Yonago Acta Medica 2023;66(1):24–35 doi: 10.33160/yam.2023.02.004 *Original Article*

Pathological Comparison of TDP-43 Between Motor Neurons and Interneurons Expressed by a Tetracycline Repressor System on the Mouse Artificial Chromosome

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ABSTRACT

Background Cytoplasmic mislocalization of TAR-DNA binding protein of 43 kDa (TDP-43) is a major hallmark of amyotrophic lateral sclerosis (ALS). TDP-43 aggregation is detected in the cortical and spinal motor neurons in most ALS cases; however, pathological mechanism of this mislocalized TDP-43 remains unknown.

Methods We generated a tetracycline-inducible TDP-43 A315T system on a mouse artificial chromosome (MAC) vector to avoid transgene-insertional mutagenesis, established a mouse embryonic stem (ES) cell line holding this MAC vector system, and investigated whether overexpressed exogenous TDP-43 A315T was mislocalized in the cytoplasm of the ES cell-derived neurons and triggered the neurotoxic effects on these cells.

Results Inducible TDP-43 A315T system was successfully loaded onto the MAC and introduced into the mouse ES cells. These ES cells could differentiate into motor neurons and interneurons. Overexpression of TDP-43 A315T by addition of doxycycline in both neurons resulted in mislocalization to cytoplasm. Mislocalized TDP-43 caused cell death of motor neurons, but not interneurons.

Conclusion Vulnerability to cytoplasmic mislocalized TDP-43 is selective on neuronal types, whereas mislocalization of overexpressed TDP-43 occurs in even insusceptible neurons. This inducible gene expression system using MAC remains useful for providing critical insights into appearance of TDP-43 pathology.

Key words doxycycline; interneuron; motor neuron; mouse artificial chromosome, TAR-DNA binding protein of 43 kDa

Amyotrophic lateral sclerosis (ALS) is characterized by muscle wasting due to the degeneration of lower and upper motor neurons, leading to paralysis and death caused by respiratory failure within 5 years from disease onset.[1] More than 90% of ALS cases exhibit the presence of cytoplasmic inclusions in affected cells, which consist of hyperphosphorylated, ubiquitinated TAR-DNA binding protein of 43 kDa (TDP-43) regardless of mutation status.[2–4] TDP-43 is an RNA-binding protein that localizes primarily in the nucleus and controls RNA transcription, splicing and transport. Both upregulation and depletion of TDP-43 expression causes neuronal loss,[5, 6] but it has not yet been established whether ALS is caused by a gain or loss of TDP-43 function. Several transgenic rodent models, which overexpressed either wild-type (WT) or ALS-linked mutant TDP-43, exhibited degeneration of motor neurons, whereas disease severity was dependent on their expression levels but not genotypes.[7–10] In many transgenic models, affected motor neurons exhibited cytoplasmic mislocalization of TDP-43, as well as sporadic form of ALS in almost all cases.[11, 12] Moreover, it has been reported that dysfunction of interneurons occurred rather than motor neurons in WT and mutant TDP-43 transgenic mice, and a mutant *Tardbp*, which codes for Tdp-43 protein, knock-in mouse.[13–15] Neural susceptibility to unphysiological state of TDP-43 is also unclear.

To unravel the role of dysfunctional TDP-43 in the disease pathogenesis, we created tetracycline-inducible human TDP-43 (hTDP-43) A315T and analyzed

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Received 2022 November 25
Accepted 2022 December 9
Online published 2023 January 16

Abbreviations: ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; CHO, Chinese hamster ovary; CNTF, ciliary neurotrophic factor; DMEM, Dulbecco's modified Eagle's medium; EB, embryoid body; ES, embryonic stem; GDNF, glial cell line-derived neurotrophic factor; KSR, Knock-Out Serum Replacement; MAC, mouse artificial chromosome; 2-ME, 2-mercaptoethanol; MEF, mouse embryonic fibroblast; MMCT, microcell-mediated chromosome transfer; PAC, P1-derived artificial chromosome; PBS, phosphate-buffered saline; SAG, Smoothened agonist; TDP-43, TAR-DNA binding protein of 43 kDa; WT, wild-type

pathogenesis of mutant TDP-43 in the mouse motor neurons and interneurons differentiated from embryonic stem (ES) cells *in vitro*. The tetracycline repressor (tetR)-regulated system is a widely used tool to specifically control gene expression in mammalian cells; thereby, involvement of overexpressed TDP-43 to differentiation process of ES cells into neural lineage can be avoided by allowing mutant TDP-43 to be expressed exclusively on differentiated neural cells. Furthermore, this conditional TDP-43 expression system is made on a mouse artificial chromosome (MAC) vector, preventing insertional mutagenesis of transgenes and intervention of promoter activity by surrounded loci at the transgene-inserted site.[16, 17] This model allows the elucidation of a correlation between TDP-43 overexpression and different neuronal subtypes. We observed distinct differences in degeneration by overexpressed mutant TDP-43 between motor neurons and interneurons derived from the mouse ES cells. Our findings suggest the preferential vulnerability of motor neurons to overexpressed TDP-43 as compared with interneurons.

