A double-edged sword: The world according to Capicua in cancer

Miwa Tanaka,¹ Toyoki Yoshimoto¹,² and Takuro Nakamura¹

¹Division of Carcinogenesis, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo; ²Department of Pathology, Toranomon Hospital, Tokyo, Japan

Key words
Capicua/CIC, CIC-DUX4, oligodendroglioma, round cell sarcoma, RTK/RAS/MAPK pathway

Correspondence
Takuro Nakamura, Division of Carcinogenesis, The Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan. Tel: +81-3-33570-0462; Fax: +81-3-33570-0463; E-mail: takuro-ind@umin.net

Funding information
Japan Society for the Promotion of Science.

Received August 29, 2017; Revised September 24, 2017; Accepted October 2, 2017

Cancer Sci 108 (2017) 2319–2325
doi: 10.1111/cas.13413

CIC/Capicua is an HMG-box transcription factor that is well conserved during evolution. CIC recognizes the T(G/C)AATG(A/G)A sequence and represses its target genes, such as PEA3 family genes. The receptor tyrosine kinase/RAS/MAPK signals downregulate CIC and relieves CIC's target genes from the transrepressoral activity; CIC thus acts as an important downstream molecule of the pathway and as a tumor suppressor. CIC loss-of-function mutations are frequently observed in several human neoplasms such as oligodendroglioma, and lung and gastric carcinoma. CIC is also involved in chromosomal translocation-associated gene fusions in highly aggressive small round cell sarcoma that is biologically and clinically distinct from Ewing sarcoma. In these mutations, PEA3 family genes and other important target genes are upregulated, inducing malignant phenotypes. Down-regulation of CIC abrogates the effect of MAPK inhibitors, suggesting its potential role as an important modifier of molecular target therapies for cancer. These data reveal the importance of CIC as a key molecule in signal transduction, carcinogenesis, and developing novel therapies.

The RTK/RAS/MAPK pathway plays a central role in cell proliferation, motility, and invasion. A number of mutations in the pathway have been identified in the broad spectrum of cancer.¹ In most of the mutations, enhancement and/or prolongation of phosphorylation was found in proteins of signal mediators in the pathway. The signal is transmitted to the nuclear proteins, such as transcription factors, cofactors, and/or chromatin regulators, and the abnormal signaling disorganizes the epigenetic status.² During malignant transformation, progression, and survival of cancer cells under therapeutic stress, the nuclear proteins and transcriptional program downstream to RTK/MAPK signaling modify cellular biological activities and their interference will be one of the critical targets of therapies.³

Multiple downstream molecules are activated in response to RTK/RAS/MAPK phosphorylation signals.⁴ The PEA3 family of ETS transcription factors, ETV1, ETV4, and ETV5, are known to act as such downstream nuclear proteins.⁵–⁷ The PEA3 family genes are involved in chromosomal translocation associated with prostate cancer and ES, and their overexpression promotes cell proliferation, motility, and invasion.⁸ As a common direct repressor of PEA3 genes, Capicua/CIC is an important RTK/MAPK downstream molecule that is contained in an ATXN1/CIC repressor complex and regulates cell proliferation.⁹–¹⁰

Capicua/CIC is an HMG-box transcriptional repressor that is well conserved during evolution. There is growing evidence that CIC is involved in a variety of human cancer. These aberrations include both loss-of-function and gain-of-function mutations, indicating the pleiotropic characteristics of CIC in cancer. This review describes the functions of CIC, its mutation spectrum in human cancer and signaling pathways, and mechanistic consequences involved in these mutations.

Structure and function of Capicua/CIC. Human CIC encodes two protein isoforms, CIC-L and CIC-S, consisting of 2517 and 1608 amino acids, respectively (Fig. 1a).¹¹ CIC is a mammalian homolog of Drosophila capicua that is well conserved in many organisms and there are no apparent homologs in mammals (Fig. 1b). CIC recognizes chromatin through its consensus T(G/C)AATG(A/G)A sequence (also called CIC octamer) using an HMG-box as a DNA-binding motif,¹² unlike other HMG-box transcription factors most of which do not bind DNA in the sequence-specific manner.¹³ The CIC HMG-box is highly conserved among species and there are also additional conserved motifs, C1 and C2, in the C-terminus and the central part, respectively.¹⁵–¹⁶ The in vitro DNA binding assay using mutant CIC constructs showed that the C1 motif is required for stable DNA binding by its interaction with the HMG box.¹³ Thus, both the HMG-box and C1 motif contribute to the core function of CIC, as is also suggested by the mutation spectrum in human cancer (see below).

