Tyrosine pre-transfer RNA fragments are linked to p53-dependent neuronal cell death via PKM2

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ABSTRACT

Fragments of transfer RNA (tRNA), derived either from pre-tRNA or mature tRNA, have been discovered to play an essential role in the pathogenesis of various disorders such as neurodegenerative disease. CLP1 is an RNA kinase involved in tRNA biogenesis, and mutations in its encoding gene are responsible for pontocerebellar hypoplasia type-10. Mutation of the *CLP1* gene results in the accumulation of tRNA fragments of several different kinds. These tRNA fragments are expected to be associated with the disease pathogenesis. However, it is still unclear which of the tRNA fragments arising from the *CLP1* gene mutation has the greatest impact on the onset of neuronal disease. We found that 5′ tRNA fragments derived from tyrosine pre-tRNA (5′ Tyr-tRF) caused p53-dependent neuronal cell death predominantly more than other types of tRNA fragment. We also showed that 5′ Tyr-tRF bound directly to pyruvate kinase M2 (PKM2). Injection of zebrafish embryos with PKM2 mRNA ameliorated the neuronal defects induced in zebrafish embryos by 5′ Tyr-tRF. Our findings partially uncovered a mechanistic link between 5′ Tyr-tRF and neuronal cell death that is regulated by PKM2.

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1. Introduction

Transfer RNAs (tRNAs) are one of the most abundant types of non-coding RNA, and are essential for protein synthesis by bringing amino acids to the translating ribosome. Current deep-sequencing technologies have revealed that various types of small RNA fragments derived from tRNAs are present in most organisms. Recent studies have shown that some tRNA fragments directly contribute to the pathogenesis of some human diseases [1–3].

CLP1 is an RNA kinase that phosphorylates the 5′ hydroxyl ends of RNA [4–6]. Human CLP1 is a component of the messenger RNA 3′-end cleavage and polyadenylation machinery [7]. Human CLP1 is also a component of the tRNA splicing endonuclease (TSEN) complex, which removes the intron present within the anticodon loop of several pre-transfer RNAs and generates tRNA exon halves [8]. Previously, we have shown that CLP1 kinase-dead knock-in mice developed progressive neurodegenerative disease [9]. We also showed that the cause of the neuronal pathogenesis is the accumulation of the 5′ exon of tyrosine pre-tRNA fragments (5′ Tyr-tRF), which comprises a 5′ leader sequence followed by the 5′ exon Tyr-tRNA. The 5′ Tyr-tRF augments the activation of p53 resulting in neuronal cell death.

Human *CLP1* mutation (p.R140H) has been identified in four Turkish families [10,11]. As was also shown in the CLP1 kinase-dead knock-in mice, human *CLP1* mutation causes a neurological syndrome. This syndrome is called pontocerebellar hypoplasia type-10

(PCH10) and involves microcephaly and axonal peripheral neuropathy. We detected an accumulation of introns derived from isoleucine pre-tRNAs in patient's fibroblasts, indicating that these tRNA introns may be a cause of the neurodevelopmental and neurodegenerative disorder. Further, the 3′ exon halves of Tyr-tRNA, in which the 5′ end was unphosphorylated, have been reported to be the most toxic in human cells [11]. However, the pathological association between these tRNA fragments and neurodegeneration remained unclear.

Here, we report the pathological significance of 5′ Tyr-tRF in neuronal development by using a zebrafish (*Danio rerio*) model. We also demonstrated by using a biochemical method that 5′ Tyr-tRF directly binds to PKM2. Neuronal defects induced in zebrafish by the accumulation of 5′ Tyr-tRF were ameliorated by microinjection of PKM2 mRNA into zebrafish embryos. These results suggest that the neuronal defects are initiated by the interaction between 5′ Tyr-tRF and PKM2 during neurogenesis.

2. Materials and methods

2.1. Cell culture

SH-SY5Y cells and HEK293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 Nutrient Mixture (DMEM/Ham's F12) with L-glutamine (Wako, Osaka, Japan), containing 10% FBS (Life Technologies, Grand Island, NY, USA) and 1% penicillin/streptomycin (Nacalai Tesque, Kyoto, Japan). Cells were maintained at 37 °C in a saturated humidity atmosphere containing 95% air and 5% CO_2. For differentiation into neuronal cells, SH-SY5Y cells were cultured with 15 μM all-trans-retinoic acid (Sigma-Aldrich, St. Louis, MO, USA) in DMEM/Ham's F-12 with L-glutamine, containing 1% FBS and 1% penicillin/streptomycin. HEK293T cells were transfected with FLAG-tagged PKM2 plasmid using the polyethylenimine (PEI) method [12].

2.2. Zebrafish maintenance

Wild-type zebrafish of the AB strain and the transgenic zebrafish line Tg[isl1:GFP] [13] were raised and maintained with standard procedures. They were kept at 28°C-29 °C and a 14-h light: 10-h dark cycle. Embryos were collected and placed at 28.5 °C. All experimental animal care was performed in accordance with institutional and national guidelines and regulations. The study protocol was approved by the institutional review board of Oita University (180506).

Please see the detailed experimental procedure in Supplementary Methods.

3. Results

3.1. Toxicity of 5′ Tyr-tRF in human SH-SY5Y cells

To assess the potential roles of RNA fragments derived from tyrosine pre-tRNA in neuronal cells, 5′ Tyr-tRF and 3′ Tyr-tRF as well as control-tRF (5′ arginine-tRF [5′ Arg-tRF]) were transfected into the SH-SY5Y human neuroblastoma cell line. The concentrations of each of the tRFs used for the cell transfection were those used in previous experiments examining tRF toxicity in human fibroblasts [11].