MATERIALS AND METHODS

Plasmids

PCR-amplified human TDP-43 A315T from a genomic DNA of a transgenic mouse: Tg(Prnp-TARDB*A315T)23Jlel/J (016143, TDP43^{A315T}) (Jackson Laboratory, Bar Harbor, ME) was cloned into SalI/MluI sites of the pTRE3G vector (TAKARA, Kusatsu, Japan), resulting in pTRE3G-hTDP-43. Next, PCR fragments of pTRE3G-hTDP-43 and EF1a-Tet3G (TAKARA) were sequentially cloned into NotI/SnaBI and AscI/KpnI sites of the pB4 vector which had modified multiple cloning site of pBSII (Stratagene, San Diego, CA),[18] respectively (pB4-Tet-hTDP-43 A315T). Finally, a KpnI/AvrII fragment of pB4-Tet-hTDP-43 A315T was inserted to KpnI/AvrII sites of pPH3-9, which was modified pPAC4 (CHORI, Oakland, CA).[18] The resulting vector was designated pPAC-Tet-hTDP-43 A315T (Fig. 1A).

Cell culture

Hprt-deficient Chinese hamster ovary (CHO) cells each bearing a MAC vector, [CHO(MAC6), CHO/MAC6/Tet-hTDP-43 A315T] were maintained at 37 °C in Ham's F-12 nutrient mixture (Thermo, Waltham, MA) supplemented with 10% fetal bovine serum (FBS) and 800 μg/mL G418 (Promega, Madison, WI). Mouse embryonic fibroblasts (MEFs), isolated from 13.5 days post-coitum wild-type embryos (C57BL/6-J), were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO) plus 10% FBS. The mouse ES cell lines, XOES9, a subclone from TT2,[19] and the microcell hybrid clones, were maintained on mitomycin C-treated Jcl:ICR (CLEA Japan, Fujinomiya, Japan) MEF feeder layers in ES medium (DMEM with 18% FBS, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine (Thermo), 0.1 mM 2-mercaptoethanol (2-ME) (Thermo), and 1000 U/mL leukemia inhibitory factor (LIF) (Merck Millipore, Burlington, MA).

Construction of MAC vectors

Transfection of pPAC-Tet-hTDP-43 A315T was performed in semi-confluent CHO(MAC6) cells in a 60-mm dish using Lipofectamine2000 (Thermo) according to manufacturer's instructions. These cells were treated with HAT (Sigma) 2 days after transfection. After 2–3 weeks, drug-resistant colonies with a functional HPRT allele were identified by genomic PCR and isolated.

Microcell-mediated chromosome transfer (MMCT)

MMCT was carried out as described previously.[20] CHO/MAC6/Tet-hTDP-43 A315T was used as microcell donor cells. In brief, microcells were harvested by centrifugation from the donor cells and fused with XOES9 by 45% polyethyleneglycol 1500 (Roche, Basel, Switzerland) and 10% dimethylsulfoxide (Sigma). Next day, fused cells were re-plated onto a feeder layer. On day 2, culture medium was replaced with ES medium containing 200 μg/mL G418, and individual G418-resistant colonies were isolated from day 12 onward.

Motor neuron induction

For differentiation, XOES9 or XOES9/Tet-hTDP-43 A315T was dissociated with 0.25% trypsin/EDTA. To remove MEF feeder layers, detached mouse ES cells were incubated in gelatin-coated dishes for 30 min. The supernatants were harvested, washed once with serum-free GMEM, and plated at the cell density of 3000 cells/150 μL/well in low-attachment, round bottom 96-well plates (SUMITOMO BAKELITE, Shinagawa, Japan) in GMEM/KSR medium (GMEM with 10% KSR, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 0.1 mM 2-ME (Thermo). After 2 days, medium was changed to GMEM/KSR medium containing 100 nM retinoic acid and 100 nM SAG. This motor neuron embryoid body (EB) induction medium was renewed every other day. At day 7, the EBs were harvested, washed once with serum-free GMEM, trypsinized, and replated at cell density of 4.0 x 10^5 cells in GMEM/KSR containing 1 μM BDNF/1 μM GDNF/1 μM CNTF on poly-L-lysine/fibronectin-coated dishes.[21]

