsewhere in the genome, notably in the acrocentric chromosomes and heterochromatic regions on 1q12 [1,2]. *DUX4* is expressed in the early embryo consistent with its likely function in stimulating **zygotic genome activation (ZGA)** (reviewed in [3], and is then repressed in the majority of adult tissues, except for the testis [4] and the thymus [5].

Aberrant *DUX4* expression is considered to be one of the major causes of the facioscapulohumeral dystrophy (FSHD), an autosomal dominant disorder characterized by progressive and asymmetric muscle weakness [6]. Recently, chromosomal *DUX4* rearrangements were implicated in the pathogenesis of **rhabdomyosarcoma (RMS)** [7], **Ewing-like sarcoma** [8] and **B-cell precursor acute lymphoblastic leukemia (BCP-ALL)** [9]. All these translocations result in the production of putative chimeric proteins implicated in oncogenesis (reviewed in [10]) (see Key Figure 1). Thus, understanding how *DUX4* expression is regulated in physiological and pathological conditions is essential for development of potential treatment strategies.

DUX4 expression and its consequences

Physiological conditions

DUX4 transcripts are detected from the oocyte stage to the 4-cell (4C) embryo and DUX4 transcriptional targets are detected from 2C to 8C [11], concordant with the major wave of

zygotic genome activation (**ZGA**), which takes place at ~ 2C in mice and 4C-8C in humans [12]. Both mouse Dux and human DUX4 activate ZGA-associated genes [13,14], and DUX family proteins have been proposed to be key ZGA-inducers in placental mammals [11]. Nevertheless, more recent studies indicate that Dux is not strictly required for the survival of mouse embryos and DUX proteins are rather non-essential ZGA synchronizers [15–18], that possibly facilitate post-implantation development [18]. In adult human tissues, except for testis [4] and thymus [5], *DUX4* is heavily repressed and its post-embryonic function (if any) remains unknown.

Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is the most extensively studied DUX4-associated pathology (see **Box 1**). The *DUX4* gene located within each D4Z4 repeat [19] is considered the major cause of FSHD [20]. DUX4 protein is cytotoxic in postnatal tissues and *DUX4* aberrant expression is associated with apoptosis [21–23], oxidative stress and DNA damage [21,24], myogenesis disruption [21,25–27], immune response [28] and impaired transcript quality control [29–31] in several cell types. Nevertheless, the exact mechanism connecting DUX4 production and specific pathological phenotypes observed in FSHD still remains to be elucidated.

Cancer

Genes aberrantly expressed in cancer may both induce oncogenic transformation, i.e. be cancer drivers, or be associated with it, i.e. serve as biomarkers. Depending on the cancer, DUX4 may be either a driver or a marker. In several cancers, chromosomal translocations produce chimeric genes with the participation of *DUX4*. In BCP-ALL, these translocations

lead to the insertion of *DUX4* ORF into the **immunoglobulin heavy chain (*IGH*)** gene locus [9,32–34] or into an intron of the **ETS-related gene (*ERG)*** [9]. In both *DUX4-IGH* and *DUX4-ERG* **fusion genes**, the *DUX4* homeodomains are preserved, while the part of the *DUX4* ORF encoding for a C-terminal transactivation domain is truncated and/or partially replaced by the non-coding parts of the host genes [9,32,33,35]. Thus, the resulting putative fusion proteins would retain the DNA-binding specificity of DUX4, but would not activate transcription in a DUX4-specific manner, which was indeed experimentally demonstrated for the DUX4-IGH protein in a cellular BCP-ALL model [36]. DUX4-involving rearrangements in BCP-ALL are accompanied by *DUX4* overexpression [9,33], as well as frequent production of an alternative ERG isoform with transforming properties, ERGalt [33], which is likely produced due to the interaction of DUX4 homeodomains with the alternative transcription initiation site in *ERG* intron 6 [33,35]. Finally, the chemotaxis and cytokine environment may favour a relapse in BCP-ALL patients carrying *DUX4-IGH* rearrangements [37]. DUX4 upregulates chemotaxis genes in myoblasts and stimulates mesenchymal stem cell migration in **CXCR4** (C-X-C motif Receptor 4)-dependent manner [38]. A similar cell migration mechanism may favour relapse in *DUX4-IGH* BCP-ALL patients.

In Ewing-like sarcomas, chromosomal translocation generates a fusion between *DUX4* and the capicua transcriptional repressor (***CIC***) gene [8,39,40], encoding for a strong tumor suppressor [41]. The resulting CIC-DUX4 fusion protein is ~90% similar to the original CIC, but gains the C-terminal transactivation domain of DUX4, thus reversing its own function to activate the formerly repressed genes, i.e. *PEA3* genes of the ETS family [8] that promote cellular proliferation and migration [42]. Overexpression of the *ETV4* gene of the PEA3 subgroup is observed in 90% of the *CIC-DUX4*–expressing tumors and distinguishes them from other sarcomas [8,43–45]. The pro-metastatic function of ETV4 demonstrated in studies with CIC inactivation [46,47] may explain the rapid progression and increased metastatic

activity of *CIC-DUX4*-positive sarcomas [48]. Other transcriptional targets of CIC-DUX4 include cell cycle regulators, notably *CCNE1* [49] and negative regulators of MAPK-ERK signaling, such as *DUSP6* [44].

In one case of embryonic RMS, t(4;22) translocation led to the *EWSR1-DUX4* fusion [7], which might have contributed to a specific striated muscle differentiation phenotype of this tumor.

DUX4 is reactivated in many solid cancers, where it inhibits the induction of MHC1 (major histocompatibility complex 1) by gamma interferon [50]. This decreases antigen presentation by cancer cells and reduces recognition by cytotoxic T cells, contributing to cancer cells immune evasion and resistance to immune checkpoint blockade [50]. DUX4 is also one of the markers predicting the transition of sinonasal papilloma to carcinoma [51]. On the other hand, in colon adenocarcinoma, DUX4 may inhibit growth and proliferation of cancer cells as it interacts with the cell cycle regulator cyclin-dependent kinase 1 (**CDK1**), blocking its activity [52]. When ectopically overexpressed in multiple cell-types, DUX4 causes cellular toxicity and cell death [21–23], though this phenomenon has not been observed in cancer cells so far, probably because of the insufficient level of its expression and/or the alterations of the *DUX4* ORF following chromosomal aberrations. Thus, an increasing body of evidence links *DUX4* to cancers of different origin, both as a driver and as a marker.

Regulation and silencing of *DUX4* expression

Gene expression is regulated by hierarchical interactions of various control elements. Enhancers activate transcription via interaction with promoters essential for transcription initiation. Insulators block the enhancer-promoter interactions. The three-dimensional folding of these control elements in the nuclear space orchestrates regulation of gene expression. All

these elements are present in the 4q35 locus and participate in the regulation of the DUX4 gene in physiological conditions, FSHD and cancer (**Figure 2**).

Promoters

The D4Z4 repeat where the *DUX4* gene is located has long been considered "junk" DNA (reviewed in[53]) until an open reading frame for a double homeobox gene with a cryptic promoter sequence was discovered inside each D4Z4 unit [2,19,54]. The *DUX4* transcripts are generated from the most distal D4Z4 unit, stabilized by the polyA provided by an adjacent 4qA sequence (see Box 1). Three major splice isoforms of the polyadenylated *DUX4* transcript exist: two *DUX4-fl* transcripts containing the entire *DUX4* ORF and one or two introns spliced in the 3'- UTR, and a *DUX4-s* transcript utilizing the cryptic splice site inside the *DUX4* ORF that conserves the double-homeobox domain without the C-terminal part of *DUX4* [4,55]. *DUX4-s* transcripts are observed in both healthy and FSHD muscles and are not associated with the pathology, while *DUX4-fl* transcripts are specific to FSHD [4,55]. The switch from the canonical to the cryptic splice site leading to the predominant DUX4-s production is possibly correlated with the accumulation of repressive chromatin modifications in the region [4]**. Nonsense-mediated decay** (**NMD**) eliminates *DUX4-fl* mRNA transcripts due to the presence of a spliced intron in the 3′ UTR of *DUX4* [29]. Importantly, NMD is inhibited in myoblasts ectopically expressing *DUX*4 [29].

Enhancers

Though each D4Z4 repeat contains a functional promoter [19,54], the muscle specificity of FSHD suggests the presence of additional tissue-specific regulatory elements modulating the transcription from the D4Z4 units. A strong myo-specific enhancer interacting with KLF15 transcription factor is located inside the D4Z4 repeat [56,57], but, surprisingly, this enhancer

activates the genes proximal to D4Z4, *DUX4c* and *FRG2*, but not *DUX4* located within the D4Z4 unit itself [57]. Two muscle-specific enhancers named **DME1** and **DME2** (for DUX4 Myogenic Enhancer) are situated proximally to the D4Z4 array (**Figure 2**) [58]. They interact directly with the *DUX4* promoter in both healthy and FSHD myoblasts, but *DUX4* activation is only observed following their association with the hypomethylated promoter in FSHD cells. DME1 and DME2 contain binding sites for several myogenic transcription factors: PAX7, Myogenin and MYOD [58].

Promoter function is conserved in some pathological conditions, e.g. in FSHD and some cancers where a 5'part of the gene fusion is composed of the *DUX4* gene. On the contrary, partner promoters (e.g. that of *CIC)* are used when the 3' part of the *DUX4* gene is involved in the fusion. DME1 and 2 only participate in the pathological regulation of *DUX4* expression in FSHD and possibly some cancers where *DUX4* overexpression is not linked to translocations. In other cases, enhancers from the host regions, e.g. *IGH* μ enhancer, activate expression of *DUX4* and its fusion variants.

Insulators

Insulators mediate long-distance intra- and inter-chromosomal interactions *via* loop formation and epigenetic modifications [59]. A region adjacent to the D4Z4 array harbors the **FR-MAR** insulator (FSHD-related nuclear matrix attachment region) [56,60,61] (**Figure 1**). This region separates the chromatin in the 4q35 locus into two distinct domains, so that the enhancers (DME1, DME2 and the enhancer within the D4Z4 repeat) and their target genes (*DUX4c*, *FRG2* and *DUX4*) can no longer interact. In FSHD myoblasts, the FR-MAR insulator is non-functional: the distinct loops fuse into one and the insulator activity is abolished, thus permitting expression of several genes within the locus [56,61]. The presence of specific

haplotypes (4A161, 4A159 and 4A168) within the insulator sequence together with the methylation of a specific CpG is important for the formation of the two distinct chromatin domains and the insulator function [60]. It is currently unclear whether the insulator is involved in DUX4 regulation in cancer.

Epigenetic regulation of DUX4 expression

DNA methylation

The CpG-rich D4Z4 array is located close to the telomere [62] in the nuclear periphery [63] and is highly methylated in most somatic tissues, including skeletal muscle [64]. Two genes associated with DNA methylation: *SMCHD1* and *DNMT3B*, are often mutated in FSHD. Thus, it is tempting to hypothesize that the hypomethylation of the D4Z4 array on chromosome 4 is one of the reasons underlying the FSHD pathology. Nevertheless, the methylation level of the D4Z4 array is highly variable in both healthy and FSHD1 individuals and seems to correlate with the number of D4Z4 units rather than the disease status (reviewed in [65]). In contrast, hypomethylation resulting from the *SMCHD1* or *DNMT3B* mutations in FSHD2 seems to be the major cause of *DUX4* derepression, though the strict correlation between *SMCHD1/DNMT3B* variants, the extent of D4Z4 hypomethylation and FSHD2 symptoms has not been established yet.