Subsequently, neuronal differentiation of the cells was induced by retinoic acid. Although none of the fragments showed any effect on cell viability under normal cell culture conditions (Fig. 1A and B), 5′ Tyr-tRF led to cell death before the cells were fully differentiated into neurons (Fig. 1C and D). We further examined the cellular responses to other tRNA fragments, such as 3′ Tyr-tRF containing an

unphosphorylated 5′ end (OH-3′ Tyr-tRF), 3′ Tyr-tRF with a CCA sequence (3′ Tyr-CCA tRF), 3′ Tyr-tRF containing an unphosphorylated 5′ end with a CCA sequence (OH-3′ Tyr-CCA tRF), and introns of isoleucine pre-tRNA (Ile intron) (Table S1). These tRNA fragments did not show any effect on cell viability during neuronal differentiation (Fig. 1D). Thus, from among the tRNA fragments expected to accumulate in CLP1 mutants, it may be 5′ Tyr-tRF that potentially exert a toxic effect on neuronal cell differentiation.

p53 activation has been linked to neuronal cell death by the accumulation of tRNA fragments [9]. To provide definitive proof of the importance of the p53 pathway in neuronal cell death by the tRNA fragments, we established p53 gene-deficient SH-SY5Y cells (Fig. S1A and B). The differentiated p53-deficient SH-SY5Y cells were resistant to 5′ Tyr-tRF toxicity (Fig. 1E and F). These results suggest that p53 plays a critical role in neuronal cell death induced by 5′ Tyr-tRF accumulation in cells during neuronal differentiation.

### 3.2. 5′ Tyr-tRFs lead to neuronal defects in zebrafish embryos

To assess the *in vivo* effect of 5′ Tyr-tRF, we used the zebrafish model. The transparency of the fish larvae enables us to monitor neuronal development after the injection of tRNA fragments into eggs. We first injected either 5′ Tyr-tRF or control-tRF (5′ Arg-tRF) into one-cell embryos of zebrafish (Fig. 2A). Although the 5′ Tyr-tRF did not affect hatching rate, microcephaly and curved tail phenotypes were observed at 48 hpf (hours post fertilization) in larvae that had been injected with 5′ Tyr-tRF (Fig. 2A).

In addition to 5′ Tyr-tRF, we injected 3′ Tyr-tRF, OH-3′ Tyr-CCA-tRF, and Ile intron into one-cell embryos of zebrafish. The tRNA fragments other than 5′ Tyr-tRF did not affect zebrafish development (Fig. 2B). Importantly, the nucleotide sequence of the 5′ Tyr pre-tRNA exon in humans, mice, and zebrafish are almost the same, except for just one nucleotide (Fig. S2), indicating that the effects of 5′ Tyr-tRF observed in zebrafish may occur even in humans and mice. Furthermore, we examined the importance of the leader sequence contained in the 5′ end of the 5′ Tyr-tRF on the phenotype. Injection of 5′ Tyr-tRF without the leader sequence induced developmental abnormalities (Fig. S3A and B), such as microcephaly (Fig. S4A and B), in zebrafish embryos to much the same extent as 5′ Tyr-tRF containing the leader sequence. Thus, the accumulation of 5′ Tyr-tRF results in developmental defects in zebrafish embryos regardless of whether the leader sequence is present.

We next assessed abnormalities of the nervous system in the 5′ Tyr-tRF-injected zebrafish during embryogenesis. Compared with control 5′ Arg-tRF injection, 5′ Tyr-tRF injection significantly induced microcephaly in zebrafish larvae, as was observed in CLP1 kinase-dead mice (Fig. 2C and D). We further analyzed the morphological changes of the motor neurons by using the Tg[isl1:GFP] transgenic zebrafish, which is useful for observing the development of motor neurons [13]. In the spinal cord, the numbers of motor neurons declined in the larvae injected with 5′ Tyr-tRF (Fig. 2E and F). Thus, these data indicate that the accumulation of 5′ Tyr-tRF *in vivo* has a high impact on the development of the nervous system during the early embryonic stage.