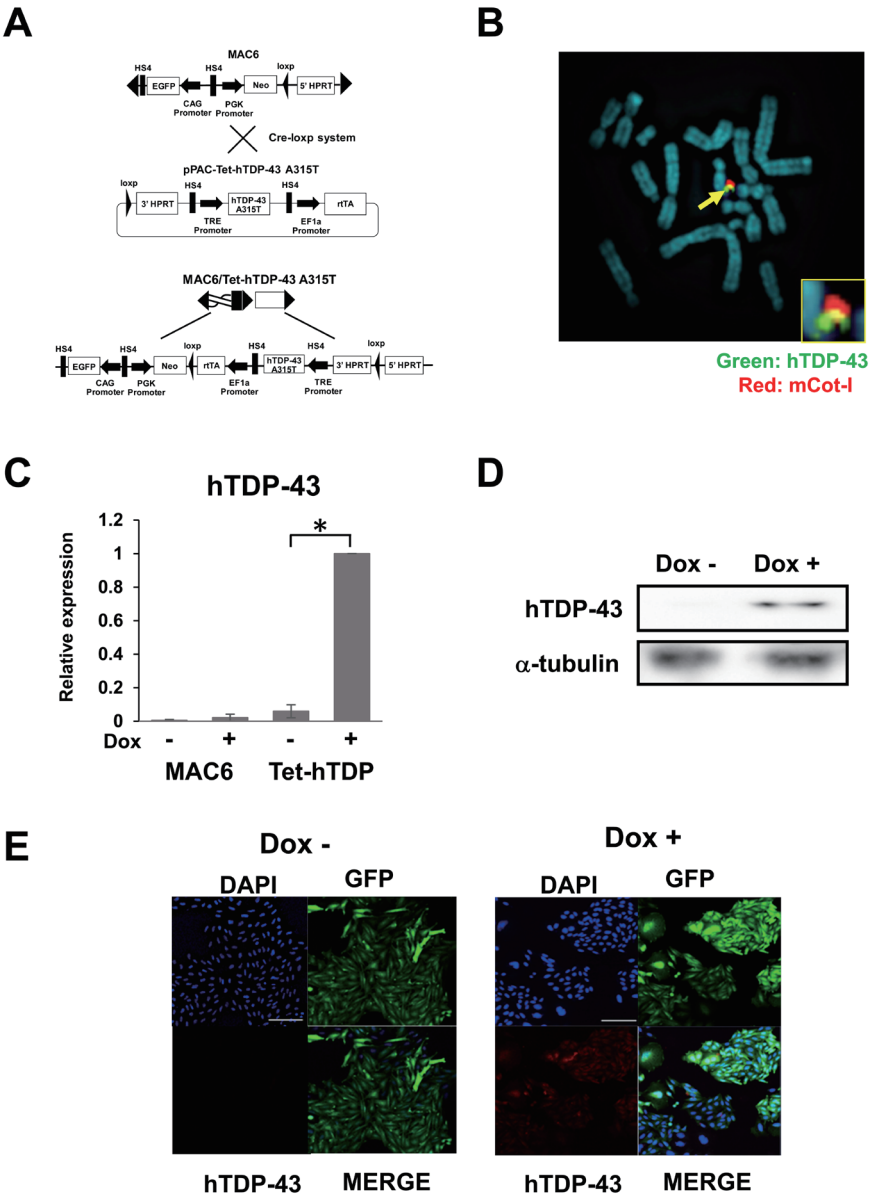

Fig. 1. Construction of a MAC6/Tet-hTDP-43 A315T vector. (**A**) A schematic diagram of the insertion of pPAC-Tet-hTDP-43 A315T containing two elements of the Tet-On 3G systems: rtTA and pTRE3G promoter, and a full-length human TDP-43 cDNA sequence carrying the A315T amino acid substitution into the MAC6 vector. The expression cassette of human TDP-43 A315T is followed by exon 3 to exon 9 of human HPRT gene flanked with a loxP site. (**B**) FISH analysis of CHO/MAC6/Tet-hTDP-43 A315T. Digoxigenin-labeled mouse Cot-1 DNA (red) was used to detect the MAC. Biotin-labeled pPAC-Tet-hTDP-43 A315T (green) was used to detect the transgene. Chromosomal DNA was counterstained with DAPI. A yellow arrow indicates the objective MAC vector. (**C**) Quantitative RT-PCR analysis, (**D**) Western blotting and (**E**) Immunostaining to detect the expression of human TDP-43 A315T in CHO/MAC6/Tet-hTDP-43 A315T cells in a doxycycline (Dox)-dependent manner. CHO *Gapdh* mRNA and α-tubulin were used as internal controls for qRT-PCR and western blotting, respectively. Data were obtained from triplicate experiment, mean ± SEM. **P* < 0.05 (Student's *t*-test) (**C**). Scale bars, 100 μm (**E**).

Interneuron induction

For differentiation to interneuron, GMEM/KSR medium containing 100 nM XAV939 and 250 nM LDN193189 was used.[22] From day 7, the same protocol of motor neuron induction was performed.

FISH analysis

FISH analyses were performed on either fixed metaphase or interphase spreads of each cell hybrid using digoxigenin-labeled (Roche) mouse COT-1 DNA (Thermo) and biotin-labeled pPAC-Tet-hTDP-43 A315T, essentially as described previously.[20] Images were

captured using the Isis system (Zeiss, Oberkochen, Germany). We counted 20 metaphases and 100 interphases for each analysis.

RT-PCR analysis

Total RNA was extracted with TriZol (Thermo) and cDNA was synthesized using an oligo(dT) primer by ReverTra Ace (TOYOBO, Osaka, Japan). Quantitative RT-PCR was performed using GoTaq qPCR System (Promega) on an StepOnePlus (Applied Biosystems, Waltham, MA). Primer sequences are listed in Appendix Table A1.

Western blot

Protein extraction from cultured cells was performed with sample buffer (100 mM Tris, 1% 2-ME and 4% SDS). Standard SDS-PAGE and blotting to PVDF membrane (GE healthcare, Chicago, IL) were performed. Membranes were blocked with 5% skim milk in Tris-buffered saline plus 0.1% Tween 20 (TBS-T) for 1 h and probed overnight at 4 °C with one of the following primary antibodies in 5% TBS-T: mouse monoclonal TDP-43 antibody recognizing human TDP-43, rabbit polyclonal TDP-43 antibody recognizing total TDP-43, and mouse monoclonal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody. In detail, primary antibodies used are listed elsewhere (Appendix Table A2). The membrane was washed 3 times for 5 min in TBS-T and then incubated with the appropriate secondary antibody for 1 h at room temperature. The membrane was again washed 3 times for 5 min in TBS-T. ECL reagent was added for 2 min, and images were acquired using LAS4000mini (GE Healthcare).

Immunocytochemistry

Cells were fixed by 4% paraformaldehyde for 15 min at room temperature and washed with 0.1% glycine/PBS. Samples were permeabilized and blocked with 5% goat serum, 5% skim milk in PBS and 0.1% Triton X-100 (PBS-T) for 1 h at room temperature and probed with primary antibodies in the same blocking solution. Used primary antibodies are listed in Appendix Table A2. The sample was washed 3 times for 5 min in PBS-T and then incubated with the appropriate secondary antibody for 1 h at room temperature.

