DUX4 Pathological Expression: Causes and Consequences in Cancer

Carla Dib, Vlada Zakharova, Ekaterina Popova, Ekaterina Kiseleva, Boris Chernyak, Marc Lipinski, Yegor Vassetzky

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Carla Dib[1,2], Vlada Zakharova[1,2,4], Ekaterina Popova[3], Ekaterina Kiseleva[5], Boris Chernyak[3], Marc Lipinski[1,2], Yegor S. Vassetzky[1,2,5,6]

[1]UMR 8126, CNRS, Univ. Paris-Sud, Université Paris Saclay, Institut Gustave Roussy, F-94805 Villejuif, France

[2]LIA 1066 LFR2O French-Russian Joint Cancer Research Laboratory, 94805 Villejuif, France

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

[4]CNRS UMR7216 "Epigénétique et Destin Cellulaire" Université Paris Diderot Paris 7

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

[6]To whom correspondence should be addressed:
Yegor S. Vassetzky,
CNRS UMR8126,
Institut Gustave Roussy,
F-94805 Villejuif, France
Tel: +(33)142116283 ;
E-mail: vassetzky@igr.fr

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Abstract

DUX4, a double homeobox transcription factor, has been mostly studied in facioscapulohumeral dystrophy (FSHD), a pathology linked to a deletion of subtelomeric repeats on chromosome 4q. More recently, however, the gene has been associated with various sarcomas and hematological malignancies. Drugs developed for FSHD could be tested on cancer cells to develop efficient treatment strategies for both pathologies.

DUX4 is a double homeobox transcription factor encoded within the D4Z4 subtelomeric repeat element on chromosome 4q. Recently, *DUX4* rearrangements were reported in a frequent paediatric subtype of B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) (reviewed in [1]), in Ewing-like sarcoma [2] and rhabdomyosarcoma (RMS) [3]. Previously, aberrant expression of *DUX4* was identified as a major factor in the aetiology of facioscapulohumeral dystrophy (FSHD), an autosomal dominant disorder. Below, we discuss features and consequences of *DUX4* gene rearrangements in malignancies and new therapeutic approaches in the context of FSHD that might prove useful for cancer treatment.

DUX4 expression and gene rearrangements

In humans, an aberrantly expressed *DUX4* has been observed in numerous malignancies including renal, breast and testicular cancers (source: Human Protein Atlas[i]). Translocations (4;19) have been observed in Ewing-like sarcomas resulting in portions of *DUX4* fused with partner genes. One translocation produced a fused protein associating the N-terminal part of CIC, an ETS family transcription factor, and the C-terminus of DUX4 (Figure 1A) resulting in a dysregulation of the transcriptional activity of the fused gene [2]. In embryonic rhabdomyosarcoma (RMS), a t(4;22) rearrangement led to the production of an EWSR1-DUX4 chimeric protein [3]. Other *DUX4* rearrangements included the insertion of a truncated copy of *DUX4* into either an intron of *ERG* [4] or the *IGH* gene locus [1] (Figure 1B,C), characteristic of a subtype of BCP-ALL. Both chimeric proteins had a DUX4 C-terminal truncation. Additionally, some DUX4 C-terminal aminoacids have been found replaced by aminoacids encoded by non-coding parts of the partner gene.

DUX4 regulation in normal and pathological conditions

Most data concerning the function and regulation of *DUX4* were generated from studies in FSHD [5]. The subtelomeric D4Z4 repeat has long been considered as "junk" DNA until *DUX4* transcripts were discovered in FSHD muscle cells following the identification of an open reading frame (ORF) encoding two homeoboxes [6]. *DUX4* expression is controlled by two *DUX4*-specific enhancers and an insulator proximal to the D4Z4 repeat [7]. Epigenetic modifications including DNA hypomethylation of D4Z4 units reported in FSHD patients appear to correlate with the severity of the disease [8]. Non-coding RNAs, miRNAs, telomere shortening, and long-range chromatin interactions also affect the expression of *DUX4*. In BCP-ALL, following the translocation, *DUX4* transcription is regulated by control elements in the partner region which further provides the poly-A signal. An additional *DUX4* activation may result from relocation from a repressed to an actively

transcribed nuclear compartment. Similar to the CIC-DUX4 chimeric transcript regulated by the CIC promoter in the t(4;19) translocation found in a Ewing-like sarcoma, the expression of the EWSR1-DUX4 fused gene in the RMS with a t(4;22) translocation probably results from the activity of the EWSR1 promoter [3]. In other cancers, additional abnormalities of *DUX4* have been observed including epigenetic alterations within the 4q35 FSHD-associated locus. Triggered by those observations, expression profiles compared FSHD and 35 different cancers revealing a significant level of similarity between FSHD and Ewing-like sarcomas [9].