Drosophila capicua was first identified as a transcriptional repressor downstream to torso, a Drosophila RTK with partial homology to mammalian RET, PDGFR, and c-kit (Fig. 1c).¹⁷–¹⁸ Capicua represses tailless and huckebein by interacting...
with *groucho* using the *capicua* C-terminus encompassing the C1 motif during *Drosophila* embryogenesis. *Capicua* also represses *mirror* expression that determines the ovarian follicle cell fate. Moreover, ATXN1 that is mutated in human SCA1 modulates the repressional activity of *capicua*, interacting with the N-terminal region of *capicua*.

Conversely, haploinsufficiency of Cic improved SCA1 disease phenotypes in *Atxn1* mutant mice. The homozygous knockout mouse for Cic-1 shows the defect of alveolar organization of the lung, and the phenotype is similar with that of the compound *Atxn1* and *Atxn1l* knockout mouse, indicating the importance of Cic/ATXN1 interaction in tissue...
In mutants, Env-4 repression by CIC is cancelled, resulting in upregulation of Mmp9 and aberration of ECM remodeling. The conditional Cic mutation lacking exons 2–6 also induced abnormal lung alveolarization with reduced alveolar surfactant protein expression. Moreover, hematopoietic lineage cell-specific knockout of Cic induced remarkable autoimmune responses with increased follicular helper T cells, which was mediated by derepression of Env5.

Interaction between Cic and ATXN1 protein family is also important for brain development. Disruption of the ATXN1/CIC complex affects thickness of cerebral cortex, inducing multiple behavioral abnormalities in mice. In human, the germline heterozygous CIC truncating mutations were reported in patients of intellectual disability, attention deficit hyperactivity disorder, and autism spectrum disorder. The CIC germline heterozygous truncating mutations are found as responsive elements for RTK signaling. There is a well-conserved MAPK-docking site (C2 motif, PEA3 family genes) that the function of CIC-S might be important in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis.

Subsequently, frequent and recurrent loss-of-function mutations in CIC have been reported in lung, stomach, and prostate cancer (Fig. 2a). No mutations have been reported at the early stage of human cancer development and survival remains unclear. Subsequently, frequent and recurrent loss-of-function mutations in CIC have been reported in lung, stomach, and prostate cancer (Fig. 2a). CIC's repressive function to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis.

Subsequently, frequent and recurrent loss-of-function mutations in CIC have been reported in lung, stomach, and prostate cancer (Fig. 2a). CIC's repressive function to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis. CIC's repressive function to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis.

CIC functions as a tumor suppressor in human cancer. CIC's repression to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). CIC functions as a tumor suppressor in human cancer. CIC's repression to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis.

Subsequently, frequent and recurrent loss-of-function mutations in CIC have been reported in lung, stomach, and prostate cancer (Fig. 2a). CIC's repression to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis. CIC functions as a tumor suppressor in human cancer. CIC's repression to the downstream targets in the RAS/MAPK signals suggests its role as a tumor suppressor gene in carcinogenesis. Indeed, CIC was found mutated in the majority of human oligodendroglioma, in which biallelic mutations and/or loss of CIC were frequently observed (Fig. 2a, Table 1). In brain tumors, CIC mutations are rather specific to oligodendroglioma and are rarely observed in astrocytic tumors. CIC mutations in oligodendroglioma are frequently associated with IDH1 and FUBP1 mutations, suggesting a cooperative role among these three genes in tumorigenesis.
acquired at the advanced stages in lung and gastric cancer. CIC mutations are not maintained in some cases of recurrent oligodendroglioma, (43) suggesting the mutation might not be required for oligodendroglioma survival.

