Biochemical and Biophysical Research Communications 450 (2014) 961–965

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Increased centrosome amplification in aged stem cells of the *Drosophila* midgut

Joung-Sun Park [a,1], Jung-Hoon Pyo [a,1], Hyun-Jin Na [a], Ho-Jun Jeon [a], Young-Shin Kim [a], Robert Arking [b,*], Mi-Ae Yoo [a,*]

[a] Department of Molecular Biology, Pusan National University, Busan 609-735, Republic of Korea
[b] Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA

ARTICLE INFO

Article history:
Received 2 June 2014
Available online 24 June 2014

Keywords:
Centrosome
Adult stem cell
Aging
PVR
EGFR
Drosophila midgut

ABSTRACT

Age-related changes in long-lived tissue-resident stem cells may be tightly linked to aging and age-related diseases such as cancer. Centrosomes play key roles in cell proliferation, differentiation and migration. Supernumerary centrosomes are known to be an early event in tumorigenesis and senescence. However, the age-related changes of centrosome duplication in tissue-resident stem cells *in vivo* remain unknown. Here, using anti-γ-tubulin and anti-PH3, we analyzed mitotic intestinal stem cells with supernumerary centrosomes in the adult *Drosophila* midgut, which may be a versatile model system for stem cell biology. The results showed increased centrosome amplification in intestinal stem cells of aged and oxidatively stressed *Drosophila* midguts. Increased centrosome amplification was detected by overexpression of PVR, EGFR, and AKT in intestinal stem cells/enteroblasts, known to mimic age-related changes including hyperproliferation of intestinal stem cells and hyperplasia in the midgut. Our data show the first direct evidence for the age-related increase of centrosome amplification in intestinal stem cells and suggest that the *Drosophila* midgut is an excellent model for studying molecular mechanisms underlying centrosome amplification in aging adult stem cells *in vivo*.

1. Introduction

Adult stem cells play a key role in tissue homeostasis and regeneration based on their ability to sustain both self-renewal and the production of differentiated cells. Therefore, age-related changes in tissue-resident stem cells may be tightly linked to aging and age-related diseases arising from these tissues.

Centrosomes are the microtubule organizing centers of most animal cells and play many important roles in key cellular processes, including cell division, cell migration and cell polarity [1]. Numerical centrosome aberrations, including amplification, are well known to be an early event in tumorigenesis and senescence [2–4]. Recently, centrosome amplification was shown to drive tumor metastasis [5]. It was established in *Drosophila* that centrosome amplification can switch asymmetric division of stem cells to symmetric division, which lead to expansion of stem cell population and hyperplasia [6]. Recent works also show that the enteroendocrine cells (EEs) of the intestine play an important role in regulating the longevity of the organism by sending longevity signals to the rest of the organism [7–9]. The homeostasis of centrosome in tissue-resident stem cells, especially in high turnover tissues such as the intestine, is a critical factor for tissue homeostasis, regeneration, and longevity.

Centrosome amplification has been known to be inducible by various genotoxic stresses, especially DNA double-strand breaks [10]. DNA damage-induced cell cycle arrest, especially in the G2 phase, is involved in centrosome amplification [11,12]. DNA damage in long-lived tissue-resident stem cells, especially in high turnover tissues such as the intestine, increases with age [13]. These facts support the hypothesis that the centrosome amplification in long-lived tissue-resident stem cells, especially in high turnover tissues such as the intestine, may increase with age, leading to tissue aging, decreased longevity, and age-related diseases such as cancer. However, evidence for age-related centrosome amplification in tissue-resident stem cells *in vivo* remains unreported.

The adult *Drosophila* midgut is a versatile model system for stem cell biology [14–17]. The *Drosophila* midgut undergoes rapid cell turnover by intestinal stem cells (ISCs), similar to the intestine

* Corresponding authors. Addresses: Department of Biological Sciences, 5047 Gullen Mall, Wayne State University, Detroit, MI 48202, USA. Fax: +1 313 577 6891 (R. Arking), Department of Molecular Biology, Pusan National University, Busan 609-735, Korea, Tel.: +82 51 510 2278; fax: +82 51 513 9258 (M.-A. Yoo).
E-mail addresses: aa2210@wayne.edu (R. Arking), mayoo@pusan.ac.kr (M.-A. Yoo).
[1] These authors contributed equally to this article.

http://dx.doi.org/10.1016/j.bbrc.2014.06.085
0006-291X/

of vertebrate [14,15,18]. ISCs are the only mitotic cells in the intestine. They produce diploid enteroblasts (EBs), which differentiate to either polyploid enterocytes or diploid EEs [14–15]. These cell types are detectable by specific markers [14,15,19]. Previous studies have described age-related changes in the midgut, such as hyperproliferation of ISCs, abnormal Delta-positive (ISC marker) and esg-GFP (ISC/EB marker) polyploid cells, DNA damage accumulation, and hyperplasia [13,20–23]. Several signaling pathways including epidermal growth factor receptor (EGFR), platelet-derived growth factor/vascular endothelial growth factor (PVF)/PVF receptor (PVR), and AKT (known as Protein Kinase B) pathways, have been demonstrated to be involved in age-related changes including hyperproliferation of ISCs and hyperplasia in the midgut [17,21,23,24]. The damage patterns induced by both intrinsic (a Catalase mutant) and extrinsic oxidatively stressed guts have been reported to mimic the age-related phenotypes [21,25], suggesting that similar mechanisms may be involved in both processes leading to loss of normal stem cell function.