3.3. Neuronal defects is mediated by p53

To examine whether the neuronal defects observed in the zebrafish injected with 5′ Tyr-tRF were p53-dependent, we injected an antisense morpholino (MO) for the p53 gene at the same time as 5′ Tyr-tRF into one-cell zebrafish embryos. As expected, p53-MO knock-down significantly prevented the abnormal embryonic development (Fig. 3A and B), microcephaly (Fig. 3C and D) and spinal motor neuron loss (Fig. 3E and F) induced by 5′ Tyr-tRF
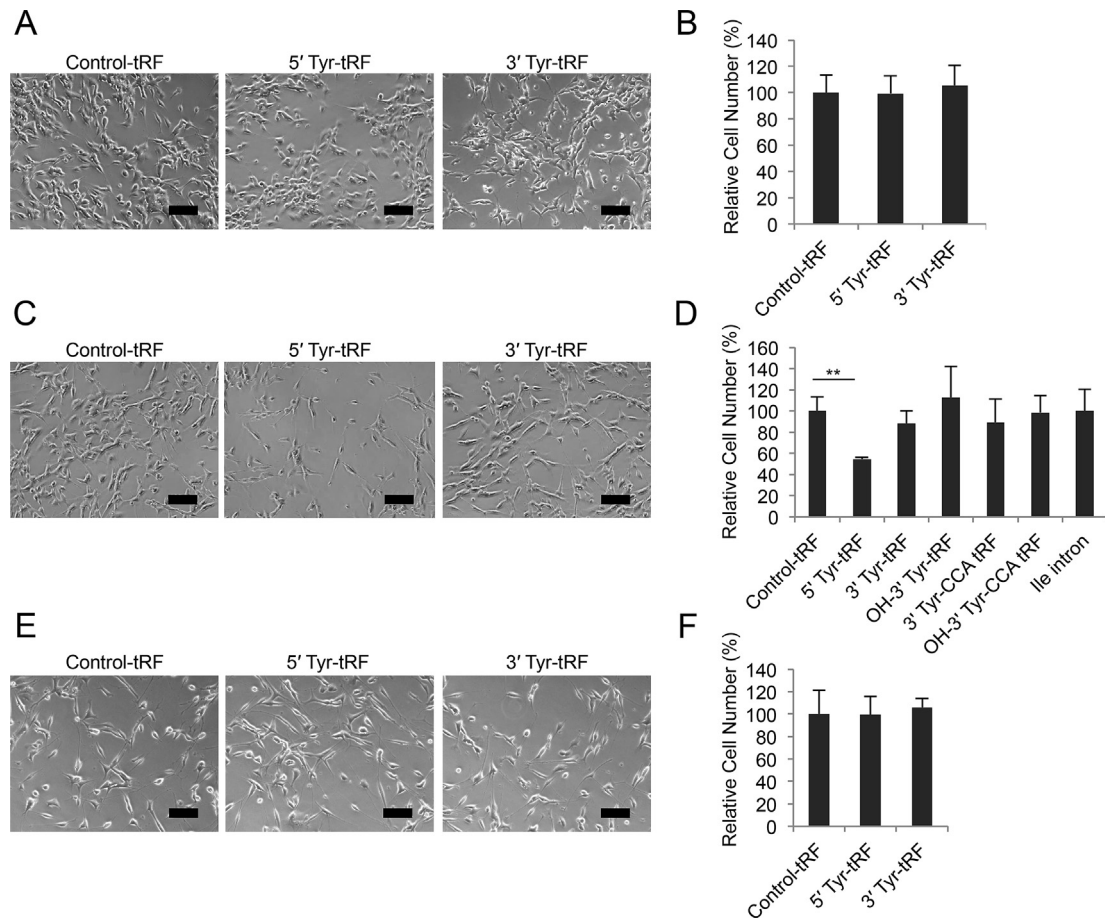
Fig. 1. 5′ Tyr-tRF induces p53-dependent toxicity in SH-SY5Y cells during neuronal differentiation. **(A, B)** SH-SY5Y cells were transfected with control-tRF, 5′ Tyr-tRF, or 3′ Tyr-tRF. The numbers of viable SH-SY5Y cells were counted 3 days later. Data represent the means ± SEM of three independent experiments. Scale bar: 100 μm. **(C, D)** SH-SY5Y cells were transfected with control-tRF or the indicated RNAs, and treated with 15 μM retinoic acid for 24 h to induce neuronal differentiation. The numbers of viable SH-SY5Y cells were counted after 48 h of retinoic acid treatment. Data represent the means ± SEM of three independent experiments. Scale bar: 100 μm $**p < 0.01$. **(E, F)** p53-knockout SH-SY5Y cells were transfected with control-tRF, 5′ Tyr-tRF, or 3′ Tyr-tRF, and neuronal differentiation was induced by retinoic acid. The numbers of viable p53-knockout SH-SY5Y cells were counted after 48 h of retinoic acid treatment. Data represent the means ± SEM of three independent experiments. Scale bar: 100 μm.

injection. These MO knock-down experiments provide direct evidence that 5′ Tyr-tRF induced neuronal defects through a p53-regulated pathway.

3.4. PKM2 is a target of 5′ Tyr-tRFs

To investigate the molecular mechanism of neuronal cell death induced by 5′ Tyr-tRF, we explored the binding targets of 5′ Tyr-tRF by using a modified version of the *drug affinity responsive target stability* (DARTS) approach. DARTS is a biochemical identification approach that analyzes the direct binding of drugs with proteins [14]. Given that the binding of drugs stabilizes or masks the protease recognition sites of proteins, the drug-binding proteins become less susceptible to proteolysis with protease treatment. In the current study, instead of a drug compound, 5′ Tyr-tRF was incubated with SH-SY5Y cell lysate and then digested with pronase. For accurate relative quantification using mass spectrometry, we applied *stable isotope labeling by amino acids in cell culture* (SILAC) labeling to the SH-SY5Y cells [15].

The modified DARTS approach revealed intense silver-stained bands between the 10 and 17 kDa MW markers (Fig. S4). We analyzed these bands by mass spectrometry and identified PKMs as the most abundant and enriched proteins present in the bands. There are two isozymes of PKM: PKM1 and PKM2, and mass spectrometry showed shared amino acid sequences in the two PKM isozymes, meaning that both PKM1 and PKM2 were both potential target molecules of 5′ Tyr-tRF.

To verify which of the two molecules was related to the effect of 5′ Tyr-tRF, we injected PKM1 and PKM2 mRNAs transcribed *in vitro* together with 5′ Tyr-tRF into one-cell embryos. Surprisingly, PKM2 mRNA specifically prevented the abnormal development from 5′ Tyr-tRF toxicity (Fig. 4A and B). On the other hand, PKM1 mRNA was not able to prevent the toxicity of 5′ Tyr-tRF. The PKM2 mRNA injection selectively prevented microcephaly (Fig. 4C and D) and spinal motor neuron loss (Fig. 4E and F) induced by 5′ Tyr-tRF injection. However, PKM1 mRNA did not have a notable effect on neuronal defects.