Hypo- or hypermethylation of the D4Z4 repeats was observed in a number of cancers [66,67], tumor cell lines [68] and in human bladder carcinoma cells treated with DNA-methyltransferase inhibitor zebularine [69], although most of these studies employed PCR-based techniques which were not specific to a particular D4Z4 array. A specific study of the 4q35-associated D4Z4 array demonstrated that D4Z4 repeats were hypomethylated in FSHD

and sperm cells, while both hypo- and hypermethylation was observed in cancer cells [70]. Interestingly, this study revealed methylation-resistant regions within the first 2.2 kb of each D4Z4 unit. These regions were not methylated in cancers with otherwise hypermethylated D4Z4 repeats and contained G-runs (several consecutive guanines) that could form **G-quadruplexes (GQs)** *in vitro* [70]. These structures might be important for the intra-array looping within the D4Z4 array of normal length, while in the contracted D4Z4, this looping would be disrupted allowing for the long-distance interactions [70]. The GQs are present in the promoter zone, the coding region, and the enhancer region of *DUX4* [71]. Treatment with a GQ-stabilizing compound berberine downregulates expression of *DUX4* and its target genes *ZSCAN4*, *TRIM43* and *MBD3L2* in FSHD myoblasts and reduces the amount of DUX4 protein in mice with exogenous *DUX4* expression [71].

Histone modifications

DUX4 transcription is also affected by histone modifications. Generally, histone acetylation is linked to transcriptional activation, while methylation can both activate (*e.g.* H3K4me2) and repress (*e.g.* H3K9me3 and H3K27me3) transcription. H3K9me3 is present within D4Z4 repeats on chromosomes 4 and 10 in healthy individuals but lost in FSHD1 patients [72,73]. The ratio of repression *vs.* activation marks (H3K9Me3 vs. H3K4Me2) is reduced in myoblasts and fibroblasts from FSHD patients; the lowest values correspond to severe symptoms [74]**.** In some regions of 4q35, both repressive and activation marks coexist: H3K27me3 and H3K27Ac were detected simultaneously at the DME1 enhancer [58].

Proteins

Certain proteins were reported to maintain the D4Z4 silencing. Each D4Z4 unit contains a region named **DBE** (D4Z4 binding element) proposed to confer copy-number-dependent

repressive activity to the D4Z4 array [75]. DBEs recruit HMGB2 chromatin-associated protein, YY1 transcriptional repressor and EZH2 protein of the Polycomb group (PcG) [75–78]. PcG proteins act to preserve the repressed chromatin state through DNA methylation, histone deacetylation and H3K27 trimethylation [79]. Intriguingly, a significant reduction of EZH2 and H3K27me3 repressive mark in the 4q35 region was observed in FSHD1 patients suggesting the loss of Polycomb-mediated silencing in the FSHD locus [77]. Recently, **NuRD** (**Nucleosome Remodeling Deacetylase**) and **CAF-1** (Chromatin Assembly Factor 1) protein complexes were also reported to play a role in *DUX4* gene repression by binding to D4Z4 repeats in healthy human muscle cells [80]. This repression is partially relieved in FSHD cells due to the DUX4-induced activation of MBD3L2 protein [80] which can replace the MBD2 and MBD3 subunits of the NuRD complex, thus possibly disrupting NuRD recruitment to its DNA targets [81].

Non-coding RNAs in DUX4 regulation

Non-coding RNAs also participate in *DUX4* regulation. Long non-coding RNA (lncRNA), *DBE-T* transcribed from the DBE of the most proximal D4Z4 unit and the NDE (Non Deleted Element), an element proximal to D4Z4 that is maintained in FSHD patients whatever is the extent of the D4Z4 contraction, may contribute to *DUX4* derepression in FSHD cells by interacting with the ASH1L protein of the Trithorax group (**trxG**) (**Figure 3**) [77]. TrxG proteins act antagonistically to those of the Polycomb group and activate genes that are normally repressed by the PcG proteins, including *DUX4. DBE-T* transcription might be affected by the chromatin conformation of 4q35 which is altered upon D4Z4 contraction. Inhibition of DNA-methyltransferases and histone deacetylases caused a mild de-repression of *DBE-T* in the CHO hybrid cells containing human chromosome 4 [82]. Specific non-coding RNAs may also participate in pathogenesis of the *DUX4* BCP-ALL subtype where a

strong correlation exists between expression of lncRNAs and the genes involved in the activation of TGF-β and Hippo signaling pathways [83].

D4Z4 units produce microRNA-sized fragments as a result of bi-directional transcription [55]. These fragments may generate siRNAs and activate the endogenous RNA interference pathway to maintain repeat-mediated epigenetic repression of the D4Z4 array. Targeting exogenous siRNAs to the region upstream of the *DUX4* transcription start site caused *DUX4* suppression in FSHD muscle cells and was accompanied by **AGO2** (Argonaute 2) recruitment and H3K9 methylation in the targeted region [84]. In contrast, knockdown of AGO2 or DICER, essential components of the RNAi pathway, induced *DUX4* expression in healthy muscle cells, suggesting that RNAi is indeed required for the D4Z4 silencing [84]. Interestingly, *DUX4* induction following AGO2 knockdown is dependent on the D4Z4 repeat number: *DUX4* was reactivated in the cells from two unaffected individuals carrying 13 D4Z4 repeats, but not in the cells from an individual carrying 74 D4Z4 units [84]. miRNome/transcriptome analysis revealed 29 microRNAs differentially expressed between the control and FSHD primary myoblasts, including those that potentially target *DUX4* [85].

Telomere length and DUX4 regulation

The 4q35 region is subtelomeric and the expression of its genes may be affected by telomere length. Indeed, *DUX4* and *FRG2* expression in the myoblasts and myotubes of FSHD1 patients is inversely proportional to telomere size; this could explain the age-related severity of FSHD [86]. Importantly, this correlation was not observed in healthy individuals, indicating that a large D4Z4 array might isolate the genes of the 4q35 region from the telomere, thus protecting them from a transcriptional deregulation due to telomere shortening. The effect of telomere shortening was greater on *DUX4*, the gene closest to the telomere, than on *FRG2* which is approximately 70Kb more proximal, and the most proximal of the three

genes, *FRG1*, was not at all affected [86]. Telomeres can also participate in long-range chromosome looping and thus influence gene expression over large distances (Telomere Position Effect-Over Long Distances - **TPE-OLD**) [87]. A telomere loop participates in the regulation of the *SORBS2* gene in the 4q35 locus. *SORBS2* is upregulated in the FSHD myoblasts with short telomeres, while its expression is low in healthy myoblasts and in FSHD myoblasts with long telomeres suggesting that the expression of this gene is altered by TPE-OLD in pathogenic conditions [88].

3D organization of the 4q35 locus

Different chromatin conformations allow genes to interact with each other and the control elements affecting their transcription (reviewed in [89]). 4q35 genes are involved both in short-range and long-range ***cis*-interactions**. In normal cells, many short-range interactions are observed, e.g D4Z4 interacts with *TUBB4Q*, *FRG1*, and *DUX4c*; *DUX4c* interacts with *FRG1*, *FRG2* and the 4qA/B region [76,90,91]. Long-range interactions in normal cells are few; they include contacts of the D4Z4 region with *ZFP42*, *FAM149A*, and *SORBS2*; *SORBS2* with *WWC2*, *FRG1* and the telomere [88,90,92]. In FSHD cells, many of the short-range contacts disappear, while some new long-range contacts emerge (**Figure 4**). Notably, 4qA/B interacts with *ANT1* and *D4S139* interacts with *FAT1* in FSHD cells [90–92]. It should be noted that there are discrepancies in the datasets from different studies. They can be explained by differences in experimental approaches used but also by the presence of the D4Z4 on other chromosomes [88]. In general, however, all results converge on a transition from short-range interactions in normal cells towards long-range interactions, in agreement with a general derepression of the locus (**Figure 4**). These changes in chromatin architecture lead to transcriptional upregulation of many genes in the 4q35 region.

In conclusion, *DUX4* is tightly regulated at the genetic and epigenetic levels to assure its timely expression in early development and repression in somatic cells. Failure to repress the *DUX4* in skeletal muscles leads to FSHD onset. In cancer, the *DUX4* gene is translocated to other chromosomes and is found in a different genomic context. In these cases, *DUX4* expression is due to the presence of control elements in the partner regions; they also provide the poly-A signal, essential to *DUX4* mRNA stabilization. The mechanisms of recently discovered *DUX4* overexpression in a panel of cancers [50] merit a separate investigation.

Feedback loops in DUX4 regulation

Even though most recent studies agree on the DUX4 involvement in FSHD pathology, this concept still raises questions. *DUX4* is believed to be expressed stochastically at a very low level [4], and is often undetectable in the biopsies of FSHD patients. How can such a rare event trigger the disease onset?

Multiple evidence suggests that DUX4 can amplify its own gene expression in a number of **feedback loops**. DUX4 induces oxidative stress and renders the cells more susceptible to it [21,24,25,93]. Inversely, oxidative stress can enhance *DUX4* expression [94] (**Figure 5**). Once transcribed, *DUX4-fl* mRNA is likely to be a substrate of NMD [29]. At the same time, DUX4 disrupts NMD by stimulating the proteolytic degradation of UPF1 protein [29], which plays a central role in the NMD pathway (reviewed in [95]). Thus, DUX4 stimulates its own production, *via* a double-negative feedback loop. A similar feedback can be observed in the interplay between DUX4 and the NuRD complex which represses *DUX4* by binding to D4Z4 repeats [80]. NuRD recruitment and subsequent *DUX4* repression could be disrupted by MBD3L2 protein, and the gene encoding for MBD3L2 is itself a DUX4 target [80]. Another feedback loop concerns the CIC-DUX4 fusion protein in Ewing sarcoma. **ERK** (Extracellular signal-regulated kinase) interacts with the wild-type CIC [96] subjecting it to the proteasome-

mediated degradation [47,97,98]. The same is true for the CIC-DUX4 fusion protein, which retains the putative ERK-interaction domains [44]. DUSP6 is a specific negative regulator of ERK (reviewed in [99]), and *DUSP6* expression is transactivated by CIC-DUX4, generating a feedback loop which enhances CIC-DUX4 protein stability. DUSP6 inhibition limits the growth of tumors bearing *CIC-DUX4* translocations in a mice xenograft model [44].

A negative feedback loop controls the murine *Dux* expression during ZGA. Dux binds to LINE1-encoding genomic loci activated at the 4C/8C stage [100]. LINE1 RNA can interact with Nucleolin and Kap1 to repress *Dux* expression [101].

