Supporting Information

Effect of the selected food phytochemicals on breast cancer metastasis based on *in vivo* capture of circulating tumor cells

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Reagents and Materials. 4T1 cell line was purchased from Cell bank of Chinese Academy of Sciences (Shanghai, China). RPMI-1640 culture media was purchased from Thermo Fisher biochemical products Co., Ltd. (Beijing, China). Polydimethylsiloxane (PDMS) prepolymer and its curing agent, Sylgard 184, were purchased from Dow Corning (Midland, U.S.). Vein indwelling needles (24 G × 0.75, 0.7 mm × 19 mm) were purchased from Suzhou Bi Di Medical Devices Co., Ltd. (Suzhou, China). Anti-EpCAM antibodies were purchased from Beijing Boosen Biological Technology Co., Ltd. (Beijing, China). Alexa Fluor 594-anti-cytokeratin 8 antibodies and fluorescein isothiocyanate (FITC)-anti-CD45 antibodies were purchased from Abcam and eBioscience respectively. 4', 6-diamidino-2-phenyl-indole (DAPI) was purchased from Sigma-Aldrich (St. Louis, U.S.). BALB/c mice were purchased from the laboratory animal center of Shandong University (Jinan, China). Procyanidine, curcumin, β-sitosterol, tea polyphenol and perillyl alcohol were purchased from Sigma-Aldrich (St. Louis, U.S.). Cremophor EL and paclitaxel purchased from Macklin biochemical technology Co., Ltd. (Shanghai, China).

Cell type and culture conditions. 4T1 cell line was cultured at 37°C in a humidified atmosphere of 5% CO_2 and grown in RPMI-1640 with 10% heat inactivated fetal bovine serum. Medium was changed every day and adherent cells were harvested by trypsinization.

Functionalization of vein indwelling needle. Functionalization of vein indwelling needle was described as our previous work.[1] In brief, indwelling needles were immersed into PDMS prepolymer mixture which curing agent ratio was 10:1, then took out and cured for 3 h in a convection oven at 65°C. The PDMS-coated needle surface was modified by anti-EpCAM antibody and stored at 4°C for CTCs' capture.

Establishment of mice tumor model. Adult (female, 10-week-old, 20 ± 2 g) BALB/c mice were

housed individually. The animals were maintained under the standard conditions of 30-50% relative humidity and 24-26°C. Mice were fed with pelleted mouse food and water ad libitum. We subcutaneously inoculated the armpit of the right anterior limb of BALB/c mice with 1×10^5, 1×10^6 and 1×10^7 4T1 mouse breast cancer cells. All animal experiments were conducted at the laboratory animal center of Shandong Normal University in compliance with the Guidelines of Shandong Normal University for the Care and Use of Laboratory Animals.

Intragastric administration of dietary factors. Procyanidine (3 mg/mL)[2], tea polyphenol (0.4 mg/mL)[3] and sucrose (8% w/w) were dissolved in ultrapure water. Curcumin (3 mg/mL)[4] and β-sitosterol (2 mg/mL)[5] were suspended in 0.5% sodium carboxymethylcellulose. 100 μL water, procyanidine, tea polyphenol and sucrose water solution, 100 μL curcumin and β-sitosterol suspension, 100 μL lard oil and 15 mg perillyl alcohol[6] were respectively delivered to different mice group (n = 10) by oral gavage every day after inoculated with cancer cells.

Injection administration of paclitaxel. Paclitaxel was dissolved in ethanol with a concentration of 10mmol/L and stored at 4°C. Paclitaxel cremophor vehicle was prepared with Cremophor EL/ethanol 1:1, diluted with PBS at 1:4. Mice were injected intraperitoneally with 10 mg/kg paclitaxel.[4]

Capture, staining and identification of CTCs. According to our previous work, at the 7, 14, 21 and 28 days after subcutaneous inoculation, functionalized needle was punctured into the caudal vein of mouse tumor model for the *in vivo* capture of CTCs. Needle was took out 2 h later, eluted with PBS to ELISA plate. Then cells were fixed with paraformaldehyde (4 wt %) for 15 min at room temperature, permeabilized with 0.5% PBS-TritonX-100 for 30 min at 37°C. Cells were stained with Alexa Fluor 594-anti-cytokeratin8 antibodies and FITC-anti-CD45 antibodies for 2 h

and DAPI for 15 min at 37°C in a moist container in dark. Cells stained with Alexa Fluor 594 and DAPI was identified as CTCs.

Measurement of the mice tumor volume and lung metastasis. Once palpable tumors were developed, their bi-directional dimensions in millimeters were measured using calipers, and the tumor volume were calculated with the formula, $(a \times b^2)/2$, where a is the longer diameter and b is the shorter diameter. At 35 days after inoculation, mice were sacrificed and the lung metastases were counted. Rate of lung metastasis of each group was calculated according to the following formula: Rate of lung metastasis = the number of mice with lung metastasis / the total number of mice in each group.

Statistical analysis. Statistical analysis was performed with the statistic package for social science (SPSS) (version 13.0 SPSS). One-way anova analysis was used to compare the lung metastasis counts in mice of different group.

References

1 H. Zhang, Z. Jia, C. Wu, L. Zang, G. Yang, Z. Chen and B. Tang, *ACS applied materials & interfaces*, 2015, **7**, 20477-20484.

2 S. Dinicola, A. Pasqualato, A. Cucina, P. Coluccia, F. Ferranti, R. Canipari, A. Catizone, S. Proietti, F. D'Anselmi and G. Ricci, *European journal of nutrition*, 2014, **53**, 421-431.

3 R. L. Thangapazham, A. K. Singh, A. Sharma, J. Warren, J. P. Gaddipati and R. K. Maheshwari, *Cancer letters*, 2007, **245**, 232-241.

4 B. B. Aggarwal, S. Shishodia, Y. Takada, S. Banerjee, R. A. Newman, C. E. Bueso-Ramos and J. E. Price, *Clinical Cancer Research*, 2005, **11**, 7490-7498.

5 Y. Zhao, S. K. Chang, G. Qu, T. Li and H. Cui, *Journal of agricultural and food chemistry*, 2009, **57**, 5211-5218.

6 N. S. Nandurkar, J. Zhang, Q. Ye, L. V. Ponomareva, Q.-B. She and J. S. Thorson, *Journal of medicinal chemistry*, 2014, **57**, 7478-7484.