Finally, we verified the interaction between the 5′ Tyr-tRF and PKM2 by a pull-down assay. Biotin-labeled 5′ Tyr-tRF showed much higher interaction with Flag-tagged PKM2 than did control 5′ Arg-tRF (Fig. 4G). Thus, 5′ Tyr-tRF interacts directly with PKM2 and may inhibit the PKM2-related signaling pathway.

4. Discussion

In this study, we investigated the role of tRNA fragments produced by *CLP1* mutation in mice and human patients. 5′ Tyr-tRF was more toxic for SH-SY5Y cells differentiation by retinoic acid
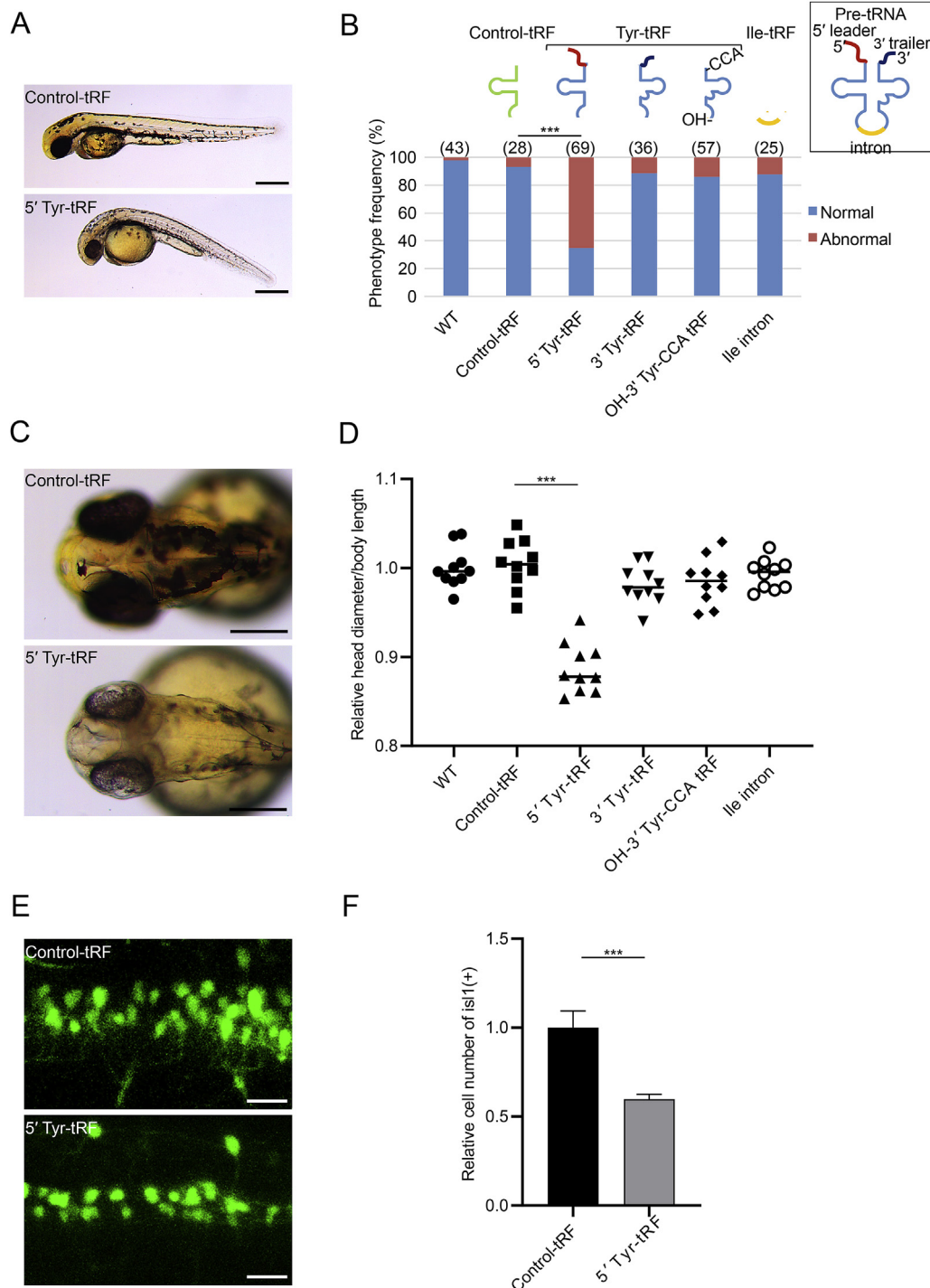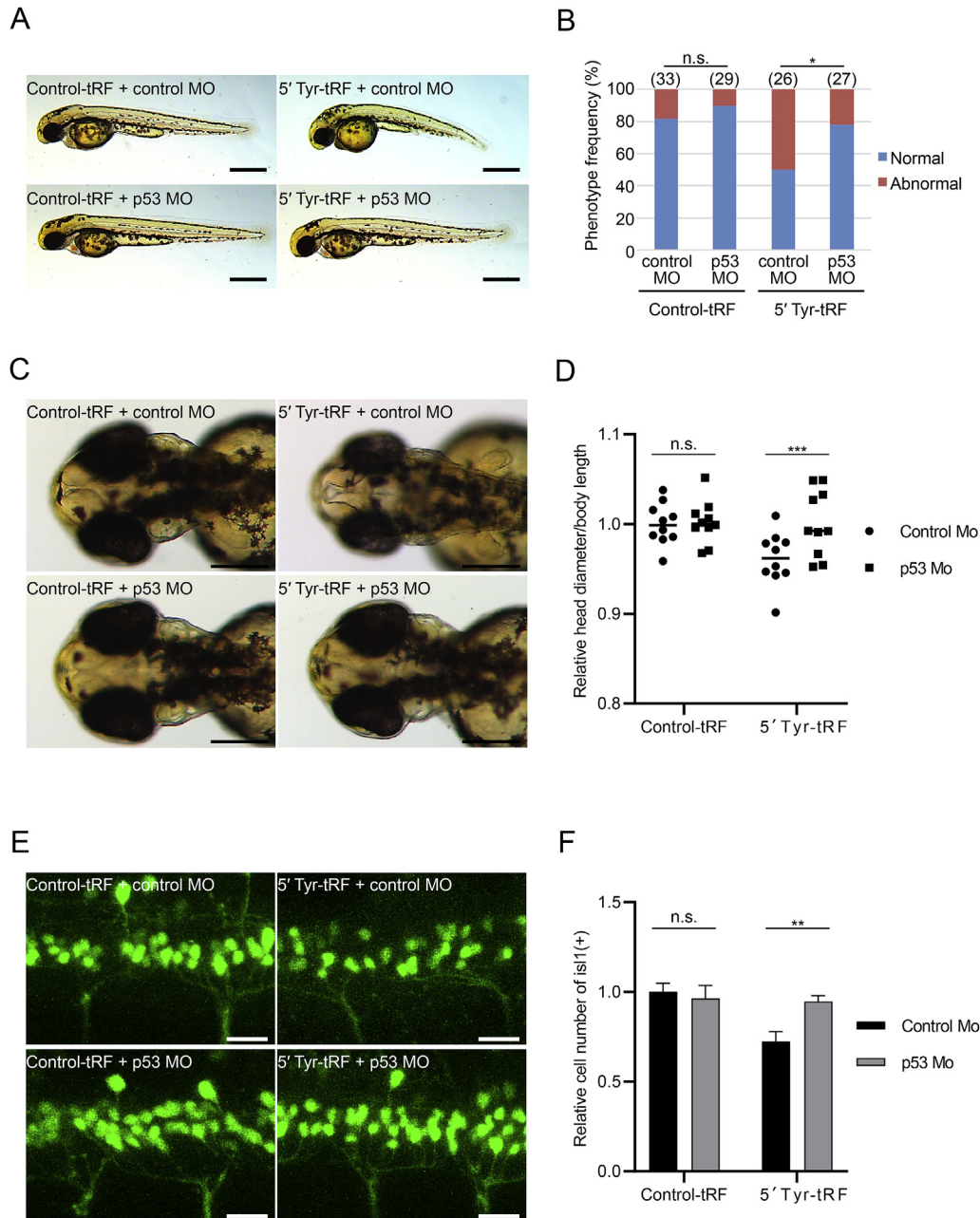
Fig. 2. 5′ Tyr-tRF led to developmental abnormalities and neuronal defects in zebrafish embryos. **(A)** Brightfield lateral views of embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF. Scale bars: 500 μm. **(B)** Relative distribution of morphological phenotypes of embryos (48 hpf) injected with control-tRF or the indicated RNAs. The numbers of zebrafish embryos are indicated at the top of each bar. ***$p < 0.001$. **(C)** Brightfield dorsal views of the heads in the embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF. Scale bars: 200 μm. **(D)** Relative head size of zebrafish embryos (48 hpf) injected with control-tRF or 5′ Tyr-tRF. n = 10 fish/group. Error bars indicate SEM. ***$p < 0.001$. **(E)** Morphological abnormality at 48 hpf in the spinal motor neurons of Tg[*isl1*:GFP] zebrafish injected with control-tRF or 5′ Tyr-tRF. Scale bars: 20 μm. **(F)** Quantification of spinal motor neuron numbers in Tg[*isl1*:GFP] zebrafish injected with control-tRF or 5′ Tyr-tRF (48 hpf). n = 5 fish/group. Error bars indicate SEM. ***$p < 0.001$.