In this study, we analyzed mitotic intestinal stem cells with supernumerary centrosomes in the adult *Drosophila* midgut using anti-γ-tubulin and anti-PH3.

2. Material and methods

2.1. Fly stock

Fly stocks were maintained at 25 °C on standard food under a ~12 h/12 h light/dark cycle. Food consisted of 79.2% water, 1% agar, 7% cornmeal, 2% yeast, 10% sucrose, 0.3% bokinin and 0.5% propionic acid. To avoid larval overpopulation in all vials, 50–60 adult flies per vial were transferred to new food vials every 2–3 days for a period of 50–60 days or longer. *esg-GAL4,UAS-GFP/CyO* flies were provided by the Drosophila Genetic Resource Center. Temperature-inducible *esg-Gal4;tub-Gal80^{ts},UAS-GFP/Cyo* (*esg^{ts} > GFP*) [16], *UAS-λPVR* [26] and *UAS-EGFR$^{\lambda Top}$* [27] were kindly provided by B. Ohlstein, P. Rørth and Z. Paroush, respectively. Catalase

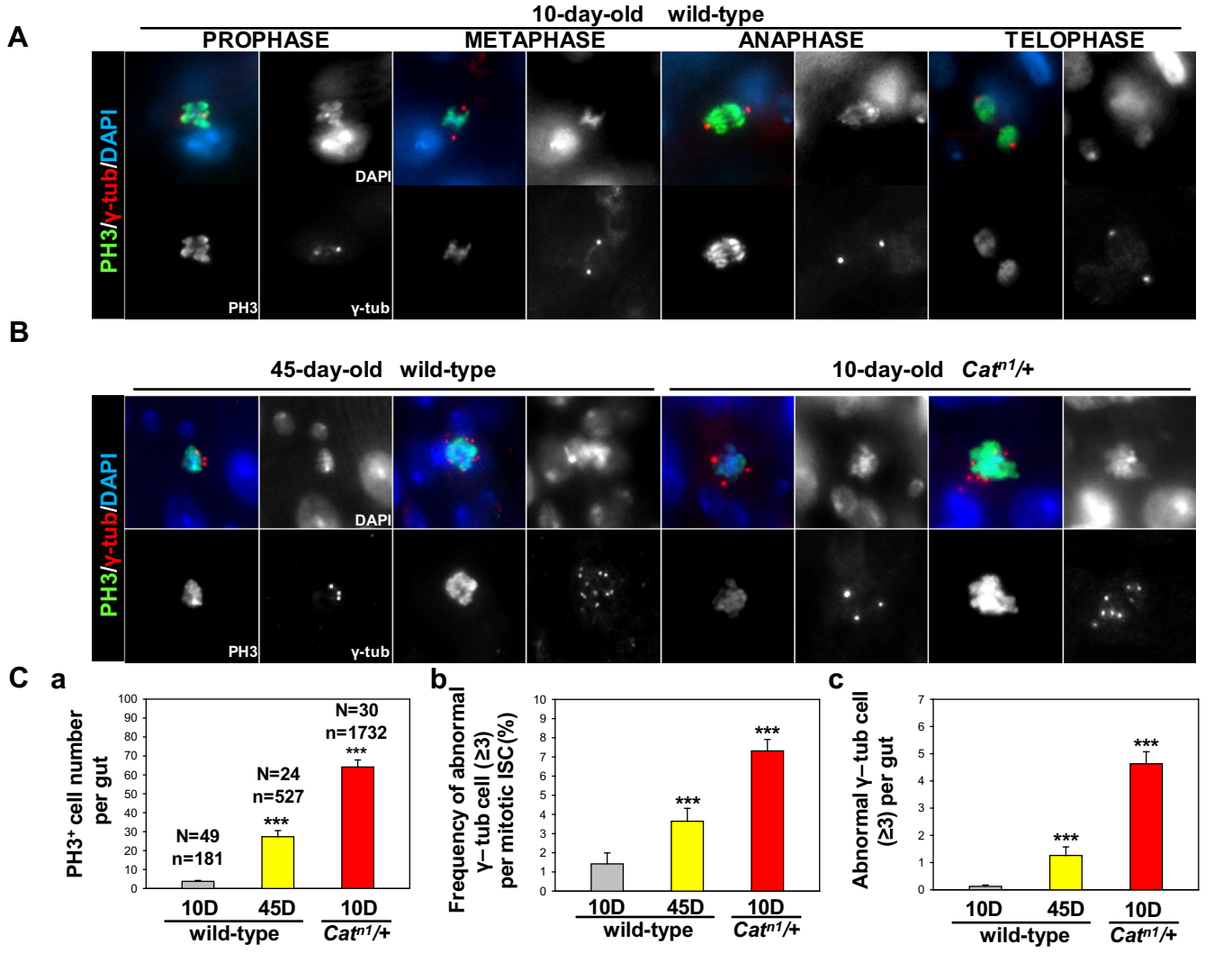

Fig. 1. Centrosome amplification in mitotic ISCs of aged and oxidative stressed guts. (A) Representative images of γ-tubulin signal in mitotic ISCs (PH3-positive cell) of 10-day-old wild-type flies. The guts of 10-day-old wild type flies were stained with anti-PH3 (green), anti-γ-tubulin (red) and DAPI (blue). (B) Images showing the mitotic ISCs with various centrosome numbers in aged wild-type flies and young Catalase mutants. The guts of 45-day-old wild type flies and 10-day-old *Cat^{n1}/+* flies were stained with anti-PH3 (green), anti-γ-tubulin (red) and DAPI (blue). Original magnification is 400×. (C) Increased number of mitotic ISCs with supernumerary centrosomes (⩾3) in the gut of 45-day-old wild-type flies and 10-day-old *Cat^{n1}/+*. (a) Age- and oxidative stress-related increases of mitotic ISCs in the midguts. (b) Frequency of abnormal γ-tubulin cell per mitotic ISC. (c) Number of abnormal γ-tubulin cell per midguts. The guts of 10-day-old and 45-day-old wild type flies and 10-day-old *Cat^{n1}/+* flies were stained with anti-PH3 (green), anti-γ-tubulin (red) and DAPI (blue). The centrosome numbers were counted in the PH3-positive cells of these guts. Data (mean ± SE) in 10-day-old and 45-day-old wild type and 10-day-old *Cat^{n1}/+* flies were collated from 181, 527 and 1732 mitotic cells of 49, 24 and 30 guts, respectively, from