The percentage of differentiated motor neurons and interneurons were calculated as the numbers of Islet1$^+$/β-tubulin III$^+$ and parvalbumin$^+$/β-tubulin III$^+$ cells divided by the number of β-tubulin III$^+$ neurons (approximately 300 cells counted visually), respectively. Similarly, cell death was determined by counting Islet1$^+$/cleaved caspase-3$^+$ and parvalbumin$^+$/ cleaved caspase-3$^+$ cells from 100 Islet1$^+$ and parvalbumin$^+$ cells, respectively.

Statistical analysis

Data are expressed as mean ± standard error. Statistical analysis was performed by using Student's *t*-test to compare neuronal subtypes. $P < 0.05$ was considered statistically significant.

RESULTS

Construction of a MAC-based tetracycline-inducible hTDP-43 A315T expression system

To clarify pathological mechanism of overexpressed TDP-43, we constructed MAC-based tetR-regulated mutant hTDP-43 expression vector. Two components of TetR system, which consisted of rtTA driven by EF1 promoter and hTDP-43 A315T driven by TRE promoter were combined in a PAC vector and introduced onto a MAC6 vector by Cre/loxP recombination. We chose MAC6 from our series of MAC vectors to carry this PAC vector because it encodes EGFP; therefore, recipient cells can be screened for the presence of a MAC6-derived EGFP fluorescence. Successfully recombined cells were cloned and designated CHO/MAC6/Tet-hTDP-43 A315T. This CHO donor cell has a single copy of recombined MAC6, confirmed by FISH analysis (Fig. 1B). Doxycycline-dependent transgene expression was also confirmed by qRT-PCR, western blotting and immunocytochemistry (Figs. 1C–E). Leaky expression of the transgene was hardly detected by all assays, and the transgene expression from most EGFP-positive cells could be induced by the treatment of doxycycline (Fig. 1E).

Establishment of mouse ES clones harboring MAC6/Tet-hTDP-43 A315T

Subsequently, this MAC6/Tet-hTDP-43 A315T was transferred into mouse ES cells, XOES9, and two ES clones, which were both EGFP-positive and G418-resistant were established and designated as ES-TDP1 and ES-TDP2 (Fig. 2A). Both ES clones held a single copy of the MAC6/Tet-hTDP-43 A315T, which was confirmed by FISH analyses (Fig. 2B) and maintained the reactivity to doxycycline, although the ES-TDP2 clone exhibited much slightly leaky expression of the transgene (Fig. 2D). Consequently, ES-TDP1 clone was used in further examinations.

Differentiation to motor neurons

To elucidate whether overexpressed TDP-43 exhibits pathological signatures to motor neurons, ES-TDP1 was differentiated into the motor neuron-lineages by

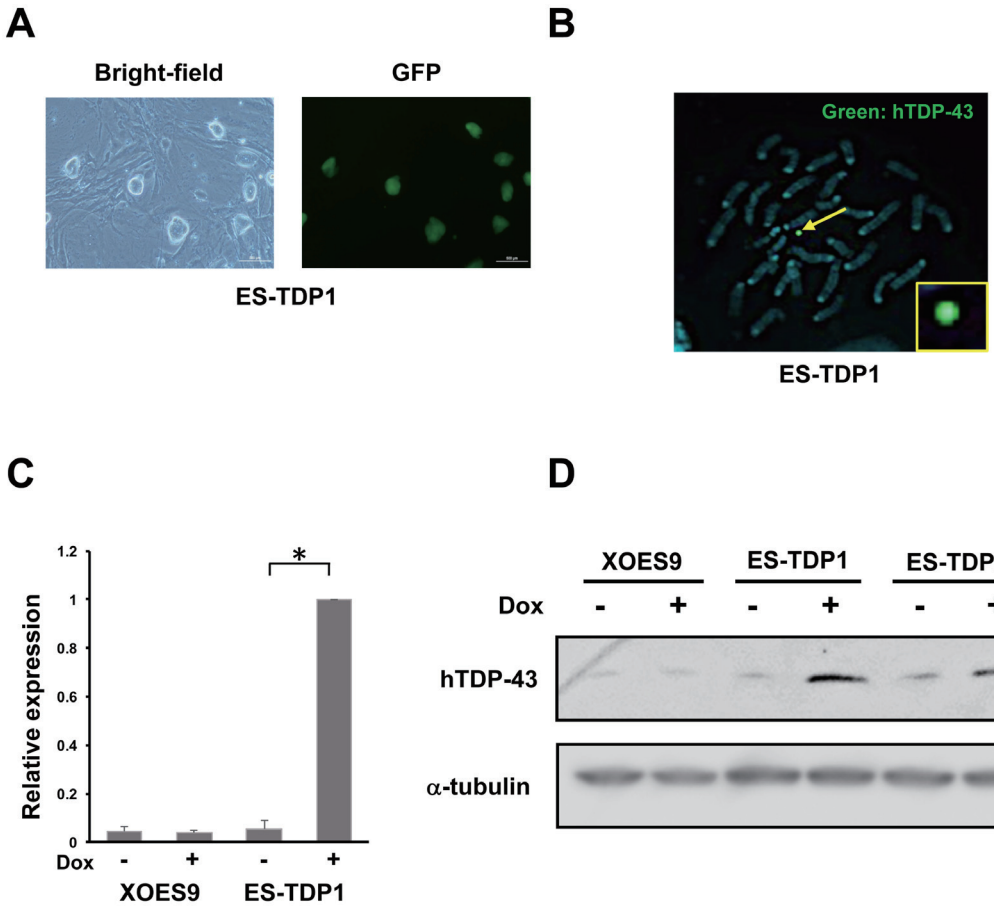

Fig. 2. Characterization of mES/Tet-hTDP-43 A315T cells. (**A**) A fluorescent image of GFP in ES-TDP1 cells. Scale bar, 500 μm. (**B**) FISH analysis of ES-TDP1 cells. A green signal is derived from MAC6/Tet-hTDP-43 A315T. A yellow arrow indicates the MAC vector. (**C**) Quantitative RT-PCR analysis of Dox-dependent expression of the transgene in the ES-TDP1 cells after 3 days of the Dox treatment. Data were obtained from triplicate experiment, mean ± SEM. **P* < 0.05 (Student's *t*-test). (**D**) Western blot analysis of the transgene expression with or without the treatment of Dox for 3 days.