stimulation than the other tRNA fragments. 5′ Tyr-tRF injection into one-cell zebrafish embryos caused more severe neuronal abnormalities than did other tRNA fragments. Furthermore, we identified PKM2 as the target molecule for 5′ Tyr-tRF. PKM2 mRNA injection prevented the developmental abnormalities induced by 5′ Tyr-tRF in zebrafish embryos, indicating that PKM2 is a crucial molecule

involved with pontocerebellar hypoplasia in patients with the *CLP1* mutation.

We showed previously that RNA fragments derived from pre-tRNA, such as 5′ Tyr-tRF and Ile intron, accumulate in cells of CLP1 kinase-dead knock-in mice and in patients with the CLP1 R140H mutation [9,10]. We also proposed that 5′ Tyr-tRF augments

Fig. 3. Developmental abnormalities and neuronal defects are mediated by p53. (A) Morphology of zebrafish embryo injected with either control-tRF or 5′ Tyr-tRF in the presence or absence of p53-MO (48 hpf). Scale bars: 500 μm. (B) Relative distribution of morphological phenotypes of zebrafish embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF together with the indicated quantity of p53-MO. The numbers of zebrafish embryos are indicated at the top of each bar. *$p < 0.05$ (C) Brightfield dorsal views of the heads in the embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF together with the indicated quantity of p53-MO. Scale bars: 200 μm. (D) Relative head size of zebrafish embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF together with the indicated quantity of p53-MO. n = 10 fish/group. Error bars indicate SEM. ***$p < 0.001$. (E) Morphological abnormality at 48 hpf in the spinal motor neurons of Tg[*isl1*:GFP] zebrafish injected with either control-tRF or 5′ Tyr-tRF together with the indicated quantity of p53-MO. Scale bars: 20 μm. (F) Quantification of spinal motor neuron numbers in Tg[*isl1*:GFP] zebrafish injected with either control-tRF or 5′ Tyr-tRF together with the indicated quantity of p53-MO. n = 5 fish/group. Error bars indicate SEM. **$p < 0.01$.

