JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2016 August 15; 36(4): 530-537
ISSN 0255-2922

EXPERIMENTAL STUDY

Breast cancer stem-like cells can promote metastasis by activating platelets and down-regulating antitumor activity of natural killer cells

Wang Shuo, Zhang Ying, Cong Weihong, Liu Jie, Zhang Yuren, Fan Huiting, Xu Yonggang, Lin Hongsheng

Wang Shuo, Oncology Department, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China; Institute of Clinical Medicine, Beijing University of Chinese Medicine, Beijing 100029, China

Zhang Ying, Liu Jie, Zhang Yuren, Fan Huiting, Lin Hongsheng, Oncology Department, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China

Cong Weihong, Research Center, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, China

Xu Yonggang, Laboratory of Hematology Department, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, China

Supported by the National Natural Science Foundation of China (the Research of Traditional Chinese Medicien Regulating the Tumor Metastasis due to Cancer Stem Cell Related Expression of TF and TF/F Ⅶ Signal Pathway Based on Traditional Chinese Medicien Theory of "FuDu", No. 81273947); the International S & T Cooperation Program Office of China (Herbal Medicine with Different Treatment Principles on Regulating the Biological Behaviour of Cancer Stem Cells and its Regulatory Factors, No. 2013DFA32540)

Correspondence to: Lin Hongsheng, Oncology Department, Guangan'men Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China. drlinhongsheng@163.com

Telephone: +86-13601188424

Accepted: April 3, 2016

Abstract

OBJECTIVE: To investigate whether cancer stem cells (CSCs) more efficiently activating platelets and evading immune surveillance than non-CSCs thus promoting metastasis.

METHODS: We enriched and identified sphere-forming cells (SFCs) and coincubated washed platelets with several platelet activators including collagen, 4T1 and SFCs. Platelet-coating tumor cells, platelet activation and TGF-β1 release were analyzed. Then natural kell cells (NK) were incubated with supernatants of different activated platelet samples what we called sample release (SR). The degranulation assay and NKG2D expression on NK cells were conducted by flow cytometry. Finally tissue factor (TF) expression of SFCs or 4T1 were evaluated by western blot.

RESULTS: Breast cancer cell line 4T1 could form spheres in serum-free medium at low adherence. Sphere-forming cells expressed high levels of the $CD24^{-/low}CD44$ + stem cell phenotype. Both sphere-forming cells or 4T1 were coated with abundant platelets while sphere-forming cells induced significantly higher expression of platelet activating receptor CD62p than 4T1 did ($P < 0.01$). And sphere-forming cells induced platelets to produce more TGF-β1 than 4T1 did ($P < 0.01$). Furthermore, sample releases induced by sphere-forming cells caused more vigorous inhibition of NK cells antitumor reactivity ($P < 0.05$) and reduced NKG2D expression ($P < 0.01$). The final results showed that sphere-forming cells expressed higher levels of TF than 4T1 ($P < 0.05$).

CONCLUSION: Our findings indicate that CSCs could efficiently activate platelets, induce platelets to secrete more TGF-β1, decrease NKG2D expression and inhibit antitumor activity of NK cell, compared with 4T1. And higher levels of TF expression of CSCs may account for this correlation of CSCs and platelets.

Key words: Breast neoplasms; Cancer stem cellNeoplastic stem cells; Metastasis Neoplasm metastasis; Platelet activation; Natural killer Killer cells, natural