Positive (or double-negative) feedback loops are often associated with bistability and the processes where a definite decision should be made, including cellular differentiation, cell cycle progression and apoptosis. Assuming its role in ZGA, it is not surprising that *DUX4* can be regulated in a bistable manner: its expression is self-amplifying in the cleavage-stage embryo to ensure the complete developmental transition, while in the adult tissues it is totally repressed. Bistability is often accompanied by **hysteresis**, which reduces the system noise sensitivity and further ensures the complete transition. In human myoblasts ectopically expressing *DUX4*, DUX4 induces the expression of the alternative histone variants, H3.X and H3.Y, which incorporate into the regions of its target-genes [102]. This likely helps to maintain an open chromatin structure in these regions, resulting in a greater perdurance of DUX4 target genes expression and their enhanced activation following the subsequent DUX4 exposure [102]. Interestingly, this "memory" effect may be more profound for the muscle fibers due to their multinucleated nature. Abnormal *DUX4* activation in a predisposed pathological condition may reactivate the amplifying feedback-loops, triggering a "pseudo-transition" in an inappropriate context, thus interfering with the normal physiological processes. If this is the case, we can expect more positive feedback loops to be soon

discovered in DUX4 signaling. Importantly, these loops can become promising therapeutic targets to control *DUX4* expression and prevent disease progression.

Therapeutic DUX4 inhibition in disease

FSHD

Several approaches have been developed to inhibit *DUX4* in skeletal muscles. They have been recently reviewed [103], therefore we will just outline these strategies to discuss their relevance for *DUX4*-related cancer treatment. Targeting *DUX4* mRNA by using antisense oligonucleotides (**AOs**), phoshporodiamidate morpholino oligomer (**PMO**) or locked nucleic acid (**LNA**) gapmer AOs targeting different parts of the *DUX4* ORF is the most straightforward approach to selectively repress *DUX4* [27,104–109]. Another strategy to silence *DUX4* is to reinforce *D4Z4* epigenetic or transcriptional repression. This can be done using RNAs targeting the *DUX4* upstream region [84] or dCas9-KRAB transcriptional inhibitor targeted to the *DUX4* promoter and exon 1 [110]. In both FSHD1 and FSHD2 myoblasts, *DUX4* expression can be repressed by ectopic expression of *SMCHD1* [111]. Several small molecules decreasing *DUX4* expression in FSHD muscle cells were identified, they include inhibitors of the bromodomain and extra-terminal (BET) family of proteins, agonists of the beta-2 adrenergic receptor [112,113] and inhibitors of p38 mitogen-activated protein kinase [114,115], although clinical trials of these compounds have not yet produced encouraging results [103]. Finally, a possible, though complicated, therapeutic strategy would be to target DUX4 protein. DUX4 toxicity is mainly attributed to its C-terminal domain, which can activate transcription in part by recruiting histone acetyltransferases p300 and **CBP** (CREB binding protein), resulting in local H3K27 acetylation [116]. A p300 inhibitor

protects cells from DUX4-mediated toxicity by reversing the global imbalance in H3 acetylation and reducing the ability of DUX4 to activate its target genes [117].

Cancer

As expression of DUX4 and chimeric proteins seems to be the major cancer driver in *DUX4*-related ALL [36] and sarcomas [44,49], it would be logical to target DUX4 in these cancers, although transcription factors were considered to be elusive targets [118], in part because many TF are essential for survival of normal cells in the organism. *DUX4* is not expressed in adult tissues and thus represents a relatively straightforward target. DUX4 can be inhibited, for example, by using siRNA to the homeodomain part of the *DUX4* ORF, conserved in most cancer cells. Another potential and more specific approach would be to target DUX4 fusion proteins. A recently developed approach for *in vivo* CRISPR/Cas9 targeting of fusion oncogenes [119] may prove useful when targeting *DUX4* fusions in BCP-ALL and sarcoma. Alternatively, the strategies targeting different domains of DUX4 protein and its interactors, such as the p300 inhibitor mentioned above could be applied to inhibit the activity of DUX4 fusion proteins in cancer cells. Other approaches include the inhibition of downstream targets of DUX4 fusion proteins, e.g. *DUSP6* [44] or the CCNE-CDK2 complex [49] in CIC-DUX4 sarcoma. Treatment of a Kitra-SRS CIC-DUX4 sarcoma cell line with the IGF-1R inhibitor, linsitinib, attenuated cell growth *in vitro* and in xenografts [120]. The Kitra-SRS cell line was also used to screen FDA-approved drugs to identify several cancer growth-inhibiting genotoxic drugs (Doxorubicin, Epirubicin, Topotecan and Camptothecin), two proteasome inhibitors (carfilzomib, bortezomib) and an inhibitor of multiple tyrosine kinases (ponatinib) [120]. Thus, a combination of approaches developed to suppress DUX4 in muscle cells and classical cancer chemotherapy may rapidly produce clinical advances in the treatment of *DUX4*-expressing tumors.

Concluding remarks

DUX4 is involved in a variety of activities in normal and pathological conditions. The effects of *DUX4* expression are tissue-specific as different subsets of genes are regulated by DUX4 in different cell types. As DUX4 is toxic to somatic cells, multiple mechanisms control its expression, from long-range enhancer-promoter interactions to feedback loops. Some of them are well known and are already targeted for development of new therapies. The feedback loops in *DUX4* regulation may be new interesting targets for therapy. Some factors and pathways that regulate DUX4 remain to be discovered (see Outstanding questions). Understanding the relationship between multiple mechanisms of DUX4 control is important for our understanding and treatment of *DUX4*-related pathologies.

Acknowledgements

The work was supported by grants from the Ministry of Science and Higher Education of the Russian Federation (075-15-2020-773), IDB RAS Government basic research program (0088-2021-0007) and AFM (CTCFSHD) to Y.V.; E.T. acknowledges the FEBS short-term fellowship.

Glossary

BCP-ALL - B-cell precursor acute lymphoblastic leukemia is the most common cancer in children, with an annual incidence of ~35 per million. A distinct subset of BCP-ALL is associated with translocations involving *DUX4*.

***Cis*-interaction** - interaction between elements located on the same molecules (chromosomes). *Trans*-interaction - interaction between elements located on different molecules (chromosomes).

D4Z4 - a macrosatellite repeat array in the 4q35 and 10q26 subtelomeric area. Each copy of D4Z4 includes a *DUX4* gene and several transcription control elements.

ETS - a family of transcription factors containing an Ets (Erythroblast Transformation Specific) domain.

Ewing-like sarcomas are a subgroup of small round blue cell sarcomas that lack a hallmark translocation between a gene of the RNA-binding TET family (*EWSR1* or *FUS*) with a gene of the ETS-transcription family (*FLI1*, *ERG*, *ETV1*, *ETV4*, or *FEV*). A subset Ewing-like sarcomas is associated with translocations involving *DUX4*.

Fusion (chimeric) protein - proteins that result from joining of two or more genes that originally coded for separate proteins.

Feedback loop - a mechanism whereby a product resulting from several reactions affects the previous steps in order to enhance or repress the overall effect of the entire process. A double-negative feedback loop is in fact a positive feedback loop made of two negative interactions, so that the two factors repress each other.

GQs (G-quadruplexes) - specific secondary DNA or RNA structures consisting of guanine-rich chains linked by Hoogsteen-type hydrogen bonds and stabilized at the center by a monovalent cation.

Hysteresis - the effect of dependence of the state of a system on its history;

NMD (Nonsense-mediated decay) - a process controlling the quality of RNA, which degrades RNAs with premature termination codons.

NuRD (Nucleosome Remodeling Deacetylase) - a protein complex with ATP-dependent chromatin remodeling and histone deacetylase activities, regulating gene transcription, genome integrity and cell cycle progression;

RMS (Rhabdomyosarcoma) - Mostly pediatric cancer that develops from skeletal muscle cells

ZGA (zygotic genome activation) - the initiation of gene expression after fertilization.

Box 1. DUX4 and facioscapulohumeral muscular dystrophy

DUX4 has been mostly studied in the context of fascioscapular humeral muscular dystrophy (FSHD), an autosomal dominant disease characterized by age-dependent muscle weakness and wasting [121]. The FSHD genetic abnormality is localized in the subtelomeric region 4q35 of chromosome 4. Its major form (FSHD1; 95% of FSHD cases) results from a combination of several genetic factors: a decrease in the number of D4Z4 units below 11 [4] and a distal haplotype 4qA [122]. It is also strongly associated with polymorphic sequences located proximally to the D4Z4 array [123]. D4Z4 contraction causes chromatin derepression and DNA hypomethylation within the short D4Z4 array allowing for the *DUX4* transcription from the most distal D4Z4 unit. The 4qA haplotype provides an intron and a polyadenylation site, stabilizing the full-length *DUX4* mRNA which is otherwise degraded. Together these lead to the production of toxic DUX4 protein in skeletal muscles. A simple sequence length polymorphism (**SSLP**), located proximally to D4Z4 harbours an insulator [56,60,61], which is less efficient in FSHD myoblasts [60]. FSHD2 comprising ~5% of total cases is phenotypically indistinguishable from FSHD1. It results from mutations in methylation-associated genes. 85% of the FSHD2 patients carry mutations in *SMCHD1* [124] , encoding for a protein involved in gene repression and X-chromosome inactivation [125]. Some cases are caused by DNA-methyltransferase 3B (*DNMT3B)* mutations [126,127]. Both *SMCHD1* and *DNMT3B* mutations lead to *DUX4* derepression and its transcription from the distal D4Z4 unit. Both FSHD2 and FSHD1 are associated with the presence of the 4qA haplotype on at least one copy of chromosome 4. In some severe FSHD cases, both D4Z4 contraction and *SMCHD1* mutations are present [128,129]. Regardless of the primary cause, all FSHD types seem to converge on DUX4 protein production.

Box 2. Evolution of DUX family genes

The genes of the Double homeobox (DUX) family are unique to placental mammals [130]. One group of DUX genes is composed of intron-containing *DUXA*, *DUXB* and *DUXC* in many placental mammals [131]. Another intron-containing variant, *Duxbl* (*DUXB*-like) exists only in mice and rats with its pseudogene forms also found in primates [130]. The second group is represented by intronless *DUX4* in primates and *Afrotheria* (elephant, hyrax and tenrec) and *Dux* in mice and rats [131].

Among double homeobox genes, only *DUX4, Dux* and *DUXC* possess a conserved C-terminal domain, responsible for transcription activation [130,132]. Similarly to *DUX4* in *Afrotheria* and primates, copies of mouse *Dux* are organized in tandem repeat arrays [132].

The intron-containing *DUXC* macrosatellite was likely present in the genome of the most recent common ancestor of all placental mammals, while the intronless *DUX4* and *Dux* arose later on in a series of independent retrotransposition events [131].

Clinician's corner

Facioscapulohumeral dystrophy

FSHD is the third most common myopathy, with a frequency ranging from 1 in 20000 to 1 in 8000 individuals. The disease affects skeletal muscles, typically those of the face (facio), shoulders (scapula) and upper arms (humerus), but can eventually spread to the other muscles of the body. The first symptoms usually manifestate in adolescence or early adulthood, though the onset of FSHD is highly variable. The molecular mechanisms leading to FSHD are outlined in **Box 1**. Currently there is no effective FSHD treatment; the potential therapeutic approaches are extensively discussed in a recent review [103]**.**

B-cell precursor acute lymphoblastic leukemia

BCP-ALL is the most common cancer in children, with an annual incidence of ~35 per million. The first symptoms may include bleeding, fatigue, or appetite loss. The disease is then confirmed by blood test and cytogenetic analysis as BCP-ALL results from chromosomal translocations. One subset of BCP-ALL is associated with translocations involving *DUX4*. The 5-year survival rate is ~90% for children younger than 15 years, ~75% for adolescents aged 15 to 19 years and ~50% for adults [133]. Current treatment options include chemotherapy with combinations of drugs, including vincristine, prednisone, cyclophosphamide, doxorubicin, L-asparaginase, cytarabine and methotrexate.