Interestingly, a glial fibrillary acidic protein-Cre-induced Cic mutation in mouse failed to induce oligodendroglioma. (24) Instead, when the same mutation was ubiquitously induced in adult mice, T-cell lymphoblastic leukemia developed at high penetrance. Although the result might be caused by the difference in genetic predisposition for cancer between human and mouse, it was consistent with the fact that mutations of CIC as well as the RTK/RAS/MAPK pathway genes were reported in human T-ALL (Table 1). (24,44)

### Table 1. CIC mutations in human cancer

| Tumor type                  | Type of alterations | Function | Reference |
|-----------------------------|---------------------|----------|-----------|
| Oligodendroglioma           | LOH(19q and/or 1p)  | LOF      | 37, 38, 41|
| Lung cancer                 | Missense: 93.9%     | LOF      | 26        |
| Gastric cancer              | Missense: 93.9%     | LOF      | 26        |
| T-ALL                       | Point mutation      | LOF      | 44        |
| CIC-rearranged sarcoma      | t(4;19)(q35;q13.1)  | GOF      | 12, 52, 57|
| CNS-PNET                    | t(15;19)(q14;q13.2) | GOF      | 63        |
| Angiosarcoma                | CIC-LEUTX fusion    | GOF      | 64        |

GOF, gain of function; LOF, loss of function.

CIC fusion genes in human cancer. CIC is also involved in human malignancies as gene fusions associated with chromosomal translocation involving 19q13. The CIC fusion to DUX4 in Ewing-like small round cell sarcoma with t(4;19)(q35;q13) translocation was first identified in 2006. (12) Most of the CIC coding region, except for the very C-terminal end, is preserved in the CIC–DUX4 fusion, and both the HMG-box and C1 domain are thus included in the fusion protein, indicating that the fusion protein possesses DNA-binding activity (Fig. 2b). Addition of the DUX4 C-terminal part induces conversion of CIC’s transrepressional activity to transactivation, resulting in drastic upregulation of target genes such as PEA3 family genes (Fig. 3a). (12) DUX4 encodes a double homeodomain protein.
and is located in the D4Z4 repeat that is distributed in the subtelomeric regions of the mammalian genome with predominant distribution in 4q and 10q.45,46 Aberrant expression of DUX4 is associated with facioscapulohumeral muscular dystrophy.47–50 The mechanisms of transcriptional activation of DUX4, by recruiting p300/CBP using its C-terminal domain that is included in the CIC–DUX4 fusion, was proposed.51 As a result of DUX4 fusion, CIC acquires transcriptional activation, perhaps through recruitment of p300/CBP, and the fusion converts transrepression activity of CIC to upregulate its target genes, thereby shows strong oncogenic activity.

CIC–DUX4-positive sarcomas are composed of small- to medium-sized, rounded to ovoid cells without any line of differentiation. CIC–DUX4-positive sarcoma shows a poor outcome; it was reported that overall survival of CDS patients was worse than that of ES patients, and phenotypes of CDS are distinct from those of ES.52–54 We have generated an ex vivo mouse model for human CDS by introducing CIC–DUX4 into embryonic mesenchymal cells.55 CIC–DUX4 expression induced small round cell sarcoma of aggressive growth with significantly shorter latency than that of the ES model.56 The model faithfully recapitulates the phenotype of human CDS with upregulation of CIC–DUX4 target genes such as PEA3 family genes.57–59 In this model, ETV4 act as a good marker of CDS,57,58 and analysis of the CDS mouse model identified CCND2 and mucin 5AC as additional biomarkers.59

The DUX4 sequences are originated from both 4q and 10q.46,53,57,59 DUX4 is also involved in translocation associated with human B-cell lymphoblastic leukemia, and the C-terminal region of DUX4 is deleted in these cases.60 suggesting the functional role of the C-terminal region might be different depends on cancer types. A CIC fusion with a non-DUX4 gene, FOXO4, was observed in a rare cases of small round cell sarcoma.61 Another cluster of CIC–NUTM1 fusion-positive tumor was found in primitive neuroectodermal tumors of the central nervous system showing a small cell phenotype.62 Moreover, CIC mutations, including CIC–LEUTX fusion, were reported in 9 of 120 cases of angiosarcoma, and PEA3 family genes were also upregulated in CIC mutated cases.63 Although it remains to be clarified whether these non-DUX4 fusions also convert CIC’s repressor function, the HMG-box was retained in both CIC–FOXO4 and CIC–NUTM1, suggesting similar functional modulation in non-DUX4 fusion proteins. Reported CIC fusion genes are summarized in Table 1.

Molecular targeted therapy using CIC and future directions. The unique mutations of CIC in human cancer are characterized as a mixture of loss-of-function and gain-of-function mutations, both of which upregulate downstream target genes such as ETV4 (Fig. 3a). Many CIC target genes upregulated in CDS are also found upregulated following CRISPR/Cas9-mediated KO in isogenic cell lines.64 The RTK/RAS/MAPK pathway is a common target of molecular targeted therapy, and acquired resistance for these therapies has been frequently observed.1 Therefore, downstream modifiers such as CIC are good alternative targets for the therapy.