DUX4 domains and functional predictions

The N-terminal part of the 424 aminoacid-long DUX4 protein harbors two homeodomains and three nuclear localization sequences (NLS). The C-terminal domain contains a domain of interaction with the histone acetyltransferase p300/CBP and two distinct transcription regulation signals required for DUX4-induced cytotoxicity. Thus, the CIC- and EWSR1-DUX4 fusion proteins should combine the DNA-binding specificity of CIC/EWSR1 with the transcriptional regulation and p300/CBP-binding capacities of DUX4. Based on experimental observations, truncation or replacement of the C-terminus should reduce/modify the transcriptional activation capacity of DUX4 [10] and inhibit interaction with p300/CBP. Indeed, the C-terminus truncated, but not the full-length copy, of DUX4 induced leukemic transformation rather than apoptosis [4].

Pathological consequences of *DUX4* expression

In FSHD myotubes, an overexpression of *DUX4* induces cellular atrophy *via* activation of MuRF1 and MAFbx/atrogin-1, two E3 ubiquitin ligases and apoptosis *via* caspase 3 and p53. DUX4 deregulates myogenic differentiation by decreasing *MYOD* expression and induces aberrant expression of genes normally expressed specifically in germ cells. *DUX4* expression additionally leads to production of reactive oxygen species (ROS) and DNA damage [11,12]. In cancer, DUX4-induced ROS may induce mutations and chromosomal aberrations that contribute to malignant transformation (Figure 1D). Significantly, FSHD cells show transcriptional profiles similar to some cancer cells [9]. In leukaemia cells, DUX4 induces expression of ERG dominant negative isoforms resulting in the loss of function of wild-type ERG which is essential for leukemogenesis [13]. In nude mice expressing *IGH-DUX4* but not *DUX4* in pro-B cells generated a B-cell leukaemia suggesting that DUX4 gains an oncogenic potential following chromosomal rearrangement [1]. In the BCP-ALL subtype, DUX4-induced DNA damage can account for the frequent deletion of ERG and additional chromosomal aberrations which constitute a hallmark in this disease. In Ewing-like sarcoma, the CIC-DUX4 fusion results in changes in CIC transcriptional activity leading to

upregulation of the PEA3 transcription factor that regulates many genes involved in oncogenesis [2].

DUX4: therapeutic opportunities

Several genetic and epigenetic approaches have been developed to inhibit *DUX4* expression in the context of FSHD, including small interfering RNAs, antisense oligonucleotides, Morpholinos impeding polyadenylation or intron splicing, as well as microRNAs directly targeting *DUX4*. The promoter and exon 1 of *DUX4* have been targeted by a dCas9-KRAB fusion protein, with KRAB significantly decreasing the expression levels of *DUX4* and downstream genes [14]. Recently, compounds with epigenetic activity including some under test in clinical trials were used in screens to identify molecules that decrease or suppress *DUX4* expression in FSHD myoblasts. Among different classes of molecules, inhibitors of the BET (bromodomain and extra-terminal) domain and beta-2 adrenergic receptor agonists have been identified [15]. Since aberrant expression of DUX4 fusion proteins induces leukemic transformation [4], some of these approaches could be potentially useful in cancers where *DUX4* is rearranged and overexpressed.

Concluding Remarks and Future Perspectives

Numerous similarities exist in the transcriptional signatures of FSHD myoblasts and several cancers [9]. Recent discovery of the role of *DUX4* in cancer provide new opportunities for both FSHD and cancer research and treatment. Cancer cells from patients with high expression of DUX4 fusion genes may provide human experimental models for DUX4-targeted drug screening. *Vice versa*, therapies developed for FSHD may prove of interest in cancer treatment. Future perspectives include the use of DUX4-targeting drugs developed for FSHD to silence its expression in cancer cells and possibly inhibit cancer cell growth. As *DUX4* is not expressed in somatic tissues, it can be considered a “safe” target for cancer therapy.

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Disclaimer Statement

The authors declare no conflict of interest.

Figure Legend

Figure 1. *DUX4 rearrangements in cancer (A-C) and physiological consequences of its aberrant expression (D).* **(A)**, in Ewing-like sarcoma, the t(4;19)(q35;q13) chromosomal translocation generates a fusion between the *DUX4* C-terminus and the *CIC* gene. This fusion results in the production of CIC-DUX4 chimeric protein under the control of the *CIC* promoter; in ALL, translocation of a truncated or complete copy of *DUX4* into an intron of *ERG* t(4;21)(q35;q22) **(B)** or into the *IGH* locus t(4;14)(q35;q32) **(C)** leads to expression of truncated or chimeric proteins. **(D),** pathological consequences of aberrant expression of *DUX4* include transcription factors deregulation, apoptosis, leukemogenesis, oxidative stress, DNA damage and deregulation of myogenesis.