using retinoic acid (RA) and SAG, Sonic Hedgehog agonist (Fig. 3A). Under this protocol, ES-TDP1 cells formed embryoid bodies and were differentiated to the neural progenitor cells of motor neuron-lineage 6 days after culture in the differentiation medium. From day 3, the expression of neurogenin 2 (Ngn 2), a proneural marker and a master regulator of neurogenesis, was induced. Continuous attached culture in the presence of neurotrophic factors: BDNF, GDNF and CNTF, allowed differentiation of the progenitor cells to motor neurons. RT-PCR analyses indicated that both wild-type ES (XOES9) and ES-TDP1 were efficiently differentiated into Nkx2.2$^+$ progenitor cells, HB9$^+$ early motor neurons and Islet1$^+$ later motor neurons over time (Fig. 3C). Consistent with these results, immunocytochemical analyses revealed that approximately 30% of β-tubulin III$^+$ neurons expressed late motor neuron marker Islet1 on day 12 (Fig. 3B). At the point of emergence of Islet1$^+$ cells (Day 9), doxycycline treatment was started. Only 6 days after treatment (Day 15), 25% of Islet1$^+$ neurons were dead as detected by immunostaining of cleaved caspase-3 (Figs. 3F and G). Prolonged treatment of doxycycline for 9 days forced a third of these neurons to be dead, while only 12% of these neurons without doxycycline were dead. Moreover, overexpressed hTDP-43 A315T localized predominantly in the cytoplasm of motor neurons (Fig. 3E), indicating that this hTDP-43 protein was misfolded. These results suggested that overexpressed TDP-43 protein mislocalizes to cytoplasm and vigorously causes neural cell death.

Differentiation to interneurons

As it is shown that motor neurons are relatively vulnerable to TDP-43 toxicity, we subsequently examined whether interneurons are vulnerable to TDP-43 toxicity, similar to the results obtained for motor neurons.