As CIC suppresses MAPK downstream signals, downregulation of CIC may be one of the resistance mechanisms for targeted therapies. Indeed, reduced expression of ATXN1L that abrogates the CIC function are found to promote resistance to MAPK pathway inhibition in KRAS mutated pancreatic cancer cells.65 In this study, Wang et al. identified CIC as a gene that modulates the sensitivity for MEK1/2 inhibitor trametinib by CRISPR/Cas9-mediated screening. The exact mechanism to explain how ATXN1L is downmodulated to reduce CIC protein and sensitivity to trametinib remains to be investigated, however, the result suggests importance of the ATXN1L–CIC axis for targeted therapy against the genetic mutations in the RTK/RAS/MAPK pathway (Fig. 3b).

To improve RTK/RAS/MAPK targeting it may be useful to assess the ATXN1L and CIC expression levels to predict the effect of inhibitory drugs, thus CIC can be used as a biological indicator of therapeutic effect. Furthermore, inhibition of CIC phosphorylation is a good alternative therapeutic approach. To this end, the reagent that mimics bicoid that blocks the CIC C2 motif from p90RSK binding might be a useful tool. The COP9 signalosome subunit 1b is another guardian of CIC that acts in an MAPK-independent manner.31 Targeting CIC mutations in carcinoma and sarcoma is more challenging, however, epigenetic therapies that modulate transcription of CIC target genes should be considered. These therapies are effective and ideal for various cancers in which CIC plays a key role in cancer cell survival as downstream of the RTK/RAS/MAPK pathway and as a causative oncogene/tumor suppressor. In addition, it may be useful to evaluate the expression of CIC and ATXN1L to predict the effects of tyrosine kinase inhibitors and MEK inhibitors. Cancer cells use multiple signaling pathways that regulate biological processes such as proliferation, immortalization, self-renewal, migration, and invasion. The CIC homozygous KO mice showed abnormal remodeling of ECM in the lung.23 This phenotype is closely recapitulated as upregulation of the ECM gene set in the CDS mouse model.55 In malignancies, mutant CIC could orchestrate biological activities of cancer cells in both cell autonomous and non-autonomous manners.

In conclusion, CIC acts as a modulator in the pathway and both loss-of-function and gain-of-function mutations of CIC dysregulate the targets, such as the PEA3 family transcription factors, CCND1/D2, and MMPs, resulting in abnormal cellular growth, invasion, and metastasis. Preservation of CIC’s tumor suppressor functions are thus of great importance for prevention and therapies against malignant disorders.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (26250029 to TN and 16K07131 to MT).

Disclosure Statement

Takuro Nakamura has received a commercial research grant from Otsuka Pharmaceutical Co. Ltd. The other authors have no conflict of interest.

Abbreviations

2HG 2-hydroxyglutarate
ATXN1 ataxin 1
ATXN1L ataxin 1 like
CCND1/D2 cyclin D1/D2
CDS CIC–DUX4-positive sarcoma
CIC Capicua transcriptional repressor
CNS-PNET primitive neuroectodermal tumors of the central nervous system
DUX4 double homeobox 4
ECM extracellular matrix
EGFR epidermal growth factor receptor
ES Ewing sarcoma
RTK receptor tyrosine kinase
SCA1 spinocerebellar ataxia type 1
TKI tyrosine kinase inhibitor
References

1 Schmitt MW, Loeb LA, Salk JJ. The influence of subclonal resistance mutations on targeted cancer therapy. *Nat Rev Clin Oncol* 2016; 13: 335–47.

2 Nabet B, Bron PO, Reyes JM, et al. Deregulation of the Ras-Erk signaling axis modulates the cancer landscape. *Cell Rep* 2015; 12: 1300–13.

3 Zawistowski JS, Bevill SM, Goulet DR, et al. Enhancer remodeling during adaptive bypass to MEK inhibition is attenuated by pharmacologic targeting of the P-TEFB complex. *Cancer Discov* 2017; 7: 303–21.

4 Simanshu DK, Nisley DV, McCormick F. RAS proteins and their regulators in human disease. *Cell* 2017; 170: 17–33.

5 Jimenez G, Shvartsman SY, Paroush Z. The Capicua repressor—a general sensor of RTK signaling in development and disease. *J Cell Sci* 2012; 125: 1383–91.

