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Alcohol inhibits the proliferation of Neuro2a cells via promoting the asymmetric cell division through down-regulation of the expression of centrosome protein-J

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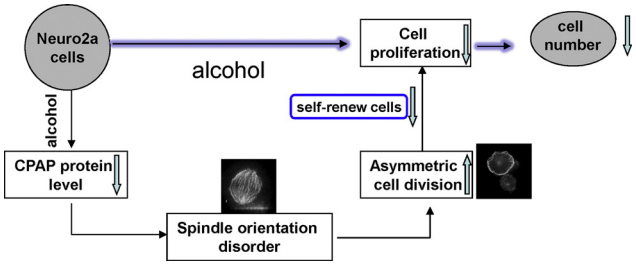
GRAPHICAL ABSTRACT



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ABSTRACT

Alcohol can decrease cell proliferation in neural cells. The proliferation of neural cells can be inhibited by the asymmetric division of neural progenitor cells. However, whether alcohol inhibits cell proliferation through inducing cell asymmetric division is not yet clear. Here, we reported that the percentage of asymmetric division was increased in alcohol-treated Neuro2a cells owing to the impaired-spindle orientation. Meanwhile, the expression of Centrosome protein-J (CPAP) which plays an important role in spindle orientation was reduced in Neuro2a cells. The overexpression of GFP-CPAP in Neuro2a cells rescued the disorder of spindle orientation and the asymmetric cell division induced by alcohol. Taken together, the results demonstrate that alcohol exposure diminished the pool of proliferative Neuro2a cells through disordering the spindle orientation and promoting the asymmetric division. And these abnormal orientation and division were due to the reduced CPAP protein level.

1. Introduction

Fetal alcohol syndrome (FAS) which develops in the fetus during pregnancy of alcoholic mothers is commonly characterized by neurological damages of the brain (i.e. physical and mental retardation). One of the main hallmarks of this disease is microcephaly, a condition in which a person's brain is significantly smaller than normal for their age and sex (May et al., 2014; Nash and Davies 2017).

In the developing brain, the first progenitor cells are polarized along the apical-basal axis and their nucleus migrated between the apical and

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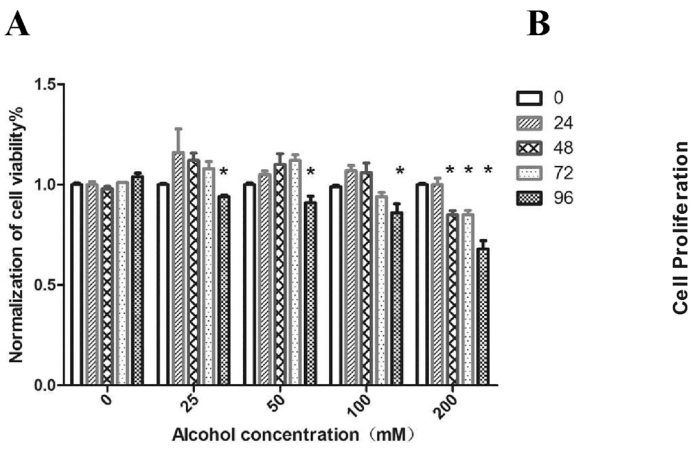

Fig. 1. Effects of alcohol on cell viability and proliferation.

(A) Neuro2a cells were treated with PBS, 25, 50, 100 or 200 mM alcohol for 0, 24, 48, 72 and 96 h, respectively. Cell viability was detected by MTT assay. * $P < 0.05$.

(B) Neuro2a cells were treated with 100 mM alcohol for 0, 24, 48 or 72 h. Cell proliferation was tested by BrdU proliferation assay. ** $P < 0.01$.

Data were obtained from three separated experiments and shown as mean ± S.D. Turkey's test was utilized to calculate p-values.

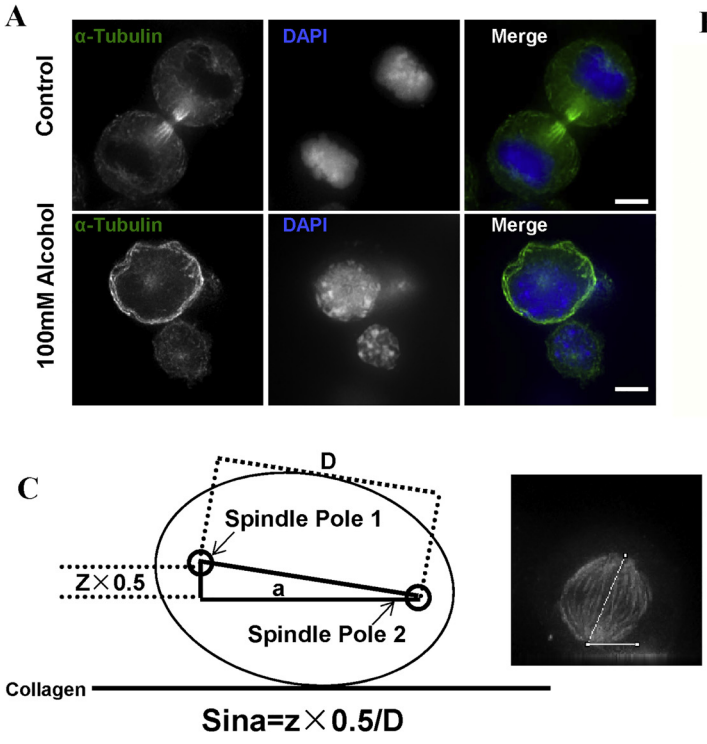

Fig. 2. Alcohol treatment results in spindle disorientation in 2-D cultured cells.

(A) Cell division was measured in cells treated with PBS or 100 mM alcohol for 48 h. Fixed metaphase Neuro2a cells were stained with α-tubulin (green) and DAPI (blue). Scale bar, 5 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(B) Quantification of the percentage of asymmetric cell division was calculated in cells treated with PBS or 100 mM alcohol for 48 h (n >30 cells from three independent experiments). Student's *t*-test was used to calculate p-values. Error bars indicate means ± SEM. ** $P < 0.01$.

(C) The formula and schematic diagram for spindle angles' calculation. Character α represents spindle angle in mitosis cells. D represents the distance between the two spindle poles. Z represents the number of stacks (0.5 μm per stack) between collagen matrix and spindle pole 1, respectively.

(D) The analysis of Z-stack and the distance between two spindle poles was measured by image-J software. Treated and untreated Neuro2a cells were stained with α-tubulin antibody. Images were taken on a Delta Vision microscope with Softworx software. Each image represents one stack.

(E) Scatter plots showed the spindle angles with calculated mean value in control and alcohol-treated cells (n>30 cells). Student's *t*-test was used to calculate p-values.

basal positions. Interestingly, the centrosomes always remain at the apical membrane during interphase and mitosis (Reiner et al., 2012). The position of centrosome is very important for the orientation of the central spindle in mitosis, which affects the cell migration as well as the fate of symmetrical or asymmetrical division (Luxton and Gundersen 2011, Yamashita and Fuller 2008). The symmetrical cell division occurs when spindle parallels to apical and provides two progenitor cells which can be still proliferating. The asymmetrical cell division who's spindles formed an angle with apical member produces one progenitor cell and one differentiated cell and hinders the further proliferation