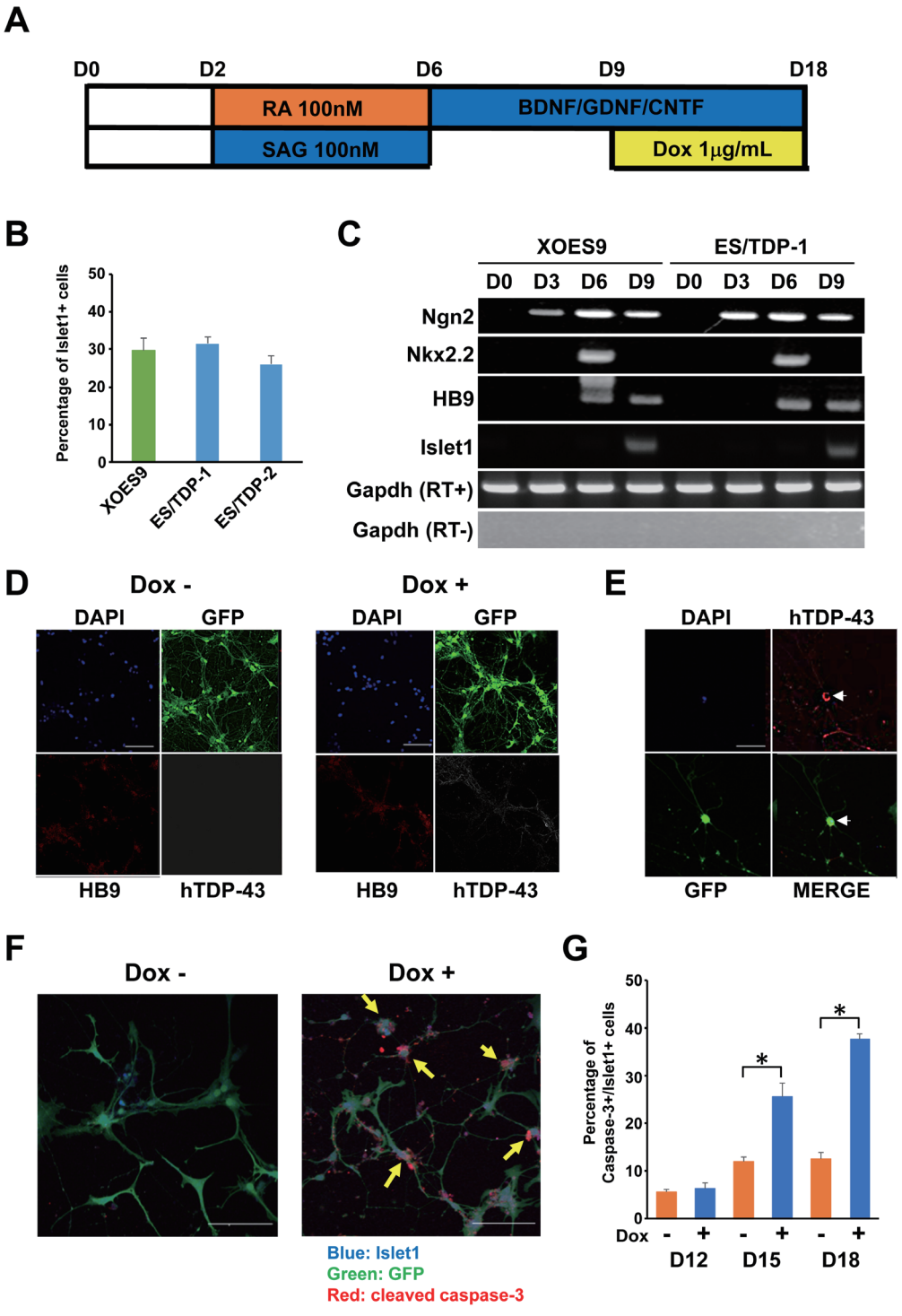

Fig. 3. Influence of exogenous hTDP-43 A315T on motor neurons differentiated from mES/Tet-hTDP-43 A315T cells. (**A**) Motor neuron differentiation scheme using the combination of retinoic acid (RA) and SAG, Sonic Hedgehog agonist. (**B**) Quantitative analysis of the number of Islet1^{+}/β-Tubulin III^{+} cells differentiated from mES/MAC6/Tet-hTDP-43 A315T clones on day 12. Data were obtained from triplicate experiment, mean ± SEM. (**C**) Time-course analysis of the expression of differentiation markers. (**D**) Immunocytochemical analyses of motor neurons for HB9 (red) and human TDP-43 (white) on day 12 in the absence and presence of Dox. Scale bar, 100 μm. (**E**) A representative image of cytoplasmic mislocalization of human TDP-43 (red) 3 days after treatment of Dox. White arrows indicate cytoplasmic localization and nuclear depletion of human TDP-43. Scale bar, 100 μm. (**F**) Immunocytochemical analyses of motor neurons for Islet1 (blue) and cleaved caspase-3 (red) on day 15 in the absence and presence of Dox. Yellow arrows indicate apoptosis of Islet1^{+} motor neurons. Scale bar, 100 μm. (**G**) Quantitative analysis of the number of cleaved caspase-3^{+}/Islet1^{+} cells. Data were obtained from triplicate experiment, mean ± SEM. *$P < 0.05$ (Student's *t*-test).

Differentiation from ES cells into interneurons was performed by using XAV939, a tankyrase/Wnt inhibitor and LDN-193189, an ALK2/3 inhibitor instead of RA and SAG (Fig. 4A). After 6 days, Nkx2.1 and Lhx6, markers for neural progenitors of ventral prosencephalon, were detected by RT-PCR, and ventral prosencephalic interneuron marker parvalbumin also emerged (Fig. 4C). Immunoreactivities against parvalbumin were demonstrated, indicating the efficacy of the differentiation protocol, although the low efficiency of approximately 12% suggests that further optimization is needed to improve the differentiation protocol to obtain parvalbumin$^+$ interneurons. Surprisingly, the treatment of doxycycline for 6 days (Day 15) had no effect on the cell death of parvalbumin$^+$ interneurons (Figs. 4E and F), whereas cytoplasmic mislocalization of hTDP-43 was detected in these cells similar to that observed in motor neurons (Fig. 4D). Prolonged treatment for up to day 18 resulted in appearance of cell death. From these results, parvalbumin$^+$ interneurons were thought to be relatively resistant to mislocalized TDP-43 toxicity, especially at early onset.

DISCUSSION

We found that exogenously expressed TDP-43 mislocalized to cytoplasm in both motor neurons and interneurons derived from the ES cells. Intriguingly, interneurons with cytoplasmic TDP-43 were slightly resistant to TDP-43 pathology rather than motor neurons. Cytoplasmic mislocalization of TDP-43 is thought to be caused by impairing nucleocytoplasmic transport, leading to increased neuronal cell death.[23–26] Therefore, our findings suggest that impairment of nucleocytoplasmic transport by overexpressed mutant TDP-43 may be independent of specific cell types; however, susceptibility to cytoplasmic TDP-43 toxicity be different among neural types.

TDP-43, primarily a nuclear protein that can shuttle between the nucleus and cytoplasm and bind thousands of mRNAs including its own transcript is implicated in repression of gene transcription and regulation of exon splicing. Thus, exogenously expressed TDP-43 has resulted in reduction of endogenous TDP-43 protein expression due to a negative feedback loop.[27] Consequently, the expression of pathological TDP-43 is linked to its accumulation in the cytoplasm, accompanied with the depletion of normal nuclear TDP-43, causing neurodegeneration. Although mouse-derived TDP-43 was not analyzed in this experimental model, it is possible that human- and mouse-derived TDP-43 interact, and the possibility that the expression level and subcellular localization of mouse-derived TDP-43 is related to cellular damage cannot be ruled out.

Recent studies have demonstrated that degeneration of interneurons occurred in both aging TDP-43 transgenic mice[14] and *Tardbp* knock-in mice[15] without distinct deficit of the motor system. In contrast, our results showed that interneurons were more resistant. It is not clear why there is a discrepancy between our *in vitro* model and these mouse models. It is conceivable that aging-related pathophysiological changes, such as mitochondrial dysfunction, inflammation, and oxidative stress[28] may influence TDP-43 pathology in the interneurons. Although the neurons differentiated *in vitro* from ES cells may rarely have age-associated characteristics, treatments with appropriate stressors, such as reactive oxygen species, ionophores and proteasome inhibitors, can recapitulate these features.[29] Future studies combining our established *in vitro* model with these circumstances may mimic the senescence that will help decode the discrepancy.