p53 activation in response to oxidative stress in that mouse model. Meanwhile, a different tRNA fragment, the OH-3′ Tyr-CCA tRF, was reported as possibly involved in neuronal pathogenesis [11]. That study indicated that OH-3′ Tyr-CCA tRF was a causative tRNA fragment for cell death in human CLP1 mutant cells in response to H_2O_2 [11]. It would be certainly reasonable because kinase-dead CLP1 cannot function to phosphorylate the 5′-terminus of the 3′-exon of the tRNA fragment, and its aberrant accumulation in neurons may contribute to the disease phenotype. In addition, Karaca et al. showed that Ile introns were the most abundantly accumulated tRNA fragment in fibroblasts of PCH patients [10]; however, it was not clear whether the accumulating Ile introns resulted in neuronal defects. In this study, we explored which of these tRNA fragments resulted in the most intense toxicity *in vitro* by using a human neuroblastoma cell line and also *in vivo* by using a zebrafish model. Our study indicated that 5′ Tyr-tRF had the most significant impact on neuronal defects during the development of zebrafish embryos.
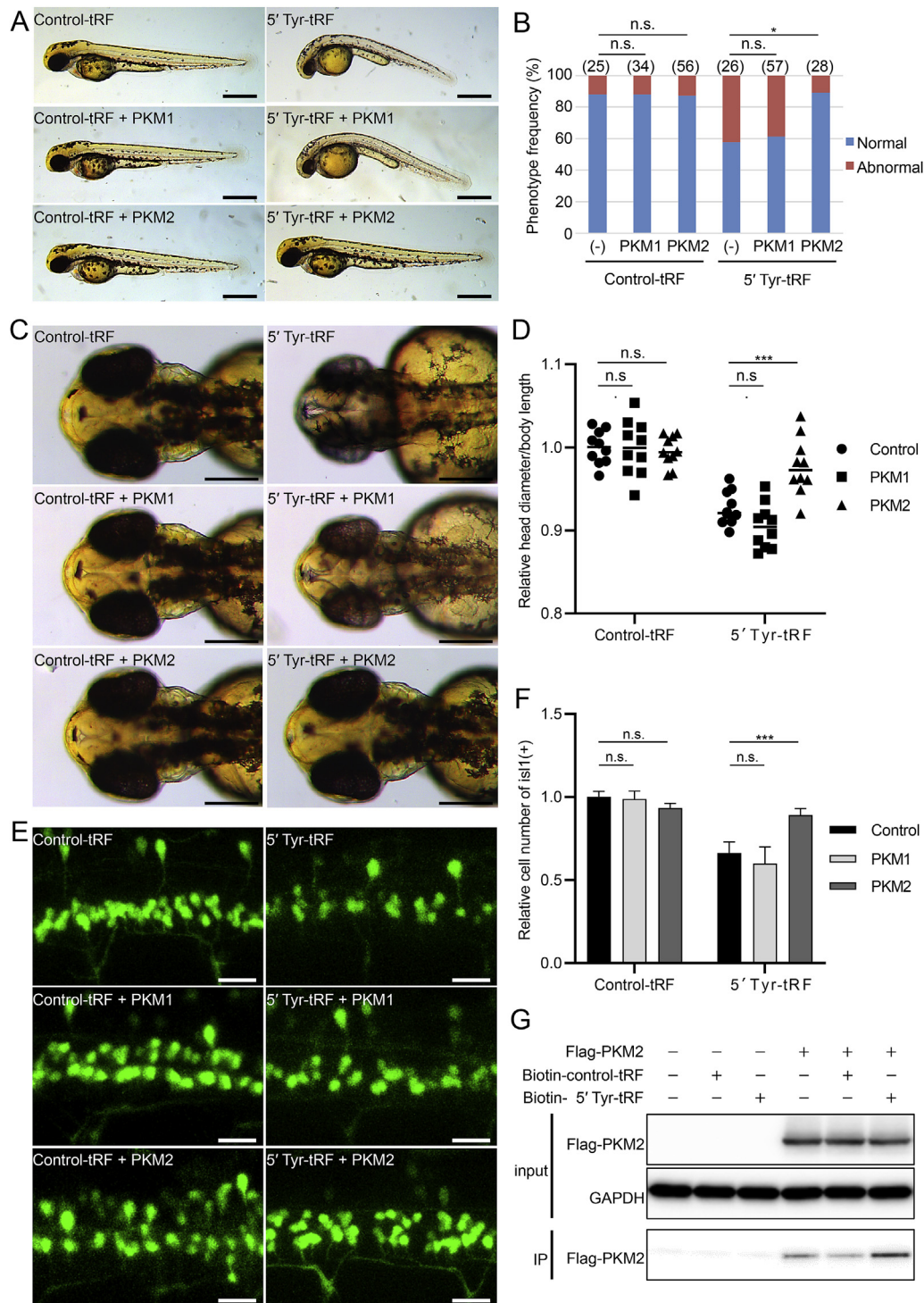
Fig. 4. PKM2 is a target of 5′ Tyr-tRF. (A) Morphology of zebrafish embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. Scale bars: 500 μm. **(B)** Relative distribution of morphological phenotypes of embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. The numbers of zebrafish embryos are indicated at the top of each bar. *$p < 0.05$. **(C)** Brightfield dorsal views of the heads in the embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. Scale bars: 200 μm. **(D)** Relative head size of zebrafish embryos (48 hpf) injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. n = 10 fish/group. Error bars indicate SEM. ***$p < 0.001$. **(E)** Morphological abnormality at 48 hpf in the spinal motor neurons of Tg[*isl1*:GFP] zebrafish injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. Scale bars: 20 μm. **(F)** Quantification of spinal motor neuron numbers in Tg[*isl1*:GFP] zebrafish injected with either control-tRF or 5′ Tyr-tRF in the presence of PKM1 mRNA or PKM2 mRNA. n = 5 fish/group. Error bars indicate SEM. ***$p < 0.001$. **(G)** Biotinylated control-tRF or 5′ Tyr-tRF was captured by streptavidin beads and then incubated with whole HEK293T cell extract expressing Flag-PKM2 (input control). Proteins bound to streptavidin beads were analyzed by Western blotting using anti-Flag antibodies.