Ewing-like sarcomas

EWS are rare and mostly pediatric soft tissue sarcomas with poor prognosis resulting from gene fusions that are distinct from that of the *FET-ETS* family rearrangements specific for Ewing sarcoma. A subset of Ewing-like sarcomas is associated with a *CIC-DUX4* translocation. In general, CIC-fused Ewing-like sarcomas have an aggressive clinical course with frequent metastases and have a 5-year survival rate of less than 50% [133]. Treatment

options include combinations of chemotherapy, such as cyclophosphamide and topotecan or irinotecan and temozolomide with or without vincristine as well as radiotherapy.

Rhabdomyosarcoma

RMS is a malignant tumor of striated muscle origin and the most prevalent soft tissue sarcoma in children, comprising ~4.5% of all childhood cancers [134]. The common symptoms are lump and swelling, and the more specific manifestations depend on the tumor location and size. The frontline therapy is currently based on the multi-modal approach combining surgical rejection, radiation therapy and chemotherapy with vincristine, actinomycin D and cyclophosphamide or isofasfamide. Such therapy is effective for over 90% of the patients with low-grade localized tumors, though the survival rates for patients with more severe metastatic or recurrent cases remain low (~20-30%) (reviewed in [135]).

Figure Legends

Key figure 1. ***DUX4 expression in early development, FSHD and cancer.***

Figure 2. ***Regulatory elements and their target genes within the 4q35 region.*** *Each D4Z4 unit contains an enhancer activating FRG2 and DUX4c genes located proximally to the D4Z4 array; two muscle specific DUX4 enhancers, DME1 and DME2, reside proximally to D4Z4; these regulatory elements are isolated from each other by an insulator (FR-MAR), which is less efficient in FSHD cells; SSLP - simple sequence length polymorphism, located within the FR-MAR sequence; the distance from the first proximal D4Z4 unit is indicated in kb.*

Figure 3. ***Non-coding RNAs in D4Z4 silencing.*** *D4Z4 units are bi-directionally transcribed generating microRNA-sized fragments which potentially produce siRNAs, activating endogenous RNA interference pathway and contributing to D4Z4 silencing. These siRNAs are less abundant upon D4Z4 contraction. Proteins of the Polycomb group bind to the DBE element inside each D4Z4 unit to repress the D4Z4 array. Chromatin relaxation on a contracted allele allows the production of a FSHD-specific lncRNA DBE-T, which attracts the ASH1L protein of the Trithorax group, acting antagonistically to PcG proteins.*

Figure 4. ***Chromatin folding and gene contacts within the 4q35 locus.***

Figure 5. ***Feedback loops in DUX4 regulation.*** *Clockwise: DUX4 is a substrate of NMD, but it also inhibits NMD. DUX4-induced oxidative stress can enhance DUX4 expression. NuRD complex which represses DUX4 by binding to D4Z4 repeats. NuRD recruitment and subsequent DUX4 repression could be disrupted by MBD3L2 protein, and the gene encoding for MBD3L2 is itself a DUX4 target.*

References

1. Lyle R, Wright TJ, Clark LN, Hewitt JE. The FSHD-associated repeat, D4Z4, is a member of a dispersed family of homeobox-containing repeats, subsets of which are clustered on the short arms of the acrocentric chromosomes. Genomics. 1995;28: 389–397. doi:10.1006/geno.1995.1166
2. Hewitt JE, Lyle R, Clark LN, Valleley EM, Wright TJ, Wijmenga C, et al. Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. Hum Mol Genet. 1994;3: 1287–1295. doi:10.1093/hmg/3.8.1287
3. Karpukhina A, Vassetzky Y. DUX4, a Zygotic Genome Activator, Is Involved in Oncogenesis and Genetic Diseases. Russ J Dev Biol. 2020;51. doi:10.1134/S1062360420030078
4. Snider L, Geng LN, Lemmers RJLF, Kyba M, Ware CB, Nelson AM, et al. Facioscapulohumeral Dystrophy: Incomplete Suppression of a Retrotransposed Gene. PLoS Genet. 2010;6. doi:10.1371/journal.pgen.1001181
5. Das S, Chadwick BP. Influence of Repressive Histone and DNA Methylation upon D4Z4 Transcription in Non-Myogenic Cells. PloS One. 2016;11: e0160022. doi:10.1371/journal.pone.0160022
6. J H, R T. Facioscapulohumeral Muscular Dystrophy: Update on Pathogenesis and Future Treatments. Neurother J Am Soc Exp Neurother. 2018;15: 863–871. doi:10.1007/s13311-018-00675-3
7. Sirvent N, Trassard M, Ebran N, Attias R, Pedeutour F. Fusion of EWSR1 with the DUX4 facioscapulohumeral muscular dystrophy region resulting from t(4;22)(q35;q12) in a case of embryonal rhabdomyosarcoma. Cancer Genet Cytogenet. 2009;195: 12–18. doi:10.1016/j.cancergencyto.2009.06.011
8. Kawamura-Saito M, Yamazaki Y, Kaneko K, Kawaguchi N, Kanda H, Mukai H, et al. Fusion between CIC and DUX4 up-regulates PEA3 family genes in Ewing-like sarcomas with t(4;19)(q35;q13) translocation. Hum Mol Genet. 2006;15: 2125–2137. doi:10.1093/hmg/ddl136
9. Lilljebjörn H, Henningsson R, Hyrenius-Wittsten A, Olsson L, Orsmark-Pietras C, von Palffy S, et al. Identification of ETV6-RUNX1-like and DUX4-rearranged subtypes in paediatric B-cell precursor acute lymphoblastic leukaemia. Nat Commun. 2016;7: 11790. doi:10.1038/ncomms11790
10. Dib C, Zakharova V, Popova E, Kiseleva E, Chernyak B, Lipinski M, et al. DUX4 Pathological Expression: Causes and Consequences in Cancer. Trends Cancer. 2019;5: 268–271. doi:10.1016/j.trecan.2019.03.001
11. De Iaco A, Planet E, Coluccio A, Verp S, Duc J, Trono D. DUX-family transcription factors regulate zygotic genome activation in placental mammals. Nat Genet. 2017;49: 941–945. doi:10.1038/ng.3858
12. Xue Z, Huang K, Cai C, Cai L, Jiang C, Feng Y, et al. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. Nature. 2013;500: 593–597. doi:10.1038/nature12364
13. Hendrickson PG, Doráis JA, Grow EJ, Whiddon JL, Lim J-W, Wike CL, et al. Conserved roles for murine DUX and human DUX4 in activating cleavage stage genes and MERVL/HERVL retrotransposons. Nat Genet. 2017;49: 925–934. doi:10.1038/ng.3844
14. Whiddon JL, Langford AT, Wong C-J, Zhong JW, Tapscott SJ. Conservation and innovation in the DUX4-family gene network. Nat Genet. 2017;49: 935–940. doi:10.1038/ng.3846

15. Chen Z, Zhang Y. Loss of DUX causes minor defects in zygotic genome activation and is compatible with mouse development. Nat Genet. 2019;51: 947–951. doi:10.1038/s41588-019-0418-7
16. Guo M, Zhang Y, Zhou J, Bi Y, Xu J, Xu C, et al. Precise temporal regulation of Dux is important for embryo development. Cell Res. 2019;29: 956–959. doi:10.1038/s41422-019-0238-4
17. Iaco AD, Verp S, Offner S, Grun D, Trono D. DUX is a non-essential synchronizer of zygotic genome activation. Development. 2020;147. doi:10.1242/dev.177725
18. Bosnakovski D, Gearhart MD, Ho Choi S, Kyba M. Dux facilitates post-implantation development, but is not essential for zygotic genome activation†. Biol Reprod. 2021;104: 83–93. doi:10.1093/biolre/ioaa179
19. Gabriëls J, Beckers MC, Ding H, De Vriese A, Plaisance S, van der Maarel SM, et al. Nucleotide sequence of the partially deleted D4Z4 locus in a patient with FSHD identifies a putative gene within each 3.3 kb element. Gene. 1999;236: 25–32. doi:10.1016/s0378-1119(99)00267-x
20. Lemmers RJLF, van der Vliet PJ, Klooster R, Sacconi S, Camaño P, Dauwerse JG, et al. A unifying genetic model for facioscapulohumeral muscular dystrophy. Science. 2010;329: 1650–1653. doi:10.1126/science.1189044
21. Bosnakovski D, Xu Z, Gang EJ, Galindo CL, Liu M, Simsek T, et al. An isogenetic myoblast expression screen identifies DUX4-mediated FSHD-associated molecular pathologies. EMBO J. 2008;27: 2766–2779. doi:10.1038/emboj.2008.201
22. Kowaljow V, Marcowycz A, Ansseau E, Conde CB, Sauvage S, Mattéotti C, et al. The DUX4 gene at the FSHD1A locus encodes a pro-apoptotic protein. Neuromuscul Disord NMD. 2007;17: 611–623. doi:10.1016/j.nmd.2007.04.002
23. Xu H, Wang Z, Jin S, Hao H, Zheng L, Zhou B, et al. Dux4 induces cell cycle arrest at G1 phase through upregulation of p21 expression. Biochem Biophys Res Commun. 2014;446: 235–240. doi:10.1016/j.bbrc.2014.02.105
24. Dmitriev P, Bou Saada Y, Dib C, Ansseau E, Barat A, Hamade A, et al. DUX4-induced constitutive DNA damage and oxidative stress contribute to aberrant differentiation of myoblasts from FSHD patients. Free Radic Biol Med. 2016;99: 244–258. doi:10.1016/j.freeradbiomed.2016.08.007
25. Barro M, Carnac G, Flavier S, Mercier J, Vassetzky Y, Laoudj-Chenivesse D. Myoblasts from affected and non-affected FSHD muscles exhibit morphological differentiation defects. J Cell Mol Med. 2010;14: 275–289. doi:10.1111/j.1582-4934.2008.00368.x
26. Bosnakovski D, Gearhart MD, Toso EA, Ener ET, Choi SH, Kyba M. Low level DUX4 expression disrupts myogenesis through deregulation of myogenic gene expression. Sci Rep. 2018;8: 16957. doi:10.1038/s41598-018-35150-8
27. Vanderplanck C, Ansseau E, Charron S, Stricwant N, Tassin A, Laoudj-Chenivesse D, et al. The FSHD atrophic myotube phenotype is caused by DUX4 expression. PloS One. 2011;6: e26820. doi:10.1371/journal.pone.0026820
28. Geng LN, Yao Z, Snider L, Fong AP, Cech JN, Young JM, et al. DUX4 Activates Germline Genes, Retroelements, and Immune Mediators: Implications for Facioscapulohumeral Dystrophy. Dev Cell. 2012;22: 38–51. doi:10.1016/j.devcel.2011.11.013
29. Feng Q, Snider L, Jagannathan S, Tawil R, van der Maarel SM, Tapscott SJ, et al. A feedback loop between nonsense-mediated decay and the retrogene DUX4 in facioscapulohumeral muscular dystrophy. eLife. 2015;4. doi:10.7554/eLife.04996
30. Shadle SC, Zhong JW, Campbell AE, Conerly ML, Jagannathan S, Wong C-J, et al. DUX4-induced dsRNA and MYC mRNA stabilization activate apoptotic pathways in

