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# Metformin inhibits age-related centrosome amplification in *Drosophila* midgut stem cells through AKT/TOR pathway

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ABSTRACT

We delineated the mechanism regulating the inhibition of centrosome amplification by metformin in *Drosophila* intestinal stem cells (ISCs). Age-related changes in tissue-resident stem cells may be closely associated with tissue aging and age-related diseases, such as cancer. Centrosome amplification is a hallmark of cancers. Our recent work showed that *Drosophila* ISCs are an excellent model for stem cell studies evaluating age-related increase in centrosome amplification. Here, we showed that metformin, a recognized anti-cancer drug, inhibits age- and oxidative stress-induced centrosome amplification in ISCs. Furthermore, we revealed that this effect is mediated via down-regulation of AKT/target of rapamycin (TOR) activity, suggesting that metformin prevents centrosome amplification by inhibiting the TOR signalling pathway. Additionally, AKT/TOR signalling hyperactivation and metformin treatment indicated a strong correlation between DNA damage accumulation and centrosome amplification in ISCs, suggesting that DNA damage might mediate centrosome amplification. Our study reveals the beneficial and protective effects of metformin on centrosome amplification via AKT/TOR signalling modulation. We identified a new target for the inhibition of age- and oxidative stress-induced centrosome amplification. We propose that the *Drosophila* ISCs may be an excellent model system for *in vivo* studies evaluating the effects of anti-cancer drugs on tissue-resident stem cell aging.



## 1. Introduction

Metformin, a biguanide drug, is clinically approved- and well-tolerated for the treatment of type 2 diabetes, and is of interest for cancer prevention and therapy (Aljada and Mousa, 2011; Baur et al., 2010; Landman et al., 2009). The direct molecular target of metformin is mitochondrial respiratory complex 1 (Owen et al., 2000). Inhibition of this protein complex decreases ATP production, which increases the AMP/ATP ratio and leads to subsequent activation of AMP-activated protein kinase (AMPK), an inhibitor of mammalian target of rapamycin (mTOR) (Owen et al., 2000). Many studies support for the anti-tumor effects of metformin (Algire et al., 2010; Berstein, 2012; Kato et al., 2012; Landman et al., 2009; Saito et al., 2013). Metformin has been shown to inhibit human cancer cells proliferation, and it can be used to prevent and treat a variety of cancers (Alimova et al., 2009; Ashinuma et al., 2012; Cantrell et al., 2010). Metformin blocks are the production of endogenous reactive oxygen species (ROS) (Halicka et al., 2011), and it inhibits pro-inflammatory responses and nuclear factor kappa B in human vascular wall cells (Isoda et al., 2006). However, the molecular mechanisms underlying the anti-tumor effects of metformin remain unclear.

The centrosome is the major microtubule-organizing center and plays an important in key cellular processes, including cell division, cell migration, and cell polarity (Bettencourt-Dias and Glover, 2007). Centrosome aberrations, such as centrosome amplification, lead to genomic instability (Rao et al., 2009). Centrosome

*Abbreviations:* 4E-BP, eukaryotic translation initiation factor 4E-binding protein; 8-oxo-dG, 8-oxo-2′-deoxyguanosine; AMPK, 5′ AMP-activated protein kinase; EBs, enteroblasts; ECs, enterocytes; EEs, enteroendocrine cells; EGFR, epidermal growth factor receptor; IGF1R, insulin-like growth factor-1 receptor; InR, insulin receptor; ISCs, intestinal stem cells; PBST, phosphate-buffered saline with 0.1% Triton X-100; PH3, phospho-histone H3; PQ, paraquat; PTEN, phosphatase and tensin homolog; Raptor, regulatory-associated protein of mTOR; Rheb, Ras homolog enriched in brain; ROS, reactive oxygen species; S6K, ribosomal protein S6 kinase; JAK/STAT, Janus kinase/signal transducers and activators of transcription; JNK, c-Jun N-terminal kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin.