three separate experiments. *p*-Values were calculated using Student's *t*-test. $^{***}p < 0.0001$ compared to that of young wild-type flies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mutant (*Cat^{n1}/TM3*) and *y^{1}w^{1118};P{UAS-Akt1.Exel}2* (*UAS-AKT*) were kindly provided by the Bloomington Stock Center. Oregon-R flies were used as the wild-type. *Cat^{n1}/+* flies were obtained from a cross of the Oregon-R males to the *Cat^{n1}/TM3* females. *esg > GFP* flies were obtained from a cross of the Oregon-R males to the *esg-GAL4,UAS-GFP/CyO* females. *esg^{ts} > GFP + UAS-λPVR*, *esg^{ts} > GFP + UAS-EGFR^{λTop}*, and *esg^{ts} > GFP + UAS-AKT* flies were obtained from a cross of the *UAS-λPVR*, *UAS-EGFR^{λTop}*, or *UAS-AKT* males to the *esg^{ts} > GFP* females, respectively. All experiments were conducted in females.

2.2. Temperature-controlled expression

For transgene expression in specific developmental stages, the Gal80^{ts} technique was used [28]. The flies were set up and maintained at 22 °C until adulthood. Three-day-old females were shifted to 29 °C for 2 days to allow expression of the transgenes in ISCs/EBs.

2.3. Immunochemistry

Intact adult guts were dissected and then fixed at room temperature. For immunostaining with anti-GFP antibody, the guts were fixed for 1 h in 4% formaldehyde (Sigma–Aldrich, St. Louis, MO, USA). For immunostaining with anti-γH2AvD and anti-Delta antibodies, the guts were fixed for 30 min in 4% paraformaldehyde (Electron Microscopy Science, USA); dehydrated for 5 min in 50%, 75%, 87.5% and 100% methanol; rehydrated for 5 min in 50%, 25% and 12.5% methanol in PBST [0.1% Triton X-100 in phosphate-buffered saline (PBS)] for postfixation; and incubated overnight with primary antibody at 4 °C. After washing with PBST, the samples were incubated for 1 h with secondary antibodies at 25 °C, washed in PBST, mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA), and analyzed using a Zeiss Axioskop 2plus microscope (Carl Zeiss Inc., Gottingen, Germany). For the quantitative analysis of abnormal γ-tubulin cells (⩾3) in the mitotic ISCs, the images were processed using PhotoshopCS5 (Adobe Systems, San Jose, CA, USA). The number of abnormal γ-tubulin cells was counted in the whole midgut.