human cell models of facioscapulohumeral dystrophy. PLoS Genet. 2017;13: e1006658. doi:10.1371/journal.pgen.1006658
31. Shadle SC, Bennett SR, Wong C-J, Karreman NA, Campbell AE, van der Maarel SM, et al. DUX4-induced bidirectional HSATII satellite repeat transcripts form intranuclear double stranded RNA foci in human cell models of FSHD. Hum Mol Genet. 2019. doi:10.1093/hmg/ddz242
32. Yasuda T, Tsuzuki S, Kawazu M, Hayakawa F, Kojima S, Ueno T, et al. Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. Nat Genet. 2016;48: 569–574. doi:10.1038/ng.3535
33. Zhang J, McCastlain K, Yoshihara H, Xu B, Chang Y, Churchman ML, et al. Deregulation of DUX4 and ERG in acute lymphoblastic leukemia. Nat Genet. 2016;48: 1481–1489. doi:10.1038/ng.3691
34. Liu Y-F, Wang B-Y, Zhang W-N, Huang J-Y, Li B-S, Zhang M, et al. Genomic Profiling of Adult and Pediatric B-cell Acute Lymphoblastic Leukemia. EBioMedicine. 2016;8: 173–183. doi:10.1016/j.ebiom.2016.04.038
35. Dong X, Zhang W, Wu H, Huang J, Zhang M, Wang P, et al. Structural basis of DUX4/IGH-driven transactivation. Leukemia. 2018;32: 1466–1476. doi:10.1038/s41375-018-0093-1
36. Tanaka Y, Kawazu M, Yasuda T, Tamura M, Hayakawa F, Kojima S, et al. Transcriptional activities of DUX4 fusions in B-cell acute lymphoblastic leukemia. Haematologica. 2018;103: e522–e526. doi:10.3324/haematol.2017.183152
37. Schroeder MP, Bastian L, Eckert C, Gökbuget N, James AR, Tanchez JO, et al. Integrated analysis of relapsed B-cell precursor Acute Lymphoblastic Leukemia identifies subtype-specific cytokine and metabolic signatures. Sci Rep. 2019;9: 4188. doi:10.1038/s41598-019-40786-1
38. Dmitriev P, Kiseleva E, Kharchenko O, Ivashkin E, Pichugin A, Dessen P, et al. Dux4 controls migration of mesenchymal stem cells through the Cxcr4-Sdf1 axis. Oncotarget. 2016;7: 65090–65108. doi:10.18632/oncotarget.11368
39. Italiano A, Sung YS, Zhang L, Singer S, Maki RG, Coindre J-M, et al. High Prevalence of CIC Fusion with Double-Homeobox (DUX4) Transcription Factors in EWSR1-Negative Undifferentiated Small Blue Round Cell Sarcomas. Genes Chromosomes Cancer. 2012;51: 207–218. doi:10.1002/gcc.20945
40. Smith SC, Buehler D, Choi E-YK, McHugh JB, Rubin BP, Billings SD, et al. CIC-DUX sarcomas demonstrate frequent MYC amplification and ETS-family transcription factor expression. Mod Pathol Off J U S Can Acad Pathol Inc. 2015;28: 57–68. doi:10.1038/modpathol.2014.83
41. Weissmann S, Cloos PA, Sidoli S, Jensen ON, Pollard S, Helin K. The Tumor Suppressor CIC Directly Regulates MAPK Pathway Genes via Histone Deacetylation. Cancer Res. 2018;78: 4114–4125. doi:10.1158/0008-5472.CAN-18-0342
42. Kherrouche Z, Monte D, Werkmeister E, Stoven L, De Launoit Y, Cortot AB, et al. PEA3 transcription factors are downstream effectors of Met signaling involved in migration and invasiveness of Met-addicted tumor cells. Mol Oncol. 2015;9: 1852–1867. doi:10.1016/j.molonc.2015.07.001
43. Le Guellec S, Velasco V, Pérot G, Watson S, Tirode F, Coindre J-M. ETV4 is a useful marker for the diagnosis of CIC-rearranged undifferentiated round-cell sarcomas: a study of 127 cases including mimicking lesions. Mod Pathol Off J U S Can Acad Pathol Inc. 2016;29: 1523–1531. doi:10.1038/modpathol.2016.155
44. Lin YK, Wu W, Ponce RK, Kim JW, Okimoto RA. Negative MAPK-ERK regulation sustains CIC-DUX4 oncoprotein expression in undifferentiated sarcoma. Proc Natl Acad Sci U S A. 2020. doi:10.1073/pnas.2009137117

45. Yoshimoto T, Tanaka M, Homme M, Yamazaki Y, Takazawa Y, Antonescu CR, et al. CIC-DUX4 induces small round cell sarcomas distinct from Ewing sarcoma. Cancer Res. 2017;77: 2927–2937. doi:10.1158/0008-5472.CAN-16-3351
46. Kim E, Kim D, Lee J-S, Yoe J, Park J, Kim C-J, et al. Capicua suppresses hepatocellular carcinoma progression by controlling the ETV4-MMP1 axis. Hepatol Baltim Md. 2018;67: 2287–2301. doi:10.1002/hep.29738
47. Okimoto RA, Breitenbuecher F, Olivas VR, Wu W, Gini B, Hofree M, et al. Inactivation of Capicua drives cancer metastasis. Nat Genet. 2017;49: 87–96. doi:10.1038/ng.3728
48. Antonescu CR, Owosho AA, Zhang L, Chen S, Deniz K, Huryn JM, et al. Sarcomas With CIC-rearrangements Are a Distinct Pathologic Entity With Aggressive Outcome: A Clinicopathologic and Molecular Study of 115 Cases. Am J Surg Pathol. 2017;41: 941–949. doi:10.1097/PAS.0000000000000846
49. Okimoto RA, Wu W, Nanjo S, Olivas V, Lin YK, Ponce RK, et al. CIC-DUX4 oncoprotein drives sarcoma metastasis and tumorigenesis via distinct regulatory programs. J Clin Invest. 2019;129: 3401–3406. doi:10.1172/JCI126366
50. Chew G-L, Campbell AE, De Neef E, Sutliff NA, Shadle SC, Tapscott SJ, et al. DUX4 Suppresses MHC Class I to Promote Cancer Immune Evasion and Resistance to Checkpoint Blockade. Dev Cell. 2019;50: 658-671.e7. doi:10.1016/j.devcel.2019.06.011
51. Bell D, Bell AH, Kupferman ME, Prieto VG, Weber RS, Hanna EY. Comparative transcriptome analysis of sinonasal inverted papilloma and associated squamous cell carcinoma: Out-HOXing developmental genes. Head Neck. 2019;41: 3090–3104. doi:10.1002/hed.25795
52. Bury M, Le Calvé B, Lessard F, Dal Maso T, Saliba J, Michiels C, et al. NFE2L3 Controls Colon Cancer Cell Growth through Regulation of DUX4, a CDK1 Inhibitor. Cell Rep. 2019;29: 1469-1481.e9. doi:10.1016/j.celrep.2019.09.087
53. Dmitriev P, Lipinski M, Vassetzky YS. Pearls in the junk: Dissecting the molecular pathogenesis of facioscapulohumeral muscular dystrophy. Neuromuscul Disord. 2009;19: 17–20. doi:10.1016/j.nmd.2008.09.004
54. Ding H, Beckers MC, Plaisance S, Marynen P, Collen D, Belayew A. Characterization of a double homeodomain protein (DUX1) encoded by a cDNA homologous to 3.3 kb dispersed repeated elements. Hum Mol Genet. 1998;7: 1681–1694. doi:10.1093/hmg/7.11.1681
55. Snider L, Asawachaicharn A, Tyler AE, Geng LN, Petek LM, Maves L, et al. RNA transcripts, miRNA-sized fragments and proteins produced from D4Z4 units: new candidates for the pathophysiology of facioscapulohumeral dystrophy. Hum Mol Genet. 2009;18: 2414–2430. doi:10.1093/hmg/ddp180
56. Petrov A, Allinne J, Pirozhkova I, Laoudj D, Lipinski M, Vassetzky YS. A nuclear matrix attachment site in the 4q35 locus has an enhancer-blocking activity in vivo: Implications for the facio-scapulo-humeral dystrophy. Genome Res. 2008;18: 39–45. doi:10.1101/gr.6620908
57. Dmitriev P, Petrov A, Ansseau E, Stankevicins L, Charron S, Kim E, et al. The Krüppel-like Factor 15 as a Molecular Link between Myogenic Factors and a Chromosome 4q Transcriptional Enhancer Implicated in Facioscapulohumeral Dystrophy. J Biol Chem. 2011;286: 44620–44631. doi:10.1074/jbc.M111.254052
58. Himeda CL, Debarnot C, Homma S, Beermann ML, Miller JB, Jones PL, et al. Myogenic Enhancers Regulate Expression of the Facioscapulohumeral Muscular Dystrophy-Associated DUX4 Gene. Mol Cell Biol. 2014;34: 1942–1955. doi:10.1128/MCB.00149-14

59. Erokhin M, Vassetzky Y, Georgiev P, Chetverina D. Eukaryotic enhancers: common features, regulation, and participation in diseases. Cell Mol Life Sci CMLS. 2015;72: 2361–2375. doi:10.1007/s00018-015-1871-9
60. Kisseljova NP, Dmitriev P, Katargin A, Kim E, Ezerina D, Markozashvili D, et al. DNA polymorphism and epigenetic marks modulate the affinity of a scaffold/matrix attachment region to the nuclear matrix. Eur J Hum Genet. 2014;22: 1117–1123. doi:10.1038/ejhg.2013.306
61. Petrov A, Pirozhkova I, Carnac G, Laoudj D, Lipinski M, Vassetzky YS. Chromatin loop domain organization within the 4q35 locus in facioscapulohumeral dystrophy patients versus normal human myoblasts. Proc Natl Acad Sci U S A. 2006;103: 6982–6987. doi:10.1073/pnas.0511235103
62. Winokur ST, Bengtsson U, Feddersen J, Mathews KD, Weiffenbach B, Bailey H, et al. The DNA rearrangement associated with facioscapulohumeral muscular dystrophy involves a heterochromatin-associated repetitive element: Implications for a role of chromatin structure in the pathogenesis of the disease. Chromosome Res. 1994;2: 225–234. doi:10.1007/BF01553323
63. Masny PS, Bengtsson U, Chung S-A, Martin JH, van Engelen B, van der Maarel SM, et al. Localization of 4q35.2 to the nuclear periphery: is FSHD a nuclear envelope disease? Hum Mol Genet. 2004;13: 1857–1871. doi:10.1093/hmg/ddh205
64. Tsien F, Sun B, Hopkins NE, Vedanarayanan V, Figlewicz D, Winokur S, et al. Methylation of the FSHD syndrome-linked subtelomeric repeat in normal and FSHD cell cultures and tissues. Mol Genet Metab. 2001;74: 322–331. doi:10.1006/mgme.2001.3219
65. Salsi V, Magdinier F, Tupler R. Does DNA Methylation Matter in FSHD? Genes. 2020;11. doi:10.3390/genes11030258
66. Cadieux B, Ching T-T, VandenBerg SR, Costello JF. Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res. 2006;66: 8469–8476. doi:10.1158/0008-5472.CAN-06-1547
67. Katargin AN, Pavlova LS, Kisseljov FL, Kisseljova NP. Hypermethylation of genomic 3.3-kb repeats is frequent event in HPV-positive cervical cancer. BMC Med Genomics. 2009;2: 30. doi:10.1186/1755-8794-2-30
68. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet. 2005;37: 391–400. doi:10.1038/ng1531
69. Cheng JC, Weisenberger DJ, Gonzales FA, Liang G, Xu G-L, Hu Y-G, et al. Continuous Zebularine Treatment Effectively Sustains Demethylation in Human Bladder Cancer Cells. Mol Cell Biol. 2004;24: 1270–1278. doi:10.1128/MCB.24.3.1270-1278.2004
70. Tsumagari K, Qi L, Jackson K, Shao C, Lacey M, Sowden J, et al. Epigenetics of a tandem DNA repeat: chromatin DNaseI sensitivity and opposite methylation changes in cancers. Nucleic Acids Res. 2008;36: 2196–2207. doi:10.1093/nar/gkn055
71. Ciszewski L, Lu-Nguyen N, Slater A, Brennan A, Williams HEL, Dickson G, et al. G-quadruplex ligands mediate downregulation of DUX4 expression. Nucleic Acids Res. 2020. doi:10.1093/nar/gkaa146
72. van Overveld PGM, Lemmers RJFL, Sandkuijl LA, Enthoven L, Winokur ST, Bakels F, et al. Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. Nat Genet. 2003;35: 315–317. doi:10.1038/ng1262
73. Zeng W, Greef JC de, Chen Y-Y, Chien R, Kong X, Gregson HC, et al. Specific Loss