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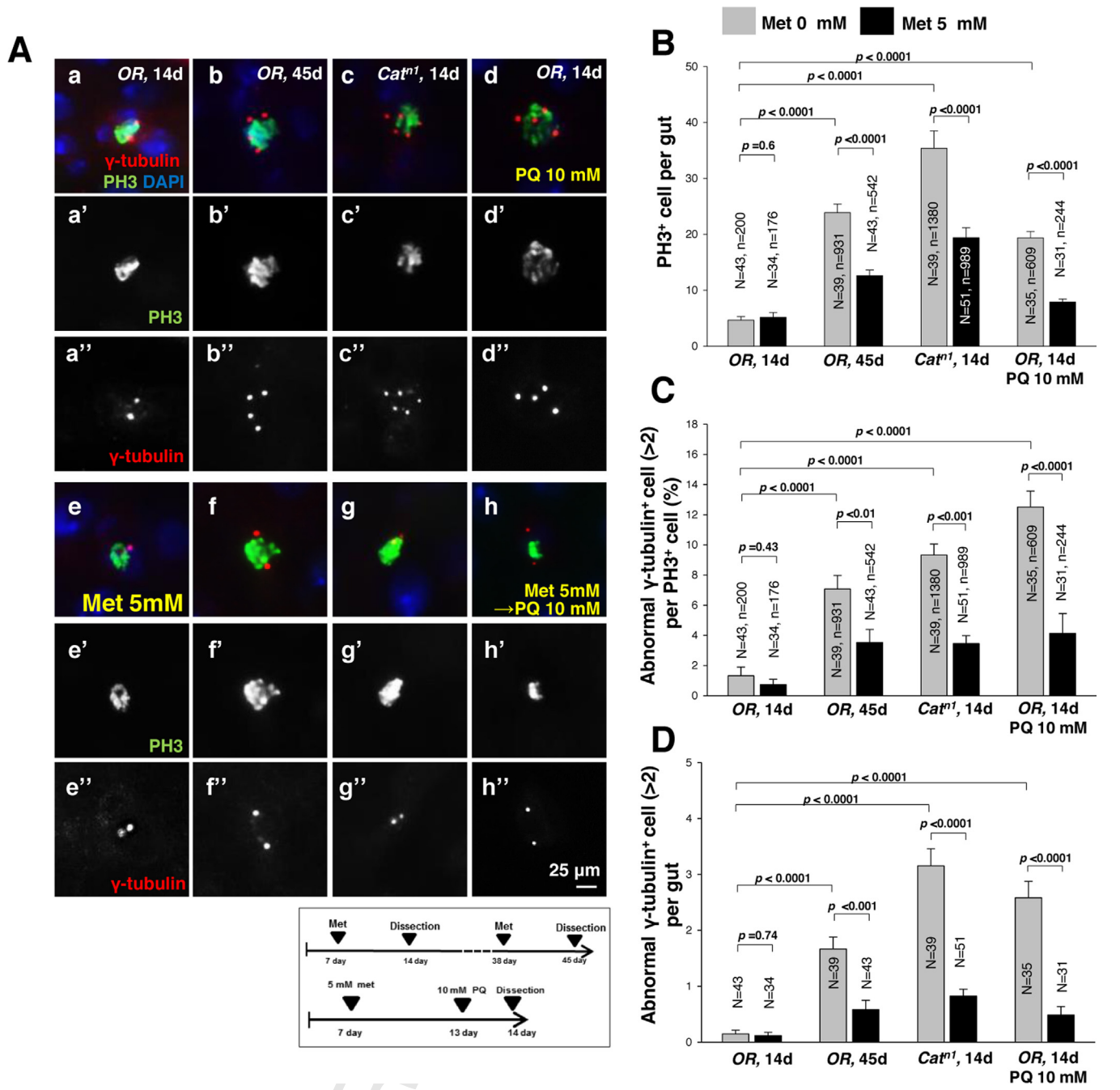


Q7 **Fig. 1.** Inhibitory effect of metformin on age-related centrosome amplification in midgut ISCs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
(A) Guts from 14-day-old wild type flies (a–a" and e–e"), 45-day-old wild type flies (b–b" and f-f"), and 14-day-old *Cat*$^{n1}$ mutant flies (c–c" and g–g") without (a–c") or with (e–g") 5 mM metformin feeding for 7 days were stained with anti-γ-tubulin (red), anti-PH3 (green), and DAPI (blue). Fourteen-day-old wild type flies without (d–d") or with (h–h") 5 mM metformin feeding for 6 days were treated with 10 mM PQ in standard media for 20 h, after which their guts were stained with anti-γ-tubulin (red), anti-PH3 (green), and DAPI (blue). a', b', c', d', e', f', g', and h' indicates enlarged PH3 staining images. a", b", c", d", e", f", g", and h" indicates enlarged images of γ-tubulin staining images. Original magnification is 400×. (B) The number of PH3-positive cells was counted in whole guts from 14-day-old wild type, 45-day-old wild type, 14-day-old *Cat*$^{n1}$ mutant, and 14-day-old PQ-treated wild type flies, with (black bars) or without (gray bars) metformin feeding for 7 days. N is the number of observed guts and n is the number of observed PH3-positive cells. (C) The frequency of supernumerary centrosome (>2) per mitotic ISC in 14-day-old wild type, 45-day-old wild type, 14-day-old *Cat*$^{n1}$ mutant, and 14-day-old PQ-treated wild type flies with (black bars) or without (gray bars) metformin feeding for 7 days guts. The centrosome numbers were counted in mitotic ISCs (PH3-positive cell) in the midgut. *N* is the number of observed guts and n is the number of observed γ-tubulin-positive cells. (D) The frequency of mitotic ISCs with supernumerary centrosome per gut in 14-day-old wild type, 45-day-old wild type, 14-day-old *Cat*$^{n1}$ mutant, and 14-day-old PQ-treated wild type flies with (black bars) or without (gray bars) metformin feeding for 7 days guts. *N* is the number of observed guts. The error bar represents standard error. *p*-values were calculated using Student's *t*-test.

amplification, which occurs when there is more than two centrosomes during mitosis, is a common feature of human cancers (D'Assoro et al., 2002; Nigg, 2002; Pihan et al., 2003). Centrosome amplification is involved in the initial stages of tumorigenesis (Leonard et al., 2013; Nakajima et al., 2004; Nam et al., 2010). Centrosome amplification is caused by DNA damage (Nigg, 2002; Xu et al., 1999). Interestingly, DNA damage incurred during G2 phase leads to greater centrosome amplification than G1 phase

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