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p53 Mutation suppresses adult neurogenesis in medaka fish (*Oryzias latipes*)

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ABSTRACT

Tumor suppressor *p53* negatively regulates self-renewal of neural stem cells in the adult murine brain. Here, we report that the *p53* null mutation in medaka fish (*Oryzias latipes*) suppressed neurogenesis in the telencephalon, independent of cell death. By using 5-bromo-29-deoxyuridine (BrdU) immunohistochemistry, we identified 18 proliferation zones in the brains of young medaka fish; *in situ* hybridization showed that *p53* was expressed selectively in at least 12 proliferation zones. We also compared the number of BrdU-positive cells present in the whole telencephalon of wild-type (WT) and *p53* mutant fish. Immediately after BrdU exposure, the number of BrdU-positive cells did not differ significantly between them. One week after BrdU-exposure, the BrdU-positive cells migrated from the proliferation zone, which was accompanied by an increased number in the WT brain. In contrast, no significant increase was observed in the *p53* mutant brain. Terminal deoxynucleotidyl transferase (dUTP) nick end-labeling revealed that there was no significant difference in the number of apoptotic cells in the telencephalon of *p53* mutant and WT medaka, suggesting that the decreased number of BrdU-positive cells in the mutant may be due to the suppression of proliferation rather than the enhancement of neural cell death. These results suggest that *p53* positively regulates neurogenesis via cell proliferation.

1. Introduction

In the adult brain of teleosts, most proliferating cells are observed in well-defined zones of the brain (called proliferation zones) [1]. The whole brain of teleosts, such as medaka (*Oryzias latipes*) [2], zebrafish (*Danio rerio*) [3], gymnotiform fish (*Apteronotus leptorhynchus*) [4], and three-spined stickleback (*Gasterosteus aculeatus*) [5], contains a large number of proliferation zones. Previously, we identified 17 proliferation zones (Zones A–Q) in the adult medaka brain using sexually mature fish (age, more than 3 months) and demonstrated that there is persistent cell proliferation in these brain regions in the adult brain, irrespective of sex, body color, or growth environment [2]. Further, the distribution of proliferation zones is largely conserved among some fish species [2], suggesting that this distribution in the adult teleost brain is important for the maintenance and development of the fundamental structure of fish brains [2].

To clarify the molecular basis underlying adult neurogenesis in teleost fish, we focused on medaka *p53* mutants [6]. p53 is a sequence-specific DNA-binding transcription factor that induces apoptosis or cell cycle arrest in response to genotoxic stress, thus preventing DNA mutations from transmitting to progeny cells [7]. In murine brains, the *p53* null mutation enhanced cell proliferation in the adult subventricular zone (SVZ) and, in association with their rapid differentiation, resulted in an increased number of newborn neurons and oligodendrocytes [8–11]. Here, we show the distribution of proliferating zones largely overlapped that of *p53*-expressing cells in the medaka brain. Furthermore, the medaka *p53* null mutant phenotype suggested that *p53* positively regulates neurogenesis.

2. Materials and methods

2.1. Fish

Medaka fish (*O. latipes*), Cab strain and *p53* mutants [6], were maintained in groups in plastic aquariums (12 × 13 × 19 cm). Sexually immature medaka fish (approximately 1 month after hatching; body length, 15 mm) without secondary sexual characteristics were used for immunohistochemistry and *in situ* hybridization studies.

2.2. Detection of mitotic cells in the young medaka brain

The detection of mitotic cells was performed as described previously [2]. Dividing cells were labeled with 5-bromo-29-deoxyuridine (BrdU), by exposure to water containing 1 g/L BrdU (Sigma Aldrich, Tokyo) for 4 h. BrdU-positive cells were detected by anti-BrdU immunohistochemistry. Paraffin sections (10-μm thick) were cut with a microtome (LR-85, Yamato Kohki, Tokyo). Immunostain-

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ing was performed following standard procedures. Cell nuclei were detected with DAPI staining (Invitrogen, Tokyo). BrdU-positive cells were counted as described previously [2].

2.3. In situ hybridization

In situ hybridization of tissue sections was performed as described previously [12,13]. The *p53* cDNA fragment was amplified with forward primer 5′-**TGTTACATTTTATAGCTGTGGAGCA**-3′ and reverse primer 5′-**TTGGGCTGAAAACAGCACAACCATAGTT**-3′ using cDNA clone number orbr44c15 (Medaka National BioResource Project [14]) as a template. The digoxigenin (DIG)-labeled riboprobes were synthesized by T7 or SP6 polymerase with a DIG labeling mix (Roche, Tokyo) from a template containing the *p53* cDNA fragment. Micrographs were obtained with a BX50 optical microscope (Olympus, Tokyo). The micrographs were processed with Photoshop software (Adobe, San Jose, CA).