2.4. Antisera

In these experiments, the following primary antibodies diluted in PBST were used: mouse anti-γ-tubulin (Sigma–Aldrich, St. Louis, MO, USA), 1:1000 dilution, rabbit anti-phospho-histone H3 (PH3) (Millipore, Billerica, MA, USA), 1:1000, rabbit anti-GFP (Molecular Probes, Eugene, OR, USA), 1:1000, and rat anti-GFP (Nacalai Tesque Inc., Kyoto, Japan), 1:1000. The following secondary antibodies diluted in PBST + 2% BSA were used: goat anti-rabbit FITC (Cappel, Solon, OH, USA), 1:400; goat anti-mouse Cy3 (Jackson ImmunoResearch, West Grove, PA, USA), 1:400; goat anti-rat FITC (Jackson ImmunoResearch), 1:400, and goat anti-rabbit Alexa Fluor® 647

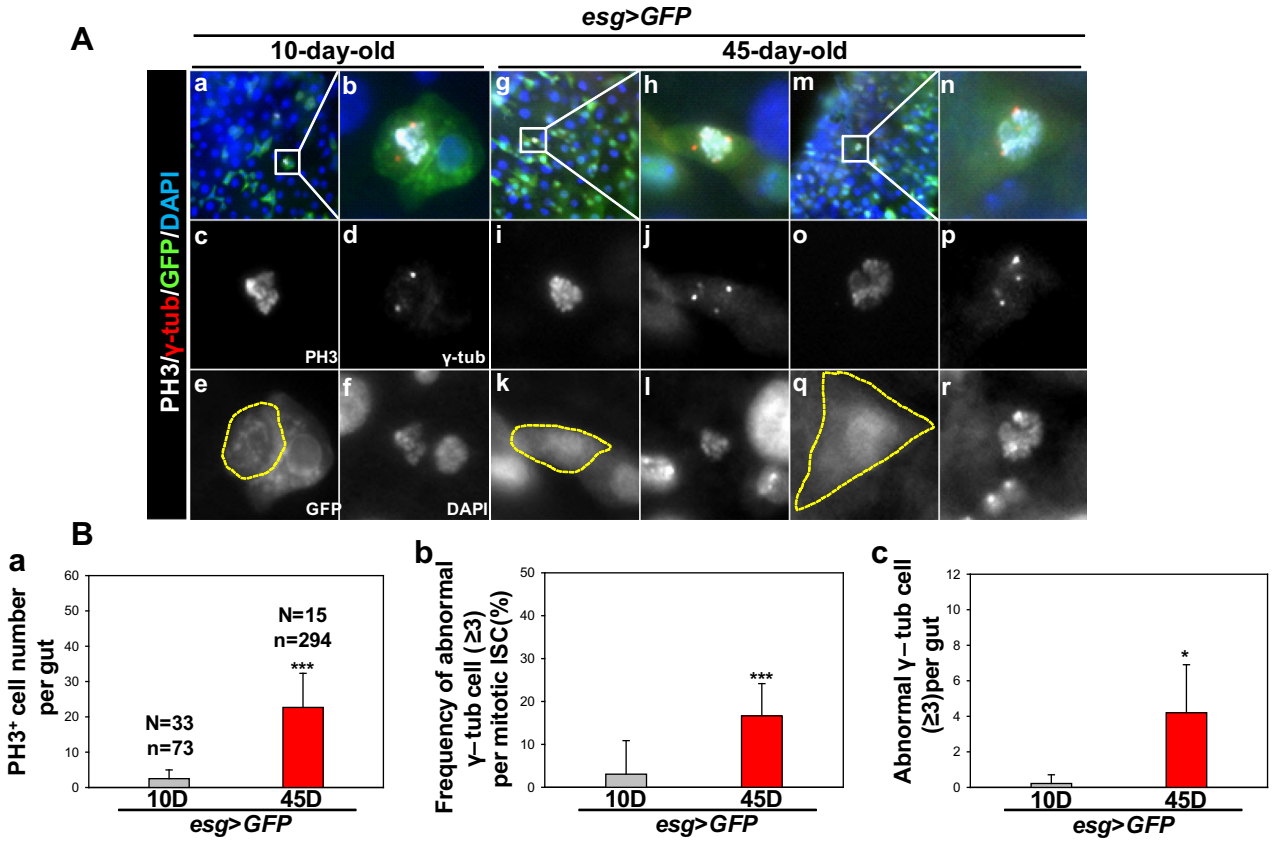

Fig. 2. Supernumerary centrosome in the *esg*-positive polyploid cell of aged midgut. (A) Images showing the mitotic ISCs with various centrosome numbers in young and aged *esg-GFP* flies. The guts of 10-day-old (a–f) and 45-day-old (g–r) *esg > GFP* flies were stained with anti-GFP (green), anti-γ-tubulin (red), anti-PH3 (white), and DAPI (blue). b–f, h–l, and n–r were enlarged image of white square in a, g, and m, respectively. Yellow dotted lines in e, k, and q indicate the area of dividing *esg*-positive cell. Original magnification is 400×. (B) Increased number of mitotic ISCs with supernumerary centrosomes (⩾3) in the gut of 10-day-old and 45-day-old *esg > GFP* flies. (a) Age-related increases of mitotic ISCs in the midguts. (b) Frequency of abnormal γ-tubulin cell per mitotic ISC. (c) Number of abnormal γ-tubulin cell per midguts. The guts of 10-day-old and 45-day-old *esg > GFP* flies were stained with anti-PH3 (green), anti-γ-tubulin (red), anti-PH3 (white), and DAPI (blue). The centrosome numbers were counted in the PH3-positive cells of these guts. Data (mean ± SE) in 10-day-old and 45-day-old *esg > GFP* flies were collated from 73 and 294 mitotic cells of 33 and 15 guts, respectively, from three separate experiments. *p*-Values were calculated using Student's *t*-test. $^{***}p < 0.0001$ and $^{*}p < 0.01$ compared to that of young *esg > GFP* flies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Jackson ImmunoResearch), 1:400. DAPI (Molecular Probes, Eugene, OR, USA) was diluted at 1:1000.