of Histone H3 Lysine 9 Trimethylation and HP1γ/Cohesin Binding at D4Z4 Repeats Is Associated with Facioscapulohumeral Dystrophy (FSHD). PLOS Genet. 2009;5: e1000559. doi:10.1371/journal.pgen.1000559
74. Balog J, Thijssen PE, de Greef JC, Shah B, van Engelen BGM, Yokomori K, et al. Correlation analysis of clinical parameters with epigenetic modifications in the DUX4 promoter in FSHD. Epigenetics. 2012;7: 579–584. doi:10.4161/epi.20001
75. Gabellini D, Green MR, Tupler R. Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. Cell. 2002;110: 339–348. doi:10.1016/s0092-8674(02)00826-7
76. Bodega B, Ramirez GDC, Grasser F, Cheli S, Brunelli S, Mora M, et al. Remodeling of the chromatin structure of the facioscapulohumeral muscular dystrophy (FSHD) locus and upregulation of FSHD-related gene 1 (FRG1) expression during human myogenic differentiation. BMC Biol. 2009;7: 41. doi:10.1186/1741-7007-7-41
77. Cabianca DS, Casa V, Bodega B, Xynos A, Ginelli E, Tanaka Y, et al. A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell. 2012;149: 819–831. doi:10.1016/j.cell.2012.03.035
78. Déjardin J, Kingston RE. Purification of Proteins Associated with Specific Genomic Loci. Cell. 2009;136: 175–186. doi:10.1016/j.cell.2008.11.045
79. Bauer M, Trupke J, Ringrose L. The quest for mammalian Polycomb response elements: are we there yet? Chromosoma. 2016;125: 471–496. doi:10.1007/s00412-015-0539-4
80. Campbell AE, Shadle SC, Jagannathan S, Lim J-W, Resnick R, Tawil R, et al. NuRD and CAF-1-mediated silencing of the D4Z4 array is modulated by DUX4-induced MBD3L proteins. eLife. 2018;7. doi:10.7554/eLife.31023
81. Jin S-G, Jiang C-L, Rauch T, Li H, Pfeifer GP. MBD3L2 interacts with MBD3 and components of the NuRD complex and can oppose MBD2-MeCP1-mediated methylation silencing. J Biol Chem. 2005;280: 12700–12709. doi:10.1074/jbc.M413492200
82. Huichalaf C, Micheloni S, Ferri G, Caccia R, Gabellini D. DNA Methylation Analysis of the Macrosatellite Repeat Associated with FSHD Muscular Dystrophy at Single Nucleotide Level. PLoS ONE. 2014;9. doi:10.1371/journal.pone.0115278
83. James AR, Schroeder MP, Neumann M, Bastian L, Eckert C, Gökbuget N, et al. Long non-coding RNAs defining major subtypes of B cell precursor acute lymphoblastic leukemia. J Hematol OncolJ Hematol Oncol. 2019;12: 8. doi:10.1186/s13045-018-0692-3
84. Lim J-W, Snider L, Yao Z, Tawil R, Van Der Maarel SM, Rigo F, et al. DICER/AGO-dependent epigenetic silencing of D4Z4 repeats enhanced by exogenous siRNA suggests mechanisms and therapies for FSHD. Hum Mol Genet. 2015;24: 4817–4828. doi:10.1093/hmg/ddv206
85. Dmitriev P, Stankevicins L, Ansseau E, Petrov A, Barat A, Dessen P, et al. Defective Regulation of MicroRNA Target Genes in Myoblasts from Facioscapulohumeral Dystrophy Patients. J Biol Chem. 2013;288: 34989–35002. doi:10.1074/jbc.M113.504522
86. Stadler G, Rahimov F, King OD, Chen JCJ, Robin JD, Wagner KR, et al. Telomere Position Effect (TPE) Regulates DUX4 in Human Facioscapulohumeral Muscular Dystrophy (FSHD). Nat Struct Mol Biol. 2013;20: 671–678. doi:10.1038/nsmb.2571
87. Robin JD, Ludlow AT, Batten K, Magdinier F, Stadler G, Wagner KR, et al. Telomere position effect: regulation of gene expression with progressive telomere shortening over long distances. Genes Dev. 2014;28: 2464–2476. doi:10.1101/gad.251041.114
88. Robin JD, Ludlow AT, Batten K, Gaillard M-C, Stadler G, Magdinier F, et al.

SORBS2 transcription is activated by telomere position effect-over long distance upon telomere shortening in muscle cells from patients with facioscapulohumeral dystrophy. Genome Res. 2015;25: 1781–1790. doi:10.1101/gr.190660.115
89. Razin SV, Vassetzky YS. 3D genomics imposes evolution of the domain model of eukaryotic genome organization. Chromosoma. 2017;126: 59–69. doi:10.1007/s00412-016-0604-7
90. Gaillard M-C, Broucqsault N, Morere J, Laberthonnière C, Dion C, Badja C, et al. Analysis of the 4q35 chromatin organization reveals distinct long-range interactions in patients affected with Facio-Scapulo-Humeral Dystrophy. Sci Rep. 2019;9. doi:10.1038/s41598-019-46861-x
91. Pirozhkova I, Petrov A, Dmitriev P, Laoudj D, Lipinski M, Vassetzky Y. A Functional Role for 4qA/B in the Structural Rearrangement of the 4q35 Region and in the Regulation of FRG1 and ANT1 in Facioscapulohumeral Dystrophy. PLOS ONE. 2008;3: e3389. doi:10.1371/journal.pone.0003389
92. Cortesi A, Pesant M, Sinha S, Marasca F, Sala E, Gregoretti F, et al. 4q-D4Z4 chromatin architecture regulates the transcription of muscle atrophic genes in facioscapulohumeral muscular dystrophy. Genome Res. 2019;29: 883–895. doi:10.1101/gr.233288.117
93. Winokur ST, Barrett K, Martin JH, Forrester JR, Simon M, Tawil R, et al. Facioscapulohumeral muscular dystrophy (FSHD) myoblasts demonstrate increased susceptibility to oxidative stress. Neuromuscul Disord NMD. 2003;13: 322–333. doi:10.1016/s0960-8966(02)00284-5
94. Sasaki-Honda M, Jonouchi T, Arai M, Hotta A, Mitsuhashi S, Nishino I, et al. A patient-derived iPSC model revealed oxidative stress increases facioscapulohumeral muscular dystrophy-causative DUX4. Hum Mol Genet. 2018;27: 4024–4035. doi:10.1093/hmg/ddy293
95. Kurosaki T, Maquat LE. Nonsense-mediated mRNA decay in humans at a glance. J Cell Sci. 2016;129: 461–467. doi:10.1242/jcs.181008
96. Futran AS, Kyin S, Shvartsman SY, Link AJ. Mapping the binding interface of ERK and transcriptional repressor Capicua using photocrosslinking. Proc Natl Acad Sci. 2015;112: 8590–8595. doi:10.1073/pnas.1501373112
97. Bunda S, Heir P, Metcalf J, Li ASC, Agnihotri S, Pusch S, et al. CIC protein instability contributes to tumorigenesis in glioblastoma. Nat Commun. 2019;10: 661. doi:10.1038/s41467-018-08087-9
98. Grimm O, Zini VS, Kim Y, Casanova J, Shvartsman SY, Wieschaus E. Torso RTK controls Capicua degradation by changing its subcellular localization. Development. 2012;139: 3962–3968. doi:10.1242/dev.084327
99. Ahmad MK, Abdollah NA, Shafie NH, Yusof NM, Razak SRA. Dual-specificity phosphatase 6 (DUSP6): a review of its molecular characteristics and clinical relevance in cancer. Cancer Biol Med. 2018;15: 14–28. doi:10.20892/j.issn.2095-3941.2017.0107
100. Bentsen M, Goymann P, Schultheis H, Klee K, Petrova A, Wiegandt R, et al. ATAC-seq footprinting unravels kinetics of transcription factor binding during zygotic genome activation. Nat Commun. 2020;11: 4267. doi:10.1038/s41467-020-18035-1
101. Percharde M, Lin C-J, Yin Y, Guan J, Peixoto GA, Bulut-Karslioglu A, et al. A LINE1-Nucleolin Partnership Regulates Early Development and ESC Identity. Cell. 2018;174: 391-405.e19. doi:10.1016/j.cell.2018.05.043
102. Resnick R, Wong C-J, Hamm DC, Bennett SR, Skene PJ, Hake SB, et al. DUX4-Induced Histone Variants H3.X and H3.Y Mark DUX4 Target Genes for Expression. Cell Rep. 2019;29: 1812-1820.e5. doi:10.1016/j.celrep.2019.10.025