2.4. TUNEL (TdT-mediated dUTP-biotin nick-end labeling) staining

Medaka brains were fixed in 4% paraformaldehyde (prepared in phosphate buffer saline) overnight and embedded in paraffin. Each brain was sliced into 10-μm sections. Apoptotic cells were detected using a DeadEnd™ Fluorometric TUNEL System (Promega, Tokyo), according to the manufacturer's protocol.

3. Results

3.1. Distribution of proliferation zones and p53-expressing cells in brains of young medaka fish

To elucidate the molecular basis underlying cell proliferation in the medaka brain, we focused on medaka *p53* [6]. *p53* is expressed in proliferating and newly formed neurons of the adult murine brain [15]. To examine whether medaka *p53*-expressing cells were

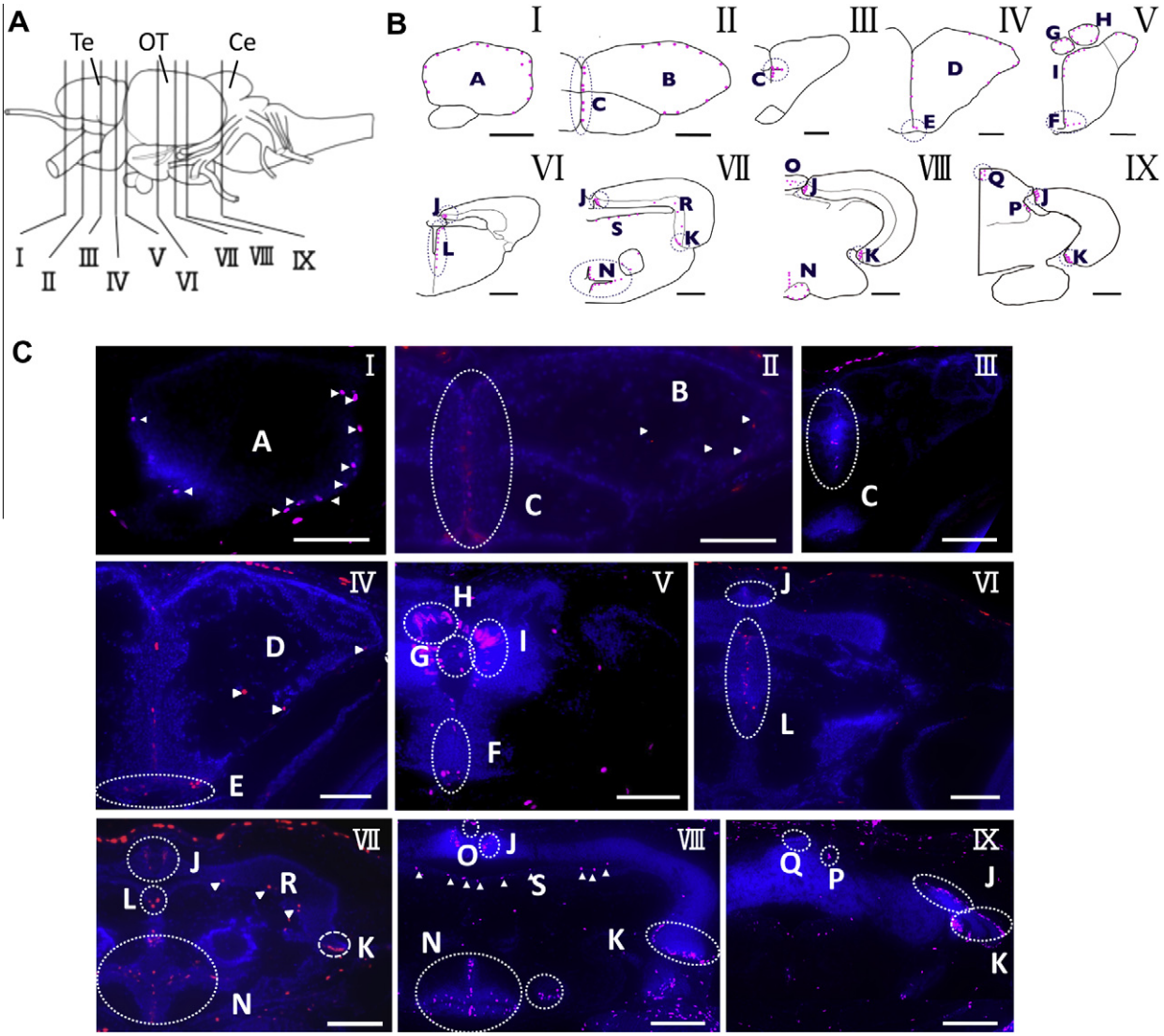

Fig. 1. Mapping proliferation zones in the brain of young medaka. (a) Schematic drawing of the lateral view of the medaka brain. The positions of sections I–IX are indicated by the lines. Te: telencephalon, OT: optic tectum, Cb: cerebellum. (b) Schematic representation of the distribution of the 18 proliferation zones. Red dots indicate proliferating cells. Zone A: marginal zones of the anterior part of the telencephalon, Zone