2.5. Statistical analyses

Quantified data are expressed as the mean ± SE values. Significance testing was conducted via Student's *t*-test (unpaired-, two-sided *t*-test).

3. Results and discussion

Using anti-γ-tubulin (a marker of centrosome) and anti-PH3 (a marker of mitotic ISC), we addressed whether centrosome duplication in mitotic ISCs is modulated by aging in the adult midgut. The midguts of 10-day-old and 45-day-old wild-type flies were examined, and 10-day-old Catalase mutant (Cat^{n1}/+) flies were used as a model of oxidatively stressed tissues.

Generally, two centrosomes were detected in the ISCs in the mitotic phase, which included prophase, metaphase, anaphase and telophase, of the 10-day-old flies (Fig. 1A), but mitotic ISCs with 3–12 abnormal centrosomes were detected in aged *Drosophila* midguts (Fig. 1B). We quantified the frequencies of these mitotic ISCs with supernumerary centrosomes (⩾3), which were 3.65% in the 45-day-old wild-type flies ($N = 24$, $n = 527$, N indicates number of guts, n indicates number of PH3-positive cells), 7.31% in the 10-day-old Catalase mutants ($N = 30$, $n = 1732$), and 1.42% in the 10-day-old wild-type flies ($N = 49$, $n = 181$) (Fig. 1C-b). The number of mitotic ISCs with supernumerary centrosomes (⩾3) per gut was 1.26 in the 45-day-old wild-type flies, 4.63 in the 10-day-old Catalase mutants, and 0.125 in the 10-day-old wild-type flies (Fig. 1C-c). Overall, these results show a higher incidence of centrosome amplification in the aged and oxidative-stressed ISC populations.

We also found supernumerary centrosomes in the *esg-GFP* positive polyploid cells (Fig. 2A-m–r) and increased centrosome amplification (Fig. 2B) in aged guts with *esg > GFP* (normally marking diploid ISC/progenitor cells). This indicates that supernumerary centrosomes can be responsible for abnormal ISC polyploid cells.

Next, we assessed how centrosome amplification is induced in aged midguts. Overexpression of PVR, EGFR, and AKT in ISCs and EBs has been demonstrated to mimic age-related changes in the midgut, such as hyperproliferation of ISCs and hyperplasia [21,23,24]. In mammals, elevated VEGF, EGFR, and AKT signaling