We uncovered an unexpected mechanistic link between 5′ Tyr-tRF and PKM2. PKM2 is an enzyme that dephosphorylates phosphoenolpyruvate to pyruvate in glycolysis [16]. Whereas homotetrameric PKM2 is an active pyruvate kinase in the cytoplasm, homodimeric PKM2 is an active protein kinase in the nucleus [17,18]. Recently, it was reported that nuclear PKM2 interacts directly with p53 and interferes with its serine 15 phosphorylation [19]. Thus, 5′ Tyr-tRF may augment the phosphorylation of p53 at serine 15 through inhibiting PKM2's interference with p53 phosphorylation. Further experimentation will be required to clarify the mechanistic link between 5′ Tyr-tRF and PKM2 on the disease pathogenesis.

Importantly, PKM2 knockout mice do not display any overt developmental abnormality in the embryonic and postnatal stages [20]. Since *Pkm2* gene deletion results in aberrant overexpression of PKM1, PKM1 may compensate for the lack of PKM2 [20,21]. Recently, a novel molecular mechanism regarding a genetic compensation response was discovered [22,23]. It was found that the upregulation of compensatory genes is specifically triggered by mutations that generate short nucleotide sequences known as premature termination codons or nonsense codons. This upregulatory response is now known as nonsense-induced transcriptional compensation (NITC) [24]. This implies that more care needs to be taken when conducting and interpreting genetic studies using gene knockout models in the future. *Pkm2* gene deletion in mice may also exert the NITC response, thereby functionally compensating for the real phenotype of the *Pkm2* gene knockout by aberrantly high PKM1 expression. From this point of view, PKM2 knockout mice may show neuronal defects if the NITC response is inhibited.

Our experiments have uncovered a mechanistic link between pathological tRNA fragments and PKM2 in the pathogenesis of neuronal disorders. The accumulation of 5′ Tyr-tRF was observed not only in CLP1 mutant cells but also in wild-type cells in response to oxidative stress [9]. It may be possible that 5′ Tyr-tRF plays a role in the pathogenesis of other neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. These neurotoxic tRNA fragments may be target molecules for therapy and biomarkers for the diagnosis and prognosis of these disorders.

Declaration of competing interest

The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.02.157.

References

[1] H. Goodarzi, X. Liu, H.C. Nguyen, S. Zhang, L. Fish, S.F. Tavazoie, Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement, Cell 161 (2015) 790–802.

[2] S. Kirchner, Z. Ignatova, Emerging roles of tRNA in adaptive translation, signalling dynamics and disease, Nat. Rev. Genet. 16 (2015) 98–112.

[3] U. Sharma, C.C. Conine, J.M. Shea, A. Boskovic, A.G. Derr, X.Y. Bing, C. Belleannee, A. Kucukural, R.W. Serra, F. Sun, L. Song, B.R. Carone, E.P. Ricci, X.Z. Li, L. Fauquier, M.J. Moore, R. Sullivan, C.C. Mello, M. Garber, O.J. Rando, Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals, Science 351 (2016) 391–396.

[4] S. Weitzer, J. Martinez, The human RNA kinase hClp1 is active on 3′ transfer RNA exons and short interfering RNAs, Nature 447 (2007) 222–226.

[5] A. Ramirez, S. Shuman, B. Schwer, Human RNA 5′-kinase (hClp1) can function as a tRNA splicing enzyme in vivo, RNA 14 (2008) 1737–1745.

[6] R. Jain, S. Shuman, Characterization of a thermostable archaeal polynucleotide kinase homologous to human Clp1, RNA 15 (2009) 923–931.

[7] H. de Vries, U. Ruegsegger, W. Hubner, A. Friedlein, H. Langen, W. Keller, Human pre-mRNA cleavage factor II(m) contains homologs of yeast proteins and bridges two other cleavage factors, EMBO J. 19 (2000) 5895–5904.

[8] S.V. Paushkin, M. Patel, B.S. Furia, S.W. Peltz, C.R. Trotta, Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation, Cell 117 (2004) 311–321.

[9] T. Hanada, S. Weitzer, B. Mair, C. Bernreuther, B.J. Wainger, J. Ichida, R. Hanada, M. Orthofer, S.J. Cronin, V. Komnenovic, A. Minis, F. Sato, H. Mimata, A. Yoshimura, I. Tamir, J. Rainer, R. Kofler, A. Yaron, K.C. Eggan, C.J. Woolf, M. Glatzel, R. Herbst, J. Martinez, J.M. Penninger, CLP1 links tRNA metabolism to progressive motor-neuron loss, Nature 495 (2013) 474–480.