B: marginal zones of the dorsolateral part of the telencephalon, Zone C: medial zones of the telencephalon, Zone D: dorsolateral part of the posterior part of the telencephalon, Zones E and F: preoptic area, Zone G: pineal body, Zone H: habenular nucleus, Zone I: ventromedial nucleus, Zones J and K: optic tectum, Zone L: anterior part of marginal zones of third ventricular zone, Zone N: hypothalamus, Zones O–Q: cerebellum, Zone R: periventricular grey zone (layer 3), and Zone S: Ependyme. Roman numerals in the panels correspond to section numbers shown in (a). Proliferation zones were determined according to the medaka fish brain atlas (Supplemental Fig. 1). (c) Distribution of BrdU-positive cells in the different proliferation zones. A magnified photo for zones P and Q (cerebellum) in panel XI is shown in Supplemental Fig. 2. Scale bars indicate 100 μm.

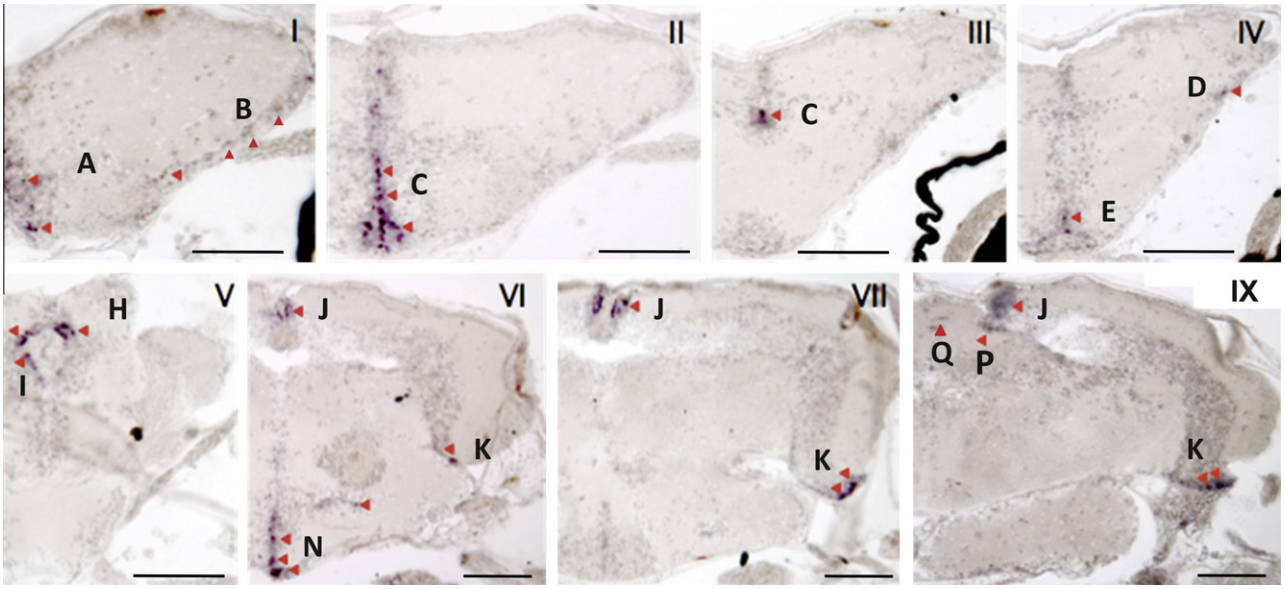

Fig. 2. Distribution of medaka *p53*-expressing cells in the brain of young medaka. Zones A–D: telencephalon, zone E: preoptic area, zone H: habenular nucleus, zone I: ventromedial nucleus, zones J and K: optic tectum, zone N: hypothalamus, zones P and Q: cerebellum. Scale bars indicate 100 μm.