103. Cohen J, DeSimone A, Lek M, Lek A. Therapeutic Approaches in Facioscapulohumeral Muscular Dystrophy. Trends Mol Med. 2020. doi:10.1016/j.molmed.2020.09.008
104. Ansseau E, Vanderplanck C, Wauters A, Harper SQ, Coppée F, Belayew A. Antisense Oligonucleotides Used to Target the DUX4 mRNA as Therapeutic Approaches in FaciosScapuloHumeral Muscular Dystrophy (FSHD). Genes. 2017;8. doi:10.3390/genes8030093
105. Chen JC, King OD, Zhang Y, Clayton NP, Spencer C, Wentworth BM, et al. Morpholino-mediated Knockdown of DUX4 Toward Facioscapulohumeral Muscular Dystrophy Therapeutics. Mol Ther J Am Soc Gene Ther. 2016;24: 1405–1411. doi:10.1038/mt.2016.111
106. Marsollier A-C, Ciszewski L, Mariot V, Popplewell L, Voit T, Dickson G, et al. Antisense targeting of 3' end elements involved in DUX4 mRNA processing is an efficient therapeutic strategy for facioscapulohumeral dystrophy: a new gene-silencing approach. Hum Mol Genet. 2016;25: 1468–1478. doi:10.1093/hmg/ddw015
107. Wallace LM, Saad NY, Pyne NK, Fowler AM, Eidahl JO, Domire JS, et al. Pre-clinical Safety and Off-Target Studies to Support Translation of AAV-Mediated RNAi Therapy for FSHD. Mol Ther Methods Clin Dev. 2018;8: 121–130. doi:10.1016/j.omtm.2017.12.005
108. Wallace LM, Liu J, Domire JS, Garwick-Coppens SE, Guckes SM, Mendell JR, et al. RNA interference inhibits DUX4-induced muscle toxicity in vivo: implications for a targeted FSHD therapy. Mol Ther J Am Soc Gene Ther. 2012;20: 1417–1423. doi:10.1038/mt.2012.68
109. Lim KRQ, Maruyama R, Echigoya Y, Nguyen Q, Zhang A, Khawaja H, et al. Inhibition of DUX4 expression with antisense LNA gapmers as a therapy for facioscapulohumeral muscular dystrophy. Proc Natl Acad Sci U S A. 2020;117: 16509–16515. doi:10.1073/pnas.1909649117
110. Himeda CL, Jones TI, Jones PL. CRISPR/dCas9-mediated Transcriptional Inhibition Ameliorates the Epigenetic Dysregulation at D4Z4 and Represses DUX4-fl in FSH Muscular Dystrophy. Mol Ther J Am Soc Gene Ther. 2016;24: 527–535. doi:10.1038/mt.2015.200
111. Balog J, Thijssen PE, Shadle S, Straasheijm KR, van der Vliet PJ, Krom YD, et al. Increased DUX4 expression during muscle differentiation correlates with decreased SMCHD1 protein levels at D4Z4. Epigenetics. 2015;10: 1133–1142. doi:10.1080/15592294.2015.1113798
112. Campbell AE, Oliva J, Yates MP, Zhong JW, Shadle SC, Snider L, et al. BET bromodomain inhibitors and agonists of the beta-2 adrenergic receptor identified in screens for compounds that inhibit DUX4 expression in FSHD muscle cells. Skelet Muscle. 2017;7: 16. doi:10.1186/s13395-017-0134-x
113. Cruz JM, Hupper N, Wilson LS, Concannon JB, Wang Y, Oberhauser B, et al. Protein kinase A activation inhibits DUX4 gene expression in myotubes from patients with facioscapulohumeral muscular dystrophy. J Biol Chem. 2018;293: 11837–11849. doi:10.1074/jbc.RA118.002633
114. Oliva J, Galasinski S, Richey A, Campbell AE, Meyers MJ, Modi N, et al. Clinically Advanced p38 Inhibitors Suppress DUX4 Expression in Cellular and Animal Models of Facioscapulohumeral Muscular Dystrophy. J Pharmacol Exp Ther. 2019;370: 219–230. doi:10.1124/jpet.119.259663
115. Rojas LA, Valentine E, Accorsi A, Maglio J, Shen N, Robertson A, et al. P38α Regulates Expression of DUX4 in Facioscapulohumeral Muscular Dystrophy. J Pharmacol Exp Ther. 2020. doi:10.1124/jpet.119.264689

116. Choi SH, Gearhart MD, Cui Z, Bosnakovski D, Kim M, Schennum N, et al. DUX4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucleic Acids Res. 2016;44: 5161–5173. doi:10.1093/nar/gkw141
117. Bosnakovski D, Silva MT da, Sunny ST, Ener ET, Toso EA, Yuan C, et al. A novel P300 inhibitor reverses DUX4-mediated global histone H3 hyperacetylation, target gene expression, and cell death. Sci Adv. 2019;5: eaaw7781. doi:10.1126/sciadv.aaw7781
118. Bushweller JH. Targeting transcription factors in cancer — from undruggable to reality. Nat Rev Cancer. 2019;19: 611–624. doi:10.1038/s41568-019-0196-7
119. Martinez-Lage M, Torres-Ruiz R, Puig-Serra P, Moreno-Gaona P, Martin MC, Moya FJ, et al. In vivo CRISPR/Cas9 targeting of fusion oncogenes for selective elimination of cancer cells. Nat Commun. 2020;11: 5060. doi:10.1038/s41467-020-18875-x
120. Nakai S, Yamada S, Outani H, Nakai T, Yasuda N, Mae H, et al. Establishment of a novel human CIC-DUX4 sarcoma cell line, Kitra-SRS, with autocrine IGF-1R activation and metastatic potential to the lungs. Sci Rep. 2019;9. doi:10.1038/s41598-019-52143-3
121. Fecek C, Emmady PD. Facioscapulohumeral Muscular Dystrophy. StatPearls. Treasure Island (FL): StatPearls Publishing; 2020. Available: http://www.ncbi.nlm.nih.gov/books/NBK559028/
122. Lemmers RJLF, de Kievit P, Sandkuijl L, Padberg GW, van Ommen G-JB, Frants RR, et al. Facioscapulohumeral muscular dystrophy is uniquely associated with one of the two variants of the 4q subtelomere. Nat Genet. 2002;32: 235–236. doi:10.1038/ng999
123. Lemmers RJLF, Wohlgemuth M, van der Gaag KJ, van der Vliet PJ, van Teijlingen CMM, de Knijff P, et al. Specific sequence variations within the 4q35 region are associated with facioscapulohumeral muscular dystrophy. Am J Hum Genet. 2007;81: 884–894. doi:10.1086/521986
124. Lemmers RJLF, Tawil R, Petek LM, Balog J, Block GJ, Santen GWE, et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. Nat Genet. 2012;44: 1370–1374. doi:10.1038/ng.2454
125. Blewitt ME, Gendrel A-V, Pang Z, Sparrow DB, Whitelaw N, Craig JM, et al. SmcHD1, containing a structural-maintenance-of-chromosomes hinge domain, has a critical role in X inactivation. Nat Genet. 2008;40: 663–669. doi:10.1038/ng.142
126. Bouwman LF, den Hamer B, Verveer EP, Lerink LJS, Krom YD, van der Maarel SM, et al. Dnmt3b regulates DUX4 expression in a tissue-dependent manner in transgenic D4Z4 mice. Skelet Muscle. 2020;10: 27. doi:10.1186/s13395-020-00247-0
127. van den Boogaard ML, Lemmers RJLF, Balog J, Wohlgemuth M, Auranen M, Mitsuhashi S, et al. Mutations in DNMT3B Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy. Am J Hum Genet. 2016;98: 1020–1029. doi:10.1016/j.ajhg.2016.03.013
128. Sacconi S, Lemmers RJLF, Balog J, van der Vliet PJ, Lahaut P, van Nieuwenhuizen MP, et al. The FSHD2 gene SMCHD1 is a modifier of disease severity in families affected by FSHD1. Am J Hum Genet. 2013;93: 744–751. doi:10.1016/j.ajhg.2013.08.004
129. Larsen M, Rost S, El Hajj N, Ferbert A, Deschauer M, Walter MC, et al. Diagnostic approach for FSHD revisited: SMCHD1 mutations cause FSHD2 and act as modifiers of disease severity in FSHD1. Eur J Hum Genet EJHG. 2015;23: 808–816. doi:10.1038/ejhg.2014.191
130. Leidenroth A, Hewitt JE. A family history of DUX4: phylogenetic analysis of DUXA, B, C and Duxbl reveals the ancestral DUX gene. BMC Evol Biol. 2010;10: 364.

doi:10.1186/1471-2148-10-364
131. Leidenroth A, Clapp J, Mitchell LM, Coneyworth D, Dearden FL, Iannuzzi L, et al. Evolution of DUX gene macrosatellites in placental mammals. Chromosoma. 2012;121: 489–497. doi:10.1007/s00412-012-0380-y
132. Clapp J, Mitchell LM, Bolland DJ, Fantes J, Corcoran AE, Scotting PJ, et al. Evolutionary conservation of a coding function for D4Z4, the tandem DNA repeat mutated in facioscapulohumeral muscular dystrophy. Am J Hum Genet. 2007;81: 264–279. doi:10.1086/519311
133. Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2017. Bethesda, MD,; 2020.
134. Dasgupta R, Fuchs J, Rodeberg D. Rhabdomyosarcoma. Semin Pediatr Surg. 2016;25: 276–283. doi:10.1053/j.sempedsurg.2016.09.011
135. Chen C, Dorado Garcia H, Scheer M, Henssen AG. Current and Future Treatment Strategies for Rhabdomyosarcoma. Front Oncol. 2019;9. doi:10.3389/fonc.2019.01458

Outstanding questions

- How is *DUX4* regulated in the cleavage embryo?
- What is the role of *DUX4* in adult tissues where it is normally expressed (testis and thymus) and how would therapeutic DUX4 inhibition affect these tissues?
- How does sporadic *DUX4* expression cause a chronic disease?
- How can the muscle specificity of DUX4-associated pathology be explained?
- What is the role of DUX4 in cancer and how DUX4 inhibition affects tumor cells?
- Why cancers associated with DUX4 are mostly pediatric?
- Can feedback loops be targeted to modulate *DUX4* expression in pathological conditions?