6 Jin Y, Ha N, Fores M, et al. The Capicua repressor capicua transcriptional repressor capicua. The Capicua repressor Capicua. *Biochem Biophys Acta* 2012; 1826: 1–12.

7 Dissanayake K, Toth R, Blakey J, et al. ERK/p90(RSK)/14-3-3 signaling has an impact on expression of PEA3 Ets transcription factors via the transcriptional repressor capicua. *Biochem J* 2011; 433: 515–25.

8 Tseng ASK, Tapon N, Kanda H, et al. Capicua regulates cell proliferation downstream of the receptor tyrosine kinase/Ras signaling pathway. *Curr Biol* 2007; 17: 728–33.

9 Chittaranjan S, Chan S, Yang C, et al. Mutations in CIC and IDH1 cooperatively regulate 2-hydroxyglutarate levels and cell clonogenicity. *Oncotarget* 2017; 8: 7960–70.

10 Kawamura-Saito M, Yamazaki Y, Kaneko K, et al. Inhibition of Capicua drives cancer metastasis. *Proc Natl Acad Sci USA* 2016; 113: 10583–8.

11 Fores M, Coppey M, Grosprêtre R, et al. MAFK substrate competition integrates patterning signals in the Drosophila embryo. *Curr Biol* 2010; 20: 446–51.

12 Jin Y, Andreu MJ, Lim B, et al. Gene regulation by MAPK substrate competition. *Dev Cell* 2011; 20: 880–7.

13 Bettgowda C, Agrawal N, Jiao Y, et al. Mutations in CIC and FUBP1 contribute to human oligodendroglioma. *Science* 2011; 333: 1453–5.

14 Yip S, Butterfield YS, Morozova O, et al. Concurrent CIC mutations, IDH2 mutations, and 1p/19q loss distinguish oligodendrogliomas from other cancers. *J Pathol* 2012; 226: 7–16.

15 Li Y, Killela PJ, Zoghbi HY, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. *Oncotarget* 2012; 3: 709–22.

16 Etienne-Spengler S, Abou-El-Ardat K, Szafranski K, et al. Novel CIC point mutations and an exon-spanning, homozygous deletion identified in oligodendroglial tumors by a comprehensive genomic approach including transcriptome sequencing. *PLoS One* 2013; 8: e76623.

17 Sahm F, Koelsche C, Meyer J, et al. CIC and FUBP1 mutations in oligodendrogliomas, oligoastrocytomas and astrocytomas. *Acta Neuropathol* 2012; 123: 853–60.

18 Choi N, Park J, Lee JS, et al. miR-93/miR-106b/miR-375-CIC-ARHBP1, a novel regulatory axis in prostate cancer progression. *Oncotarget* 2015; 6: 23533–47.

19 Ahara K, Mukasa A, Nagae G, et al. Genetic and epigenetic stability of oligodendrogliomas at recurrence. *Acta Neuropathol Commun* 2017; 5: 18.

20 Atak ZK, Gianifievi L, Hulselmans G, et al. Comprehensive analysis of transcriptome variation uncovers known and novel driver events in T-cell acute lymphoblastic leukemia. *PLoS Genet* 2013; 9: e1003997.

21 Ding H, Beckers MC, Plaisance S, Marynen P, Colfen D, Belaye A. Characterization of a double homoeodomain protein (DUX1) encoded by a cDNA homologous to 3.3 kb dispersed repeated elements. *Hum Mol Genet* 1998; 7: 1681–94.

22 van Overveld PGM, Lemmers RJFL, Deidda G, et al. Interchromosomal repeat array interactions between chromosomes 4 and 10: a model for subtelomeric plasticity. *Hum Mol Genet* 2000; 9: 2879–84.

23 Gabelini D, Green MR, Tupler R. Inappropriate gene activation in FSHD: a novel transcriptional repeat deleted in dystrophic muscle. *Cell* 2002; 110: 339–48.

24 Zeng W, de Greef CH, Chen YY, et al. Specific loss of histone H3 lysine 9 trimethylation and HP1gamma/cohesin binding at D4Z4 repeats is associated with facioscapulohumeral dystrophy (FSHD). *PLoS Genet* 2009; 5: e1000559.