It is possible that cytoplasmic mislocalized TDP-43 could disturb several cellular responses, such as the formation of stress granules,[30] axonal transport,[31] mitochondrial function,[32] ER stress and the unfolded protein response[33] and autophagy.[34] Further studies to analyze these downstream responses after overexpression of TDP-43 will lead to elucidate factors associated with different vulnerability to toxicity of mislocalized TDP-43 between motor neurons and interneurons. The MAC vector on which we constructed the cassette of tetracycline-inducible TDP-43 A315T will assist in directing efforts toward clarifying molecular mechanisms underlying TDP-43 pathology.

Acknowledgments: We are grateful to Dr. Hiroyuki Kugoh, Dr. Hiroyuki Satofuka, Dr. Kanako Kazuki, Dr. Takashi Moriwaki, Dr. Yosuke Hiramuki and Dr. Takahito Ohira at Tottori University for critical discussions. This study was supported in part by Research Support Project for Life Science and Drug Discovery (BINDS) from AMED under Grant Number JP22ama121046 (Y.K.), Joint Research of the Exploratory Research Center on Life and Living Systems (ExCELLS) (ExCELLS program No, 21-101) (Y.K.), and JST, CREST Grant Number JPMJCR18S4, Japan (K.T. and Y.K.). This research was partly performed at the Tottori Bio Frontier managed by Tottori Prefecture.

The authors declare no conflict of interest.