present in the proliferation zones, we mapped the proliferation zones and performed *in situ* hybridization for detecting *p53* transcripts. In the present study, we used young medaka before they developed secondary sexual characteristics, because the smaller brain of the young medaka makes it easier to quantify newborn cells within a specific brain structure such as the telencephalon [2]. As a detailed description of the cell proliferation zones in the whole brain of sexually immature medaka is not available, we mapped the proliferation zones by identification of mitotic cells as determined by BrdU uptake. Based upon the distribution of DAPI staining and the medaka brain atlas [16], we identified the locations of the paraffin sections in the whole brain. We then mapped the BrdU-positive cells and identified 18 proliferation zones, A–L and N–S (Fig. 1, Supplemental Fig. 1). Sixteen zones (A–L and N–Q), were identical to those previously identified in sexually mature medaka [2]. In the present study, we could not confirm that there is a proliferation zone in the pituitary gland (zone M) previously identified in mature fish, as the pituitary gland is likely to be separate from the whole brain in the young fish. The 16 zones (A–L and N–Q) were mapped to the telencephalon (zones A–D), preoptic area (zones E and F), pineal body (zone G), habenular nucleus (zone H), ventromedial nucleus (zone I), optic tectum (zones J and K), marginal zone of the third ventricular zone (zone L), hypothalamus (zone N), and cerebellum (zones O–Q) (Supplemental Fig. 2). The two additional zones (R and S) were identified in the periventricular grey zone (layer 3) and ependyme, respectively, which were not previously found in the mature fish [2], suggesting that these two proliferation zones might disappear or integrate into the surrounding proliferation zones during the sexual maturation (Fig. 1). Next, to examine whether *p53* is expressed in proliferating zones in the medaka brains, we performed *in situ* hybridization. We demonstrated that medaka *p53* expressed selectively in at least 12 zones (zones A–E, H–K, N, P, and Q) (Fig. 2).

3.2. The p53 mutation had no effect on either the distribution of the proliferating zones or the number of proliferating cells

To examine whether *p53* is involved in cell proliferation in the medaka brain, we mapped proliferation zones using two *p53* mutant strains [6]. The $p53^{E241X}$ allele has a G to T substitution that changes Glu241 to a stop codon, and the $p53^{Y186X}$ allele has a T to A substitution that changes Tyr186 to a stop codon [6]. The two mutated *p53* genes encode truncated proteins that terminate within a DNA-binding domain. These proteins lack the nuclear localization signal and tetramerization domain required for full activity. Thus, these nonsense mutations probably lead to a null phenotype [6]. We found the 18 proliferation zones in the two mutant strains, $p53^{Y186X/Y186X}$ (Supplemental Fig. 3) and $p53^{E241X/Y186X}$ (data not shown), indicating that loss of *p53* has no effect on the distribution of proliferation zones. To examine whether the number of proliferating cells was affected by the *p53* null mutation, we counted the number of BrdU-positive cells in the entire telencephalon (zones A–D). There was no significant difference in BrdU-positive cells between the wild-type (WT) (average ± SE, 2316 ± 598; $n = 4$), $p53^{Y186X/Y186X}$ mutant (1849 ± 248; $n = 4$), or $p53^{E241X/E241X}$ mutant (1728 ± 366; $n = 3$) (Fig. 3D and F).

3.3. The p53 mutation led to decreased numbers of differentiated progenitors 1 week after BrdU exposure

To determine whether *p53* mutation affects survival and/or proliferation of progeny cells, we compared the distribution pattern of differentiated newborn cells in the brains of WT (Cab strain) and $p53^{Y186X/Y186X}$ mutant medaka. One week after BrdU exposure, BrdU-positive cells migrated from the proliferation zones (Fig. 3E) in the telencephalon of both WT and mutant strains, suggesting that there is no substantial difference in the migration pattern between the two strains. However, in some brain regions, such as the telencephalon (zone C) (Fig. 3E) and hypothalamus (zone N) (Supplemental Fig. 4B), the number of BrdU-positive neurons seemed to reduce in the mutant strain compared to the WT. Next, we quantified the number of BrdU-positive cells in WT (Cab strain), $p53^{E241X/E241X}$, and $p53^{Y186X/Y186X}$ in the telencephalon (zones A–D). In the WT, the number of BrdU-positive cells 1 week after BrdU exposure (6300 ± 535, average ± S.E, $n = 4$) increased over twofold (Fig. 3F), suggesting proliferation of the migrated progenitors. In contrast, there was no significant increase in BrdU-positive cells 1 week after BrdU exposure in $p53^{Y186X/Y186X}$ or $p53^{E241X/E241X}$ mutants (3596 ± 572 and 2378 ± 560, respectively). These results raised two possibilities: (1) the *p53* mutation enhanced cell death