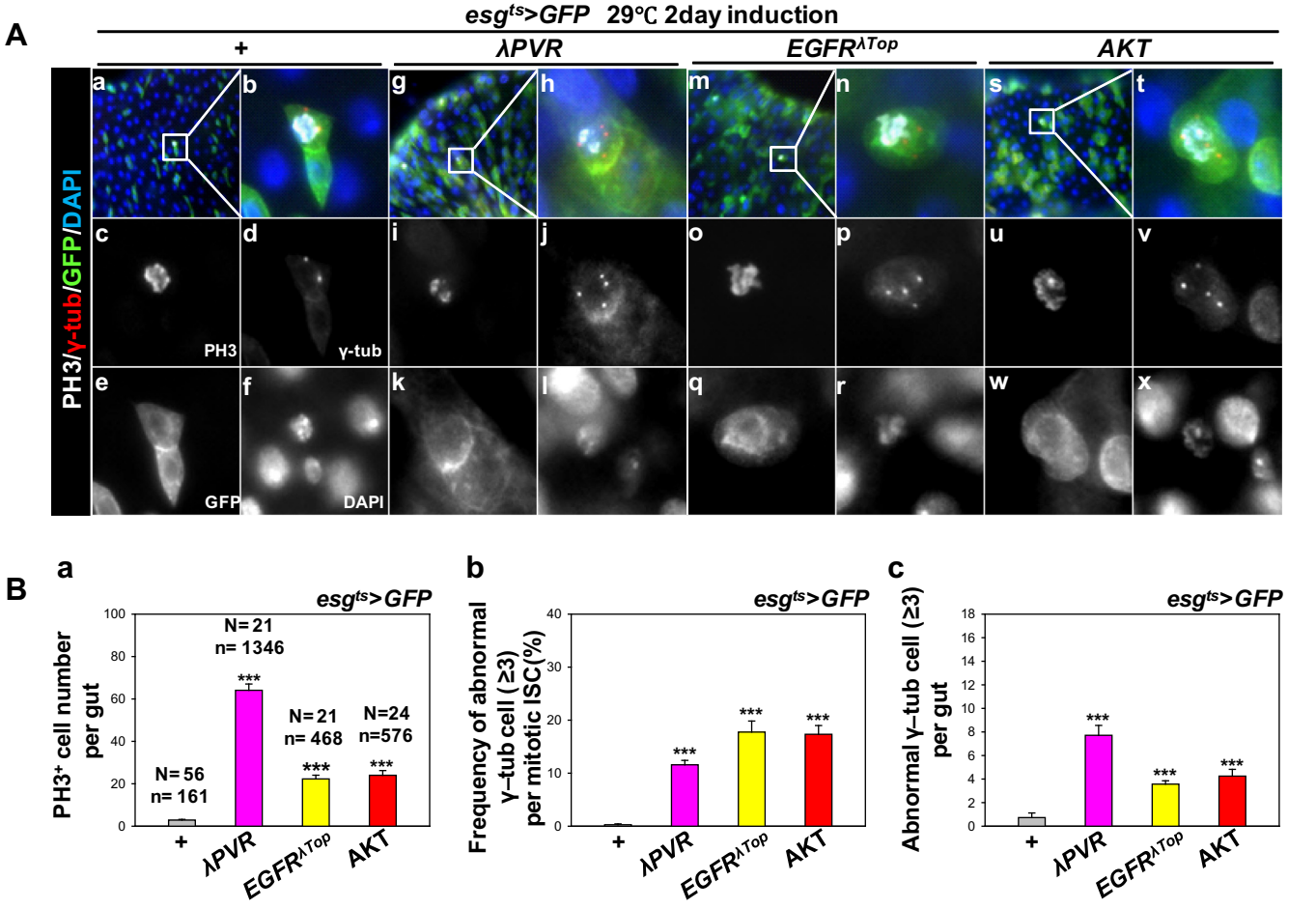

Fig. 3. Increased centrosome amplification by the ISCs/EBs-specific overexpression of γPVR, EGFR active form, and AKT. (A) Images showing the mitotic ISCs with various centrosome numbers in $esg^{ts} > GFP$ (a–f), $esg^{ts} > GFP + UAS\text{-}\lambda PVR$ (g–l), $esg^{ts} > GFP + UAS\text{-}EGFR^{\lambda Top}$ (m–r), and $esg^{ts} > GFP + UAS\text{-}AKT$ (s–x) flies. Three-day-old females were shifted to 29 °C for 2 days and dissected guts were immunostained with anti-GFP (green), anti-γ-tubulin (red), anti-PH3 (white), and DAPI (blue). b–f, h–l, n–r, and t–x were enlarged image of white square in a, g, m, and s, respectively. Original magnification is 400×. (B) Increased number of mitotic ISCs with supernumerary centrosomes (⩾3) in the gut of $esg^{ts} > GFP + UAS\text{-}\lambda PVR$, $esg^{ts} > GFP + UAS\text{-}EGFR^{\lambda Top}$, and $esg^{ts} > GFP + UAS\text{-}AKT$ flies. (a) ISCs/EBs-specific γPVR, $EGFR^{\lambda Top}$, or AKT overexpression-induced increases of mitotic ISCs in the midguts. (b) Frequency of abnormal γ-tubulin cell per mitotic ISC. (c) Number of abnormal γ-tubulin cell per midguts. Three-day-old females were shifted to 29 °C for 2 days and dissected guts were immunostained with anti-GFP (green), anti-γ-tubulin (red), anti-PH3 (white), and DAPI (blue). The centrosome numbers were counted in the PH3-positive cells of these guts. Data (mean ± SE) in $esg^{ts} > GFP$, $esg^{ts} > GFP + UAS\text{-}\lambda PVR$, $esg^{ts} > GFP + UAS\text{-}EGFR^{\lambda Top}$, and $esg^{ts} > GFP + UAS\text{-}AKT$ flies were collated from 161, 1346, 468, and 576 mitotic cells of 56, 21, 21, and 24 guts, respectively. *p*-Values were calculated using Student's *t*-test. $^{***}p < 0.0001$ compared to that of $esg^{ts} > GFP$ flies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reportedly are involved in centrosome amplification [29–31]. Therefore, we examined whether overexpression of PVR, EGFR, or AKT in ISCs and EBs induces centrosome amplification in adult *Drosophila* midgut by using esg^{ts} > *GFP*, esg^{ts} > *GFP* + *UAS-λPVR*, esg^{ts} > *GFP* + *UAS-EGFR*$^{\lambda Top}$, and esg^{ts} > *GFP* + *UAS-AKT* flies. The mitotic ISCs with supernumerary centrosomes ($\geqslant 3$) were detected in the ISCs/EBs-specific λPVR, *EGFR*$^{\lambda Top}$, and AKT overexpressed midguts (Fig. 3A). Significant increases of mitotic ISCs with supernumerary centrosomes ($\geqslant 3$) were detected in esg^{ts} > *GFP* + *UAS-λPVR*, esg^{ts} > *GFP* + *UAS-EGFR*$^{\lambda Top}$, and esg^{ts} > *GFP* + *UAS-AKT* flies (Fig. 3B). These results indicate that activation of PVR, EGFR, and AKT signaling in ISCs/EBs can induce mitotic ISCs with supernumerary centrosomes ($\geqslant 3$).