Key Figure 1

Click here to access/download;Key Figure;Key_figure1.jpg

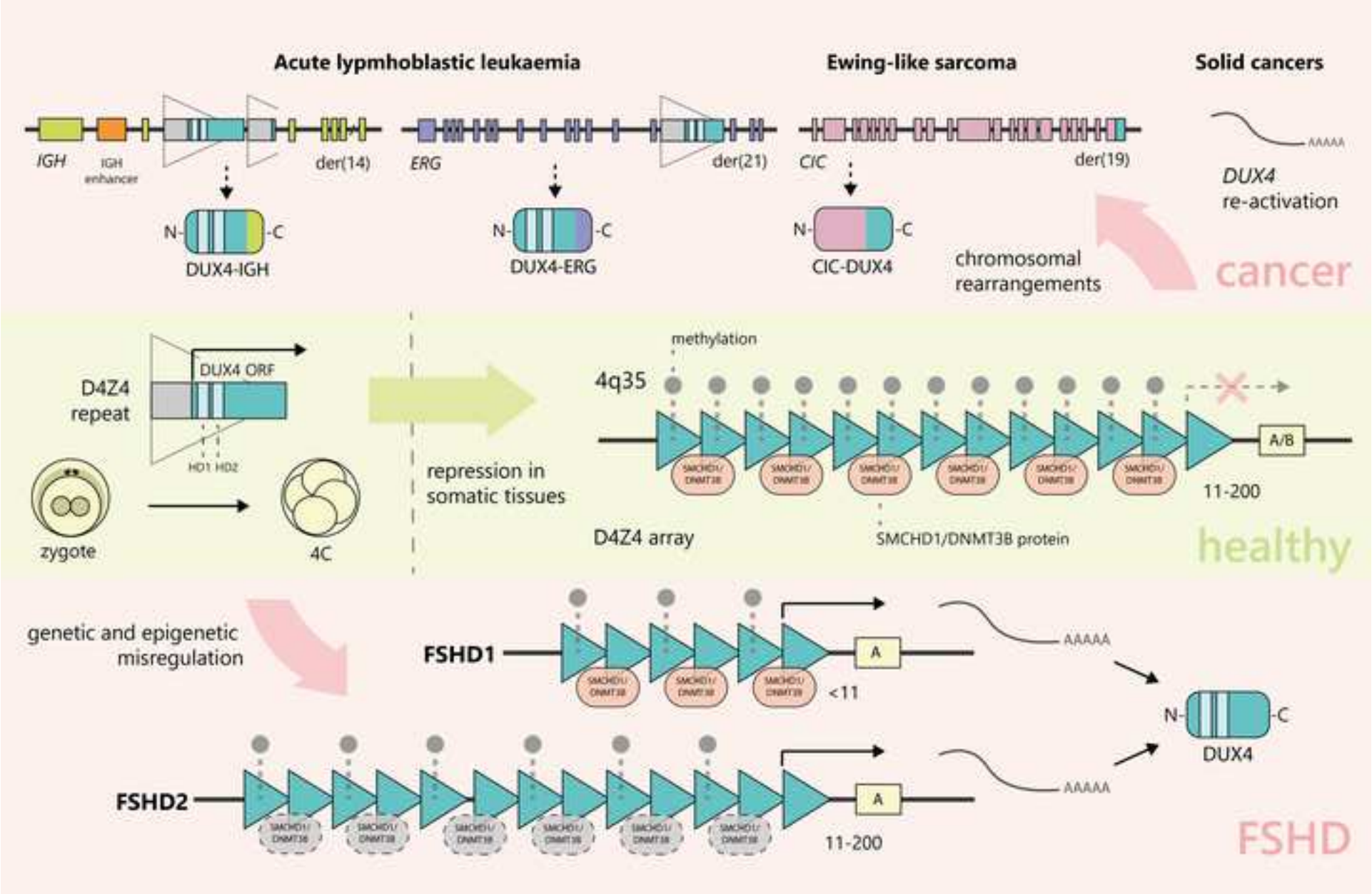


Figure 2

Click here to access/download;Figure;Figure2_2021.jpg

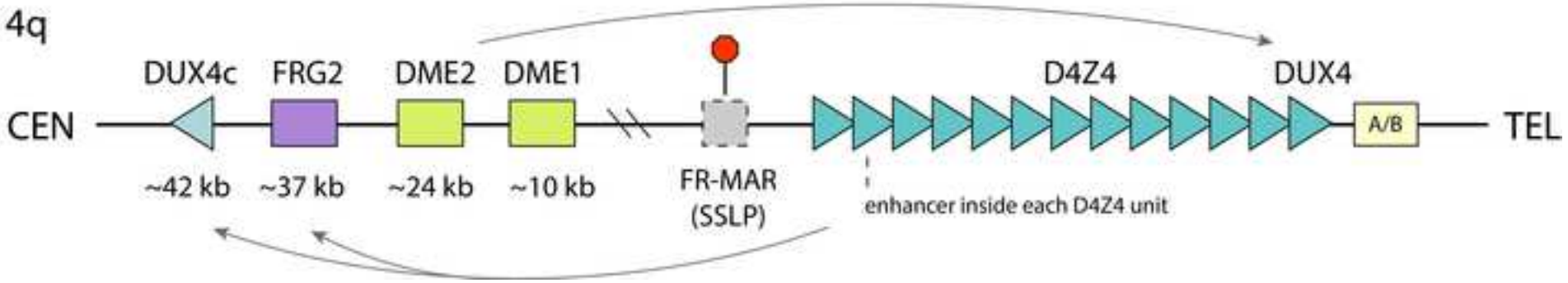


Figure 3

Click here to access/download;Figure;Figure3_2021.jpg

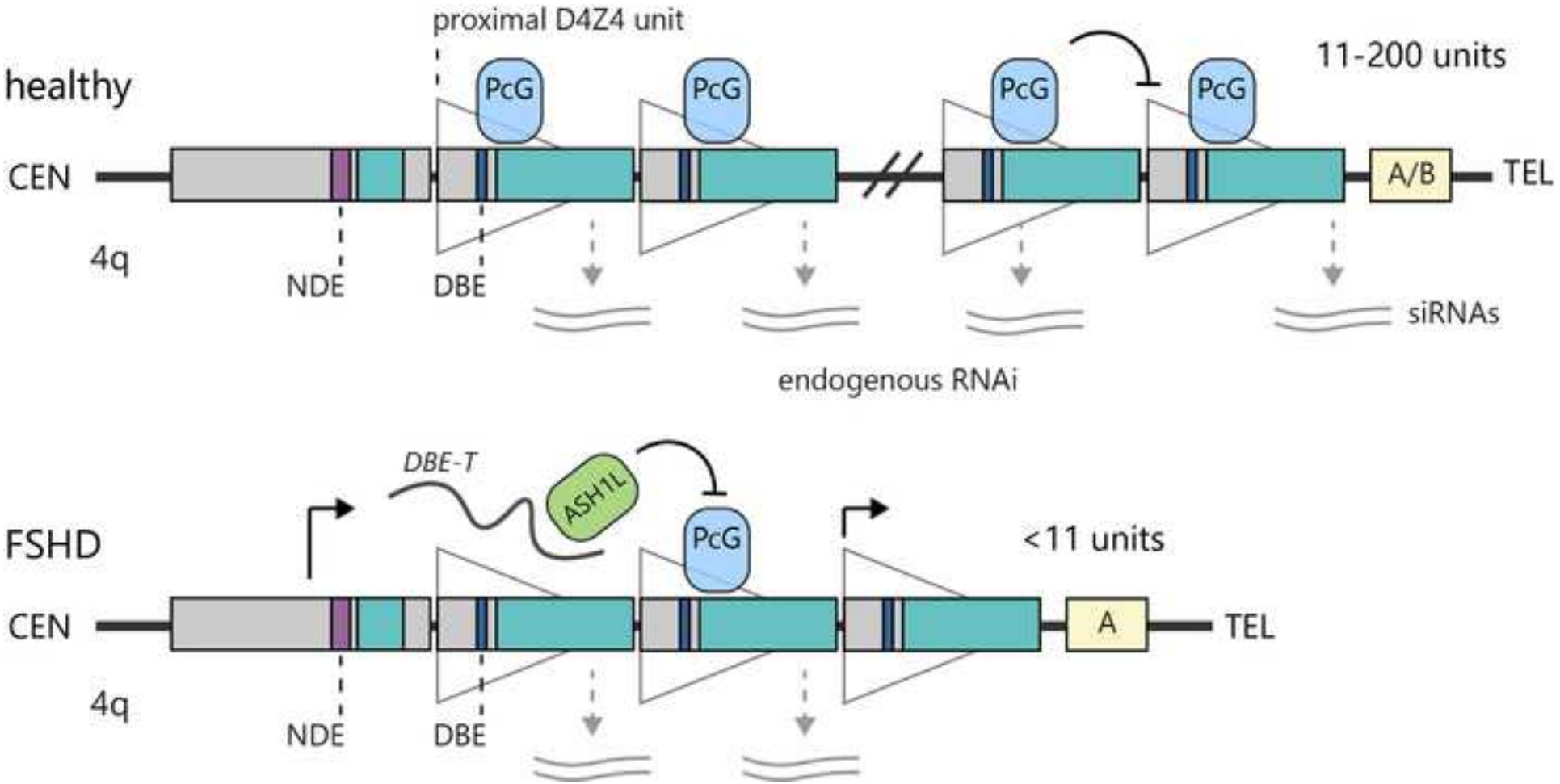


Figure 4

Click here to access/download;Figure;Figure4_2021.jpg

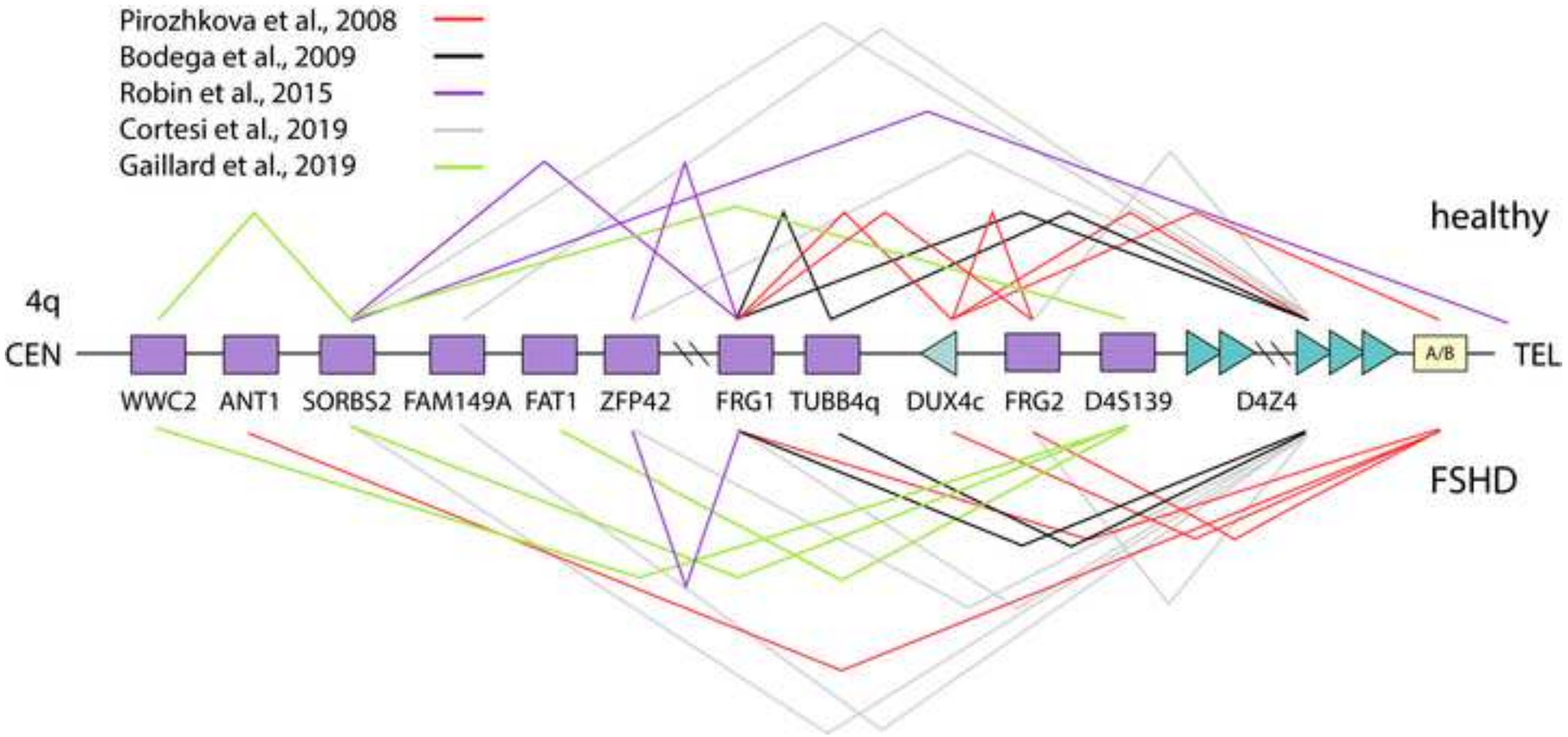


Figure 5

Click here to access/download;Figure;Figure5_2021.jpg