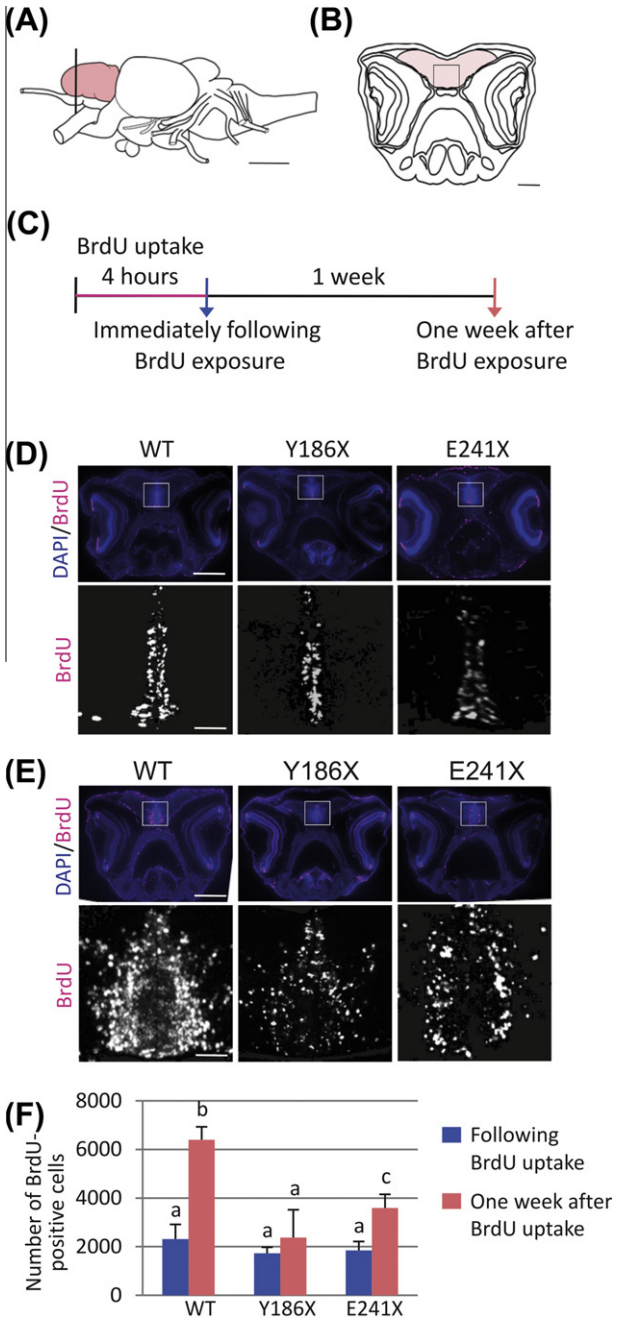

Fig. 3. Comparison of the distribution and number of newborn cells in WT and *p53* mutants. (A) Schematic drawing of the medaka brain and position of the telencephalon in the brain. (B) Schematic drawing of the transverse section of the medaka head. The section of images in (D) and (E), are indicated by a line and a square in (A) and (B), respectively. The pink area represents the medaka telencephalon. (C) The time schedule of this experiment. (D and E) Anti-BrdU immunohistochemistry of paraffin sections from wild-type (WT) medaka (Cab strain) and *p53* mutants (Magenta). Nuclei were stained with DAPI (Blue). The upper row indicates the transverse sections (Scale bars, 200 μm) and the lower row indicates the magnified view of the proliferation zone (Zone E; Scale bars, 40 μm), represented by the white rectangles in the upper row. (D) Immunohistochemistry was performed immediately after BrdU exposure. (E) Immunohistochemistry was performed 1 week after BrdU exposure. (F) Number of BrdU-positive cells in the telencephalon of WT and *p53* mutants medaka brains. Significant differences were observed between a and b, and b and c ($p < 0.01$ and $p < 0.05$, respectively; ANOVA with Bonferroni–Dunn post hoc test; $n = 3$–4 per group).

of differentiated progenitors (neuroblasts) or (2) the *p53* mutation repressed neuroblast proliferation and/or repressed differentiation of stem cells to an active, proliferating, neuroblast subpopulation.