Here, we show the first direct evidence for the age-related increase of centrosome amplification in ISCs. Centrosomal abnormalities are strongly correlated with tumorigenesis and tumor progression [2] and have been reported to interfere with asymmetric stem cell division, which leads to expansion of the stem cell population and hyperplasia [30]. Therefore, our data support the view that centrosome amplification of tissue-resident stem cells could be a cause of chronic age-related diseases such as cancer. Our data also suggest that supernumerary centrosomes can be a useful marker for aging stem cells.

We also show that overexpression of PVR, EGFR, and AKT in ISCs/EBs can induce mitotic ISCs with supernumerary centrosomes ($\geqslant 3$). These pathways are involved in age-related changes in the midgut, such as hyperproliferation of ISCs and hyperplasia [21,23,24]. Therefore, our data suggest that PVR, EGFR and AKT pathways may contribute to the increased centrosome amplification in aged *Drosophila* and also that centrosome amplification may lead to hyperplasia in the midgut. We also observed increased phospho-AKT signal in ISCs and EBs of esg^{ts} > *GFP* + *UAS-λPVR* and esg^{ts} > *GFP* + *UAS-EGFR*$^{\lambda Top}$ flies (data not shown), suggesting that AKT can be a potential mediator in activated PVR and EGFR-induced centrosome amplification in ISCs. However, the mechanisms of centrosome amplification of stem cells *in vivo* remain largely unknown. Therefore, further studies on the factors and signals leading to centrosome amplification in tissue-resident stem cells are required for increased longevity and the prevention and treatment of age-related diseases, including cancer. Our findings suggest that the *Drosophila* midgut is an excellent model for studying the molecular mechanisms-underlying centrosome amplification in adult stem cells *in vivo*, and so increase our ability to therapeutically intervene in these processes in the future.

Acknowledgments

We thank Prof. Byung P. Yu (University of Texas Health Science Center at San Antonio, Texas) for invaluable comments on the manuscript. We would also like to thank the Developmental Studies Hybridoma Bank for antibodies, and the Bloomington Stock Center. We would like to thank the Brain Pool Program sponsored by the Korean Federation for Science and Technology Societies for its support of Dr. Robert Arking's Visiting Professorship at Pusan National University. This work was supported by a Bio-Scientific Research Grant funded by Pusan National University (PNU-2011-101-261).