[10] E. Karaca, S. Weitzer, D. Pehlivan, H. Shiraishi, T. Gogakos, T. Hanada, S.N. Jhangiani, W. Wiszniewski, M. Withers, I.M. Campbell, S. Erdin, S. Isikay, L.M. Franco, C. Gonzaga-Jauregui, T. Gambin, V. Gelowani, J.V. Hunter, G. Yesil, E. Koparir, S. Yilmaz, M. Brown, D. Briskin, M. Hafner, P. Morozov, T.A. Farazi, C. Bernreuther, M. Glatzel, S. Trattnig, J. Friske, C. Kronnerwetter, M.N. Bainbridge, A. Gezdirici, M. Seven, D.M. Muzny, E. Boerwinkle, M. Ozen, T. Clausen, T. Tuschl, A. Yuksel, A. Hess, R.A. Gibbs, J. Martinez, J.M. Penninger, J.R. Lupski, Human CLP1 mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function, Cell 157 (2014) 636–650.

[11] A.E. Schaffer, V.R. Eggens, A.O. Caglayan, M.S. Reuter, E. Scott, N.G. Coufal, J.L. Silhavy, Y. Xue, H. Kayserili, K. Yasuno, R.O. Rosti, M. Abdellateef, C. Caglar, P.R. Kasher, J.L. Cazemier, M.A. Weterman, V. Cantagrel, N. Cai, C. Zweier, U. Altunoglu, N.B. Satkin, F. Aktar, B. Tuysuz, C. Yalcinkaya, H. Caksen, K. Bilguvar, X.D. Fu, C.R. Trotta, S. Gabriel, A. Reis, M. Gunel, F. Baas, J.G. Gleeson, CLP1 founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration, Cell 157 (2014) 651–663.

[12] O. Boussif, F. Lezoualc'h, M.A. Zanta, M.D. Mergny, D. Scherman, B. Demeneix, J.P. Behr, A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7297–7301.

[13] S. Higashijima, Y. Hotta, H. Okamoto, Visualization of cranial motor neurons in live transgenic zebrafish expressing green fluorescent protein under the control of the islet-1 promoter/enhancer, J. Neurosci. 20 (2000) 206–218.

[14] B. Lomenick, R. Hao, N. Jonai, R.M. Chin, M. Aghajan, S. Warburton, J. Wang, R.P. Wu, F. Gomez, J.A. Loo, J.A. Wohlschlegel, T.M. Vondriska, J. Pelletier, H.R. Herschman, J. Clardy, C.F. Clarke, J. Huang, Target identification using drug affinity responsive target stability (DARTS), Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 21984–21989.

[15] S.E. Ong, B. Blagoev, I. Kratchmarova, D.B. Kristensen, H. Steen, A. Pandey, M. Mann, Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics, Mol. Cell. Proteomics 1 (2002) 376–386.

[16] H.R. Christofk, M.G. Vander Heiden, N. Wu, J.M. Asara, L.C. Cantley, Pyruvate kinase M2 is a phosphotyrosine-binding protein, Nature 452 (2008) 181–186.

[17] W. Yang, Structural basis of PKM2 regulation, Protein Cell 6 (2015) 238–240.

[18] S. Amin, P. Yang, Z. Li, Pyruvate kinase M2: a multifarious enzyme in non-canonical localization to promote cancer progression, Biochim. Biophys. Acta Rev. Canc 1871 (2019) 331–341.

[19] L. Xia, X.R. Wang, X.L. Wang, S.H. Liu, X.W. Ding, G.Q. Chen, Y. Lu, A novel role for pyruvate kinase M2 as a corepressor for P53 during the DNA damage response in human tumor cells, J. Biol. Chem. 291 (2016) 26138–26150.

[20] T.L. Dayton, V. Gocheva, K.M. Miller, W.J. Israelsen, A. Bhutkar, C.B. Clish, S.M. Davidson, A. Luengo, R.T. Bronson, T. Jacks, M.G. Vander Heiden, Germline loss of PKM2 promotes metabolic distress and hepatocellular carcinoma, Genes Dev. 30 (2016) 1020–1033.

[21] W.J. Israelsen, T.L. Dayton, S.M. Davidson, B.P. Fiske, A.M. Hosios, G. Bellinger, J. Li, Y. Yu, M. Sasaki, J.W. Horner, L.N. Burga, J. Xie, M.J. Jurczak, R.A. DePinho, C.B. Clish, T. Jacks, R.G. Kibbey, G.M. Wulf, D. Di Vizio, G.B. Mills, L.C. Cantley, M.G. Vander Heiden, PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells, Cell 155 (2013) 397–409.

[22] M.A. El-Brolosy, Z. Kontarakis, A. Rossi, C. Kuenne, S. Gunther, N. Fukuda, K. Kikhi, G.L.M. Boezio, C.M. Takacs, S.L. Lai, R. Fukuda, C. Gerri, A.J. Giraldez, D.Y.R. Stainier, Genetic compensation triggered by mutant mRNA degradation, Nature 568 (2019) 193–197.

[23] Z. Ma, P. Zhu, H. Shi, L. Guo, Q. Zhang, Y. Chen, S. Chen, Z. Zhang, J. Peng, J. Chen, PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components, Nature 568 (2019) 259–263.

[24] M.F. Wilkinson, Genetic paradox explained by nonsense, Nature 568 (2019) 179–180.