To examine whether cell death was enhanced in the *p53* mutant strains, we compared TUNEL-positive cells in the telencephalon of WT and *p53* mutants. The number of TUNEL-positive cells was far less than the number of BrdU-positive cells in both WT and *p53* mutants, with no difference between the WT and *p53* mutants (Fig. 4A and B). We confirmed that TUNEL-positive signals were localized in nuclei stained with DAPI (Fig. 4A), and numerous TUNEL-positive cells were detected when using medaka pancreas sections, which are known to be susceptible to apoptosis [17] (Supplemental Fig. 4).

4. Discussion

In the present study, we demonstrated that the *p53* mutation did not affect the number of BrdU-positive cells immediately after BrdU exposure. In the SGZ of murine brains, adult neurogenesis originates from radial glia-like stem cells (Type 1 cells) through a proliferating stage (Type 2 cells) generating neuroblasts (Type 3 cells) and dentate granule interneurons [18]. Our finding strongly suggests that loss of medaka *p53* did not affect highly proliferating progenitors, which correspond to Type 1 and 2 cells. This seems inconsistent with a previous study indicating that genetic ablation of *p53* enhanced proliferation of stem cells in the adult murine brain [11]. There was no defect in stem cells in the *p53* mutant medaka brain. Most mice, zebrafish, and medaka with *p53* function defects develop without any obvious morphological defects [6,18,19–23], as p53 family proteins are redundant and can compensate for each other in various organs. Our results imply that other *p53* family members may compensate for a *p53* deficiency in medaka brain stem cells.

Furthermore, we showed that the number of newborn cells that migrate from the proliferation zones increased during the 1-week period after BrdU exposure in a *p53*-dependent manner. These data suggested that *p53* positively regulated the number of migrating progenitors, which may correspond to Type 3 cells (neuroblasts). Dividing neuroblasts are also found in the cerebellum (zone Q) of the zebrafish adult brain [22]. The shift in the distribution of BrdU-positive cells from the proliferation zone into the granule cell layers is accompanied by an increase in the number of labeled cells [23]. In the murine brain, there is some evidence for the proliferation of migrating neuroblasts [24], which originate from stem cells located in the SVZ of the lateral ventricles, moving along the rostral migratory stream. To determine which subpopulation of progenitor cells is regulated by *p53*, it will be crucial to characterize the subtype and maturation sequence of progenitor cells in the medaka brain.

Positive regulation of *p53* in adult medaka brain neurogenesis appears to be the opposite of what is observed in murine *p53* mutants [11,20], where *p53* negatively regulates neurogenesis. One possible explanation is that the p53 N-truncated isoform, which has the opposite effect, may function in the medaka brain. In mice and zebrafish, the *p53* family genes (including *p63* and *p73*) have 2 isoforms—full length and N-truncated—with an alternative transcriptional start site [10,20,25]. Because the latter isoform lacks a transactivation domain, it is thought to function in a dominant-negative fashion to inhibit the transcriptional activity of full-length p53 family members. In the murine brain, p53 family proteins interact with each other in a cell-type/stage-specific manner and coordinated expression of the two isoforms is required for stem cell maintenance in adult neurogenesis [10,20,25,26]. As positive regulation of *p53* in neurogenesis has not been indicated in the murine brain, a *p53* study using medaka may shed a light on a novel mechanism underlying adult neurogenesis.