References

[1] M. Bettencourt-Dias, D.M. Glover, Centrosome biogenesis and function: centrosomics brings new understanding, Nat. Rev. Mol. Cell Biol. 8 (2007) 451–463.
[2] E.A. Nigg, Centrosome aberrations: cause or consequence of cancer progression?, Nat Rev. Cancer 2 (2002) 815–825.
[3] D.G. Albertson, C. Collins, F. McCormick, J.W. Gray, Chromosome aberrations in solid tumors, Nat. Genet. 34 (2003) 369–376.
[4] G.A. Pihan, Centrosome dysfunction contributes to chromosome instability, chromoanagenesis, and genome reprograming in cancer, Front. Oncol. 3 (2013) 277.
[5] A. Ogden, P.C. Rida, R. Aneja, Heading off with the herd: how cancer cells might maneuver supernumerary centrosomes for directional migration, Cancer Metastasis Rev. 32 (2013) 269–287.
[6] R. Basto, K. Brunk, T. Vinadogrova, N. Peel, A. Franz, A. Khodjakov, J.W. Raff, Centrosome amplification can initiate tumorigenesis in flies, Cell 133 (2008) 1032–1042.
[7] B. Lemaitre, I. Miguel-Aliaga, The digestive tract of *Drosophila melanogaster*, Annu. Rev. Genet. 47 (2013) 377–404.
[8] S. Li, T. Torre-Muruzabal, K.C. Søgaard, G.R. Ren, F. Hauser, S.M. Engelsen, M.D. Pødenphanth, A. Desjardins, C.J. Grimmelikhuijzen, Expression patterns of the *Drosophila* neuropeptide CCHamide-2 and its receptor may suggest hormonal signaling from the gut to the brain, PLoS One 8 (2013) e76131.
[9] M. Rera, M.J. Azizi, D.W. Walker, Organ-specific mediation of lifespan extension: more than a gut feeling?, Ageing Res Rev. 12 (2013) 436–444.
[10] C. Saladino, E. Bourke, P.C. Conroy, C.G. Morrison, Centriole separation in DNA damage-induced centrosome amplification, Environ. Mol. Mutagen. 50 (2009) 725–732.
[11] C. Wong, T. Stearns, Centrosome number is controlled by a centrosome-intrinsic block to reduplication, Nat. Cell Biol. 5 (2003) 539–544.
[12] B. Inanç, H. Dodson, C.G. Morrison, A centrosome-autonomous signal that involves centriole disengagement permits centrosome duplication in G2 phase after DNA damage, Mol. Biol. Cell 21 (2010) 3866–3877.
[13] J.S. Park, S.H. Lee, H.J. Na, J.H. Pyo, Y.S. Kim, M.A. Yoo, Age- and oxidative stress-induced DNA damage in *Drosophila* intestinal stem cells as marked by Gamma-H2AX, Exp. Gerontol. 47 (2012) 401–405.
[14] C.A. Micchelli, N. Perrimon, Evidence that stem cells reside in the adult *Drosophila* midgut epithelium, Nature 439 (2006) 475–479.
[15] B. Ohlstein, A. Spradling, The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells, Nature 439 (2006) 470–474.
[16] B. Ohlstein, A. Spradling, Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential notch signaling, Science 315 (2007) 988–992.
[17] E.M. Lucchetta, B. Ohlstein, The *Drosophila* midgut: a model for stem cell driven tissue regeneration, Wiley Interdiscip. Rev. Dev. Biol. 1 (2012) 781–788.
[18] Y. Apidianakis, L.G. Rahme, Drosophila melanogaster as a model for human intestinal infection and pathology, Dis. Model. Mech. 4 (2011) 21–30.
[19] S.R. Singh, M.K. Mishra, M. Kango-Singh, S.X. Hou, Generation and staining of intestinal stem cell lineage in adult midgut, Methods Mol. Biol. 879 (2012) 47–69.
[20] B. Biteau, C.E. Hochmuth, H. Jasper, JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut, Cell Stem Cell 3 (2008) 442–455.
[21] N.H. Choi, J.G. Kim, D.J. Yang, Y.S. Kim, M.A. Yoo, Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor, Aging Cell 7 (2008) 318–334.
[22] J.S. Park, Y.S. Kim, M.A. Yoo, The role of p38b MAPK in age-related modulation of intestinal stem cell proliferation and differentiation in *Drosophila*, Aging (Albany NY) 1 (2009) 637–651.
[23] H.J. Na, J.S. Park, J.H. Pyo, S.H. Lee, H.J. Jeon, Y.S. Kim, M.A. Yoo, Mechanism of metformin: inhibition of DNA damage and proliferative activity in *Drosophila* midgut stem cell, Mech. Ageing Dev. 134 (2013) 381–390.
[24] H. Jiang, M.O. Grenley, M.J. Bravo, R.Z. Blumhagen, B.A. Edgar, EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in *Drosophila*, Cell Stem Cell 8 (2011) 84–95.
[25] A. Amcheslavsky, J. Jiang, Y.T. Ip, Tissue damage-induced intestinal stem cell division in *Drosophila*, Cell Stem Cell 4 (2009) 49–61.
[26] P. Duchek, K. Somogyi, G. Jékely, S. Beccari, P. Rørth, Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor, Cell 107 (2001) 17–26.
[27] P. Hasson, N. Egoz, C. Winkler, G. Volohonsky, S. Jia, T. Dinur, T. Volk, A.J. Courey, Z. Paroush, EGFR signaling attenuates Groucho-dependent repression to antagonize Notch transcriptional output, Nat. Genet. 37 (2005) 101–105.
[28] S.E. McGuire, G. Roman, R.L. Davis, Gene expression systems in Drosophila: a synthesis of time and space, Trends Genet. 20 (2004) 384–391.
[29] S.M. Taylor, K.R. Nevis, H.L. Park, G.C. Rogers, S.L. Rogers, J.G. Cook, V.L. Bautch, Angiogenic factor signaling regulates centrosome duplication in endothelial cells of developing blood vessels, Blood 116 (2010) 3108–3117.
[30] L. Li, A. Dutra, E. Pak, J.E. Labrie 3rd, R.M. Gerstein, P.P. Pandolfi, L.D. Recht, A.H. Ross, EGFRvIII expression and PTEN loss synergistically induce chromosomal instability and glial tumors, Neuro Oncol. 11 (2009) 9–21.
[31] H.J. Nam, S. Chae, S.H. Jang, H. Cho, J.H. Lee, The PI3K-Akt mediates oncogenic Met-induced centrosome amplification and chromosome instability, Carcinogenesis 31 (2010) 1531–1540.