Resources

[i] https://www.proteinatlas.org/ENSG00000260596-DUX4/pathology

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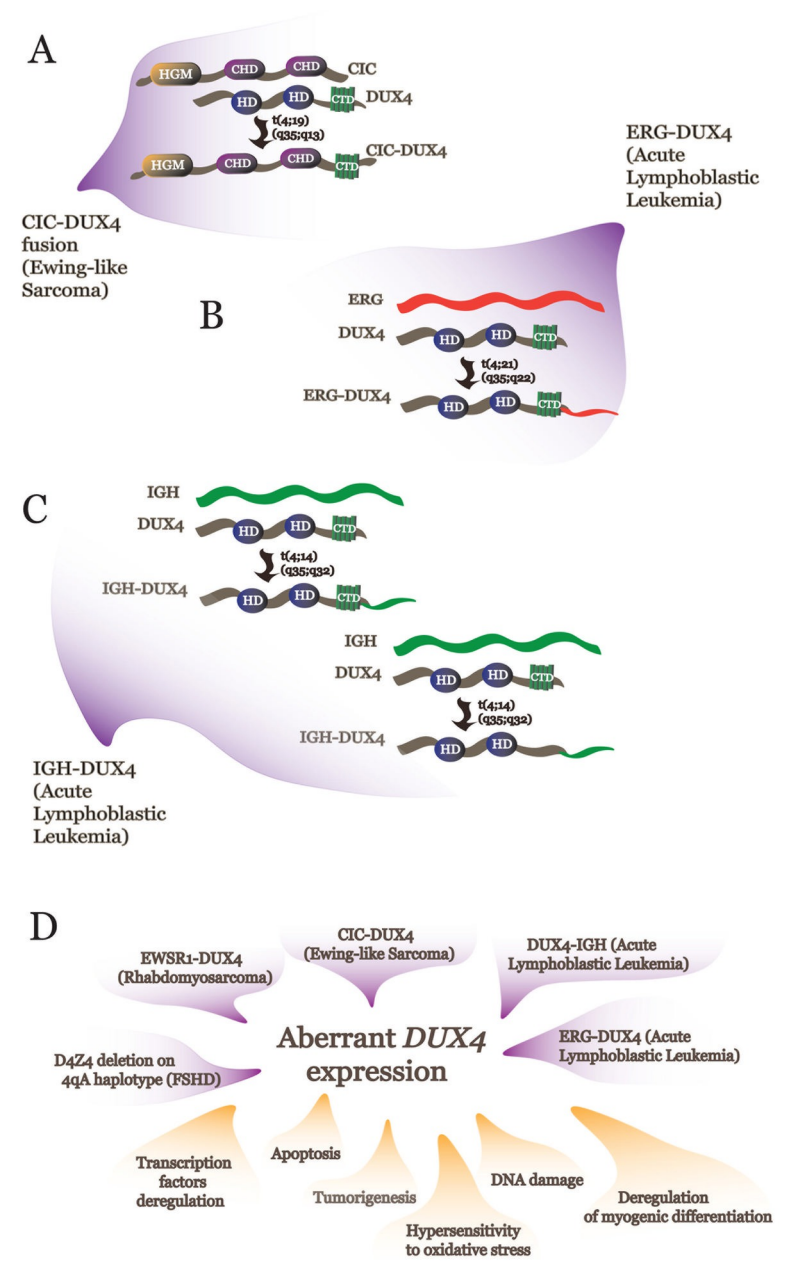
A
HGM
CHD
CHD
CIC
HD
HD
CTD
DUX4
t(4;19)
(q35;q13)
HGM
CHD
CHD
CTD
CIC-DUX4
CIC-DUX4
fusion
(Ewing-like
Sarcoma)
ERG-DUX4
(Acute
Lymphoblastic
Leukemia)
B
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DUX4
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t(4;21)
(q35;q22)
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(Rhabdomyosarcoma)
CIC-DUX4
(Ewing-like Sarcoma)
DUX4-IGH (Acute
Lymphoblastic Leukemia)
D4Z4 deletion on
4qA haplotype (FSHD)
Aberrant DUX4
expression
ERG-DUX4 (Acute
Lymphoblastic Leukemia)
Transcription
factors
deregulation
Apoptosis
Tumorigenesis
DNA damage
Deregulation
of myogenic differentiation
Hypersensitivity
to oxidative stress