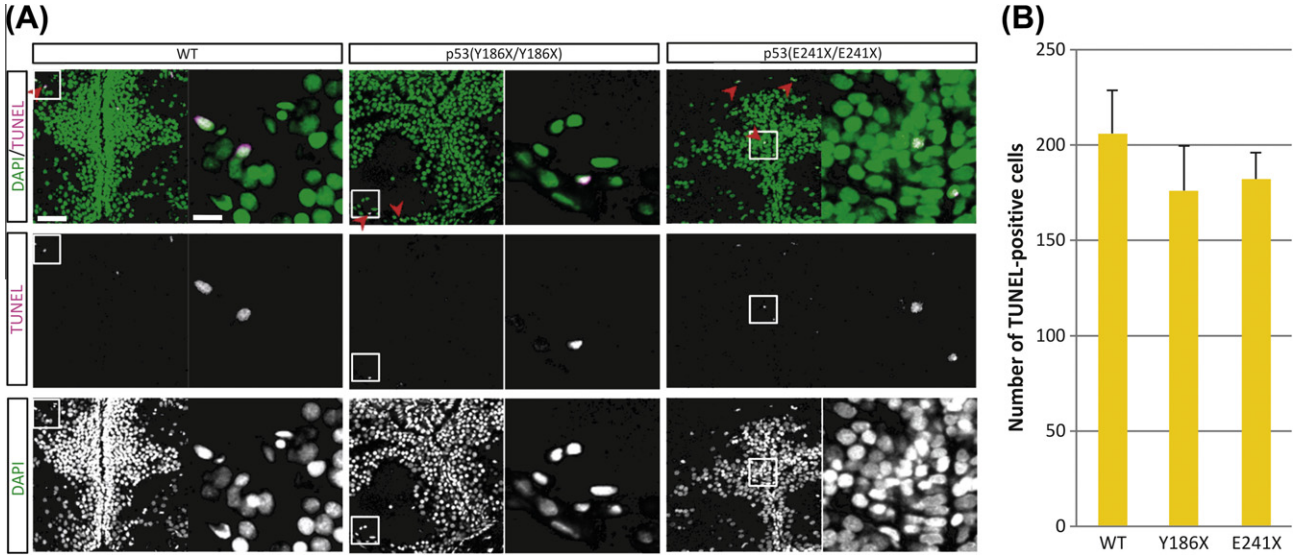

Fig. 4. Cell death in the telencephalon of young medaka. (A) Confocal images show double-labeling of TUNEL (Magenta) and DAPI (Green) in Zone D. Red arrow head indicate TUNEL-positive cells. For each strain, images in the right column are the magnified images of the region outlined by the white rectangle in the left column images. Scale bars indicate 80 μm (Left) and 20 μm (Right) (B) quantification of TUNEL-positive cells. No significant difference was detected (ANOVA with a Bonferroni–Dunn post hoc test; $n = 3–4$ per group).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.125.