25 van Overveld PG, Lemmers RJFL, Sandkuijl LA, et al. Hypomethylation of D4Z4 in 4q-linked facioscapulohumeral muscular dystrophy. *Nat Genet* 2003; 35: 315–7.

26 Dixit M, Ansseau E, Tassin A, et al. DUX4, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of PITX1. *Cell* 2008; 132: 1535–47.

27 Kim E, Lu HC, Zogbhi HY, Song JJ. Structural basis of protein complex formation and reconfiguration by polyglutamine disease protein Ataxin-1 and Capicua. *Genes Dev* 2013; 27: 590–5.

28 Fryer JD, Yu P, Kang H, et al. Exercice and genetic rescue of SCARA1 via the transcriptional repressor Capicua. *Science* 2011; 334: 600–3.

29 Lee Y, Fryer JD, Kang H, et al. ATXN1 protein family and CIC regulate extracellular matrix remodeling and lung alveolarization. *Dev Cell* 2011; 21: 746–57.

30 Simon-Carrasco L, Grana O, Salomon M, et al. Inactivation of Capicua in adult mice causes T-cell lymphoplastic lymphoma. *Genes Dev* 2017; 31: 1–13.

31 Park S, Lee S, Lee CG, et al. Capicua deficiency induces immunodeficiency and promotes follicular helper T cell differentiation via derepression of ETV5. *Nat Commun* 2017; 8: 16037.

32 Lu HC, Tan Q, Rousseaux MWC, et al. Disruption of the ATXN1-CIC complex causes a spectrum of neurobehavioral phenotypes in mice and humans. *Nat Genet* 2017; 49: 527–36.

33 Kim E, Park S, Choi N, et al. Deficiency of Capicua disrupts bile acid homeostasis. *Sci Rep* 2015; 5: 8272.

34 Okimoto RA, Breitenbucher F, Olivas VR, et al. Inactivation of Capicua drives cancer metastasis. *Nat Genet* 2017; 49: 87–96.

35 Lim B, Samper N, Lu H, Rushlow C, Jimenez G, Shvartsman SY. Kinetics of gene derepression by ERK signaling. *Proc Natl Acad Sci USA* 2013; 110: 10330–5.
56 Tanaka M, Yamazaki Y, Kanno Y, et al. Ewing’s sarcoma precursors are highly enriched in embryonic osteochondrogenic progenitors. J Clin Invest 2014; 121: 3061–74.

57 Italiano A, Sung YS, Zhang L, et al. High prevalence of CIC fusion with double homeobox (DUX4) transcription factors in EWSR1-negative undifferentiated small blue round cell sarcomas. Genes Chromosom Cancer 2012; 51: 207–18.

58 Le Guellec S, Velasco V, Perot G, et al. 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 2016; 29: 1523–31.

59 Gambarotti M, Benini S, Gamberi G, et al. CIC-DUX4 fusion-positive round-cell sarcomas of soft tissue and bone: a single-institution morphological and molecular analysis of seven cases. Histopathol 2016; 69: 624–34.

60 Yasuda T, Tsuzuki S, Kawazu M, et al. Recurrent DUX4 fusions in B-cell lymphoblastic leukemia of adolescents and young adults. Nat Genet 2016; 48: 569–74.

61 Sugita S, Arai Y, Tonooka A, et al. A novel CIC-FOXO4 gene fusion in undifferentiated small round cell sarcoma: a genetically distinct variant of Ewing-like sarcoma. Am J Surg Pathol 2014; 38: 1571–6.

62 Sturm D, Orr BA, Toprak UH, et al. New brain tumor entities emerge from molecular classification of CNS-PNETs. Cell 2016; 164: 1060–72.

63 Huang SC, Zhang L, Sung YS, et al. Recurrent CIC gene abnormalities in angiosarcomas. A molecular study of 120 cases with concurrent investigation of PLCG1, KDR, MYC, and FLT4 gene alterations. Am J Surg Pathol 2016; 40: 645–55.

64 LeBlanc VG, Firme M, Song J, et al. Comparative transcriptome analysis of isogenic cell line models and primary cancers links capicua (CIC) loss to activation of the MAPK signalling cascade. J Pathol 2017; 242: 206–20.

65 Wang B, Krall EB, Aguirre AJ, et al. ATXN1L, CIC, and ETS transcription factors modulate sensitivity to MAPK pathway inhibition. Cell Rep 2017; 18: 1543–57.