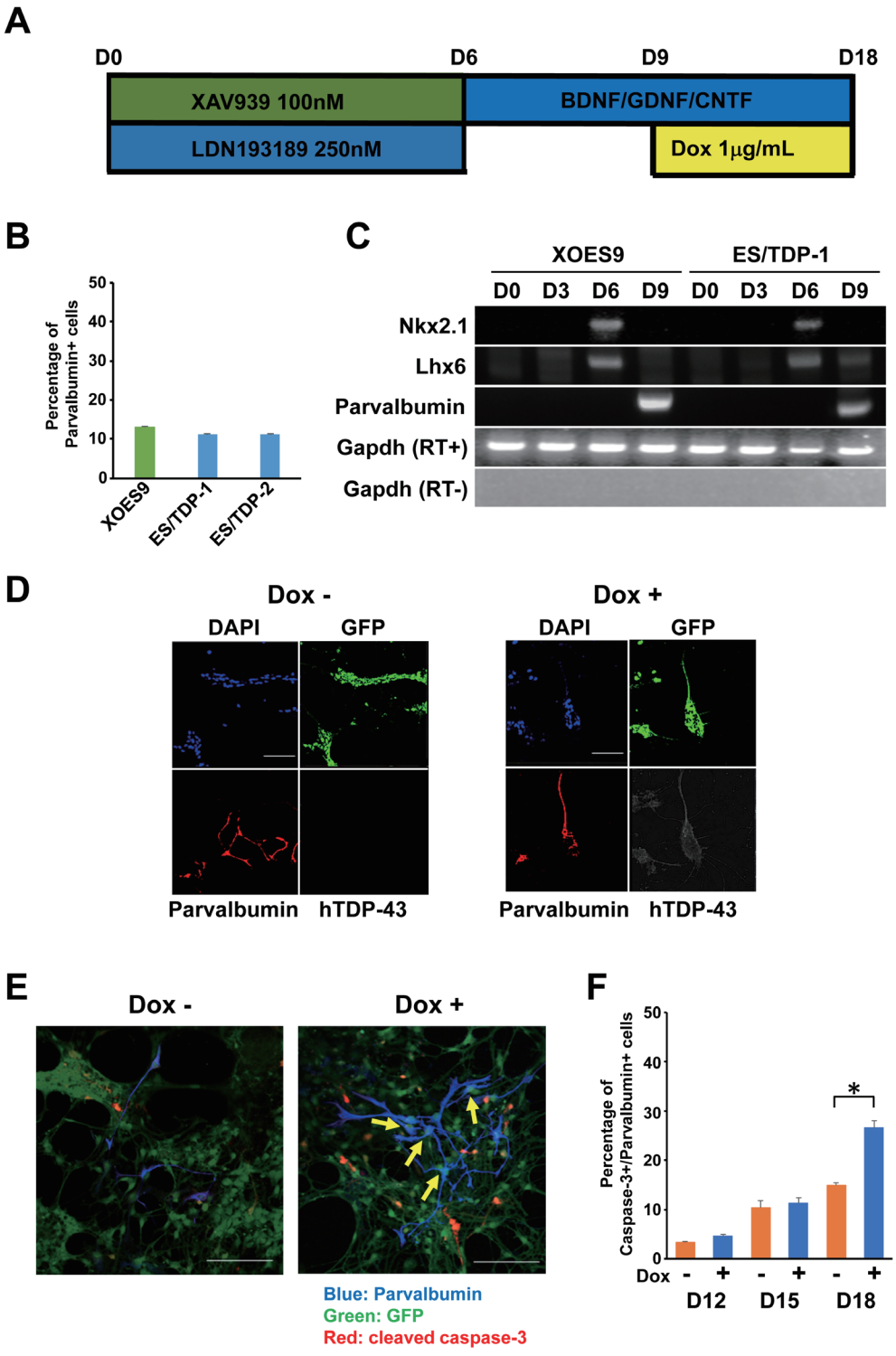

Fig. 4. Influence of exogenous hTDP-43 A315T on interneurons differentiated from mES/Tet-hTDP-43 A315T cells. (**A**) Interneuron differentiation scheme using the combination of AV939, a tankyrase inhibitor and LDN193189, an ALK2/3 inhibitor. (**B**) Quantitative analysis of the number of parvalbumin^{+}/β-tubulin III^{+} cells differentiated from mES/MAC6/Tet-hTDP-43 A315T clones on day 12. Data were obtained from triplicate experiment, mean ± SEM. (**C**) Time-course analysis of the expression of differentiation markers. (**D**) Immunocytochemical analyses of interneurons for parvalbumin (red) and human TDP-43 (white) on day 12 in the absence or presence of Dox. Scale bar, 100 μm. (**E**) Immunocytochemical analyses of interneurons for parvalbumin (blue) and cleaved caspase-3 (red) on day 15 in the absence or presence of Dox. Yellow arrows indicate parvalbumin^{+} interneurons, which demonstrate no sign of cell death. Scale bar, 100 μm. (**F**) Quantitative analysis of the number of cleaved caspase-3^{+}/parvalbumin^{+} cells. Data were obtained from triplicate experiment, mean ± SEM. *$P < 0.05$ (Student's *t*-test).

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APPENDIX

Table A1. A list of primers

ID	Sequence
TDP-43 _sal1_Fw	AAGTCGACATGTCTGAATATATTCGGGTAA
TDP-43 _mlu1_Rv	TTACGCGTCTACATTCCCCAGCCAGAAGAC
TREG-TDP43_Not1_Fw	AAGCGGCCGCctcgagtttactccctatca
TREG-TDP43_SnaB1_Rv	TTTACGTAgcagtgaaaaaaatgctttatt
EF1a-Tet_Asc1_Fw	AAGGCGCGCCctcgaggagcttggccca
EF1a-Tet_Kpn1_Rv	TTGGTACCatacttcccgtccgcca
EF1a1.6K Fw	CGATGTGAGAGGAGAGCACA
EF1a 1.9K Rv	TAGAAGACACCGGGACCGATC
EF1a 2.2K Fw	CCGGTACTCCGTGGAGTCAG
EF1a 2.6K Rv	AGTCTTGTAAATGCGGGCCA
EF1a 3.3K Rv	GAGCTTATCGCGATGCTAGC
TRE TDP 6.0K	CTCTAGAGCCGCAGACATGA
TRE TDP 6.7K Rv	GGAACTGATGAATGGGAGCA
TDP7K Fw	GAGCCAAAGCCTCCATTAAAACCAC
TDP 7.5K Rv	TTGGTGGTAATCCAGGTGGC
hTDP-43 Fw	Acgttcagcattaatccagcc
hTDP-43 Rv	gcatgttgccttggttttgg
mTDP-43 Fw	TGATAGATGGGCGATGGTGTG
mTDP-43 Rv	TGTCCTCTGTACAACGTCCA
GAP452F	ACCACAGTCCATGCCATCAC
GAP452R	TCCACCACCCTGTTGCTGTA
CHO_GAP_qRT_F	AGAAGGTGGTGAAGCAGGCAT
CHO_GAP_qRT_R	AGGTCCACCACTCTGTTGCTGT
mGAPDH_F_RIKEN	TGACCACAGTCCATGCCATC
mGAPDH_R_RIKEN	GACGGACACATTGGGGGTAG
mNgn2_RT_Fw	GCTGGCATCTGCTCTATTCC
mNgn2_RT_Rv	ATGAAGCAATCCTCCCTCCT
mNkx2_2_RT_Fw	AACCGTGCCACGCGCTCAAA
mNkx2_2_RT_Rv	AGGGCCTAAGGCCTCCAGTCT
mHB9_Fw	CTCATGCTCACCGAGACTCA
mHB9_Rv	TCA GCT CCT CTT CCG TCT TC
m2_Isl1_RT_Fw	AGATATGGGAGACATGGGCGAT
m2_Isl1_RT_Rv	ACACAGCGGAAACACTCGATG
mPax6_RT_Fw	AGGGGGAGAGAACACCAACT
mPax6_RT_Rv	CATTTGGCCCTTCGATTAGA
mNkx2_1_RIKEN_F	TACTGCAACGGCAACCTG
mNkx2_1_RIKEN_R	GCCATGTTCTTGCTCACGTC
mLHX6_Fw	CCGATTTGGAACCAAGTGCG
mLHX6_Rv	AATGTAGCCGTGCAAGGTGA
mParvalbumin_Fw	AAGAGTGCGGATGATGTGAAG
mParvalbumin_Rv	AGCCATCAGCGTCTTTGTTT

Table A2. A list of antibodies

Resource	Source	Identifier	Concentration
Mouse monoclonal anti-human specific TDP-43	Proteintech	Cat#60019-2-Ig	1/10000
Mouse monoclonal anti-alpha tublin	Abcam	Cat#ab7291	1/10000
Mouse monoclonal anti-Islet-1	DSHB	Cat#39.4D5	1/200
Goat polyclonal anti-Islet-1	R&D systems	Cat#AF1837	10 ng/ml
Rabbit polyclonal anti-HB9	Millipore	Cat#ABN174	1/200
Rabbit polyclonal anti-cleaved Caspase-3	Cell signal technology	Cat#9661	1/200
Rabbit polyclonal anti-Parvalbumin	Abcam	Cat#ab11427	1/200
Mouse monoclonal anti-Parvalbumin	Millipore	Cat#MAB1572	1/200
Chicken monoclonal anti-Beta III tublin	Millipore	Cat#AB9354	1/1000