References

[1] P. Chapouton, R. Jagasia, L. Bally-Cuif, Adult neurogenesis in non-mammalian vertebrates, Bioessays 29 (2007) 745–757.
[2] Y. Kuroyanagi, T. Okuyama, Y. Suehiro, H. Imada, A. Shimada, K. Naruse, H. Takeda, T. Kubo, H. Takeuchi, Proliferation zones in adult medaka (*Oryzias latipes*) brain, Brain Res. 1323 (2010) 33–40.
[3] G.K. Zupanc, Adult neurogenesis and neuronal regeneration in the teleost fish brain, J. Physiol. Paris 102 (2008) 357–373.
[4] G.K. Zupanc, I. Horschke, Proliferation zones in the brain of adult gymnotiform fish: a quantitative mapping study, J. Comp. Neurol. 353 (1995) 213–233.
[5] P. Ekstrom, C.M. Johnsson, L.M. Ohlin, Ventricular proliferation zones in the brain of an adult teleost fish and their relation to neuromeres and migration (secondary matrix) zones, J. Comp. Neurol. 436 (2001) 92–110.
[6] Y. Taniguchi, S. Takeda, M. Furutani-Seiki, Y. Kamei, T. Todo, T. Sasado, T. Deguchi, H. Kondoh, J. Mudde, M. Yamazoe, M. Hidaka, H. Mitani, A. Toyoda, Y. Sakaki, R.H. Plasterk, E. Cuppen, Generation of medaka gene knockout models by target-selected mutagenesis, Genome Biol 7 (2006) R116.
[7] C.J. Sherr, J.M. Roberts, Inhibitors of mammalian G1 cyclin-dependent kinases, Genes Dev. 9 (1995) 1149–1163.
[8] S. Gil-Perotin, M. Marin-Husstege, J. Li, M. Soriano-Navarro, F. Zindy, M.F. Roussel, J.M. Garcia-Verdugo, P. Casaccia-Bonnefil, Loss of p53 induces changes in the behavior of subventricular zone cells: implication for the genesis of glial tumors, J. Neurosci. 26 (2006) 1107–1116.
[9] J. Li, C.A. Ghiani, J.Y. Kim, A. Liu, J. Sandoval, J. DeVellis, P. Casaccia-Bonnefil, Inhibition of p53 transcriptional activity: a potential target for future development of therapeutic strategies for primary demyelination, J. Neurosci. 28 (2008) 6118–6127.
[10] S. Gil-Perotin, J.D. Haines, J. Kaur, M. Marin-Husstege, M.J. Spinetta, K.H. Kim, M. Duran-Moreno, T. Schallert, F. Zindy, M.F. Roussel, J.M. Garcia-Verdugo, P. Casaccia, Roles of p53 and p27(Kip1) in the regulation of neurogenesis in the murine adult subventricular zone, Eur. J. Neurosci. 34 (2011) 1040–1052.
[11] K. Meletis, V. Wirta, S.M. Hede, M. Nister, J. Lundeberg, J. Frisen, P53 suppresses the self-renewal of adult neural stem cells, Development 133 (2006) 363–369.
[12] T. Okuyama, Y. Suehiro, H. Imada, A. Shimada, K. Naruse, H. Takeda, T. Kubo, H. Takeuchi, Induction of c-fos transcription in the medaka brain (*Oryzias latipes*) in response to mating stimuli, Biochem. Biophys. Res. Commun. 404 (2011) 453–457.
[13] Y. Suehiro, A. Yasuda, T. Okuyama, H. Imada, Y. Kuroyanagi, T. Kubo, H. Takeuchi, Mass spectrometric map of neuropeptide expression and analysis of the gamma-prepro-tachykinin gene expression in the medaka (*Oryzias latipes*) brain, Gen. Comp. Endocrinol. 161 (2009) 138–145.
[14] T. Sasado, M. Tanaka, K. Kobayashi, T. Sato, M. Sakaizumi, K. Naruse, The national bioresource project Medaka (NBRP Medaka): an integrated bioresource for biological and biomedical sciences, Exp. Anim. 59 (2010) 13–23.
[15] M.V. Campagne, R. Gill, Tumor-suppressor p53 is expressed in proliferating and newly formed neurons of the embryonic and postnatal rat brain: Comparison with expression of the cell cycle regulators p21(Waf1/Cip1), p27(Kip1), p57(Kip2), p16(Ink4a), cyclin G1, and the proto-oncogene Bax, J. Comp. Neurol. 397 (1998) 181–198.
[16] R. Anken, F. Bourrat, Brain atlas of the medakafish, *Oryzias latipes*, INRA, Paris, 1998.
[17] A.I. Sarela, C.S. Verbeke, J. Ramsdale, C.L. Davies, A.F. Markham, P.J. Guillou, Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma, Br. J. Cancer 86 (2002) 886–892.
[18] B. Seri, J.M. Garcia-Verdugo, L. Collado-Morente, B.S. McEwen, A. Alvarez-Buylla, Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus, J. Comp. Neurol. 478 (2004) 359–378.
[19] N.Y. Storer, L.I. Zon, Zebrafish models of p53 functions, Cold Spring Harbour Perspect Biol. 2 (2010) a001123.
[20] W.B. Jacobs, D.R. Kaplan, F.D. Miller, The p53 family in nervous system development and disease, J. Neurochem. 97 (2006) 1571–1584.
[21] N. Danilova, K.M. Sakamoto, S. Lin, P53 family in development, Mech. Dev. 125 (2008) 919–931.
[22] G.K. Zupanc, R.F. Sirbulescu, Adult neurogenesis and neuronal regeneration in the central nervous system of teleost fish, Eur. J. Neurosci. 34 (2011) 917–929.
[23] G.K. Zupanc, K. Hinsch, F.H. Gage, Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain, J. Comp. Neurol. 488 (2005) 290–319.
[24] A. Saghatelyan, A. de Chevigny, M. Schachner, P.M. Lledo, Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain, Nat. Neurosci. 7 (2004) 347–356.
[25] F. Talos, A. Abraham, A.V. Vaseva, L. Holembowski, S.E. Tsirka, A. Scheel, D. Bode, M. Dobbelstein, W. Bruck, U.M. Moll, P73 is an essential regulator of neural stem cell maintenance in embryonal and adult CNS neurogenesis, Cell Death Differ. 17 (2010) 1816–1829.
[26] R.B. Fletcher, M.S. Prasol, J. Estrada, A. Baudhuin, K. Vranizan, Y.G. Choi, J. Ngai, P63 regulates olfactory stem cell self-renewal and differentiation, Neuron 72 (2011) 748–759.