INTRODUCTION

Metastasis is the primary cause of cancer treatment failure and patient death. Understandings of Traditional Chinese Medicine (TCM) for tumor metastasis began from Qin and Han dynasties,[1] of which TCM "Fudu" theory[2] was an important one. The theory holds that "Fudu" is a kind of delitescent pathogenic *Qi* remained after cancer treatment such as surgery, radiotherapy or chemotherapy. The residual pathogenic *Qi* cannot be eliminated by deficient *Qi* and accumulates to generate the pathogenic factor "Fudu". It may spread to other parts of the body and forms a new one.[2] On the other hand, with the progress of stem cell biology and the deepening of pathogenesis of cancer research in recent years, accumulating evidence suggests that not all tumor cells metastasize, but a small part of cancer stem cells (CSCs) with stem-like properties determine tumor recurrence and metastasis.[3] CSCs are usually in a relatively static state and able to successfully escape destruction of radiochemotherapy, thus become the source of tumor metastasis.[4-6] It elucidates that "Fudu" theory is very similar to modern medicine understandings of tumor metastasis by CSCs. Moreover, TCM therapeutic method "Fuzheng Qudu" (strengthening body resistance and dispelling toxicity) for tumor, based on "Fudu" theory, is clinically proven to have effects on tumor metastasis.[7,8]

According to "Fudu" theory, "Fudu" differs from general pathogenic *Qi* and has greater ability to transfer along channels and blood vessels.[2] Modern medicine also focuses on the issue how tumor cells leave the primary tumor, invade into blood vessels and travel to other parts of the body to finally generate metastatic tumors. Numerous studies show that platelets could promote tumor progression and metastasis.[9,10] After tail vein injection of tumor cells in mice with thrombocytopenia, their pulmonary metastases decreased which could be reversed by platelet transfusion.[11] *In vivo* and vitro studies reveal that thrombocytopenia could reduce tumor cell spread, which may be due to destruction of nature killer (NK) cells.[12-15] Thus, platelets would adhere to tumor cells by which platelet-tumor cell interaction caused platelet activation and release of platelet granule contents, and finally tumor cells with help of platelets escaped immune surveillance and elimination.[9] This raises the question whether CSCs, as the origin of metastasis, may be more effective to active platelets and evade immune surveillance than non-CSCs? It prompts us to explore the mechanisms of CSCs giving rise to tumor metastasis from the perspective of CSCs, platelets and NK cells.

MATERIALS AND METHODS

Animal and cell

Female BALB/c mice, weighted 19 to 21g, were purchased from Beijing Charles River Laboratory Animal Technology Co., Ltd., [certification No. SCXK (Jing) 2012-0001] (Beijign, China) and fed in the Laboratory Animal Center of Guangan'men Hospital. The tumor cell line 4T1 (breast cancer cell) was so kindly sent us by Laboratory of Molecular Immunology Adjustment, National Cancer Institute.

Reagents

Anti-CD24-PE, anti-CD44-APC, Anti-CD61-PE, Anti-CD62p-FITC, Anti-CD49b-FITC, Anti-CD107a-APC, Anti-CD314-PE were perchased from BD (Franklin Lakes, NJ USA) together with the corresponding isotype controls. Anti-CD41 antibody, Anti-Tissue Factor antibody, Anti-beta Actin antibody and Goat Anti-Rabbit IgG H & L (FITC) were from Abcam (Cambridge, UK). Mouse Spleen Mononuclear Cell Isolation Kit was from TBD (Tianjin, China). Protein Transport Inhibitor (Containing Monensin) was from BD (Franklin Lakes, NJ, USA). Mouse TGF-β1 ELISA kits was from R & D (Minneapolis, MN, USA). Goat Anti-Rabbit IgG H & L (HRP) was from Jackson (Baltimore Pike, MD, USA).

Cell culture and tumor sphere enriching

Grown were 4T1in RPMI-1640 medium containing 10% fetal bovine serum (FBS), penicillin and streptomycin at 37 ℃ in a 5% CO_2 incubator. 4T1 in logarithmic growth phase (passage 5-8) were cultured in serum-free DMEM/F12 medium supplemented with 10 ng/mL human recombinant basic fibroblast growth factor (bFGF), 20 ng/mL human recombinant epidermal growth factor (EGF), 1∶50 B27, 5 μg/mL insulin, 0.4% BSA, 2% KnockOut, l% penicillin and streptomycin in Ultra-Low Attachment 6-Well Plates. After 5-7 days, Tumor spheres were collected by centrifugating at 1000 rpm for 5 min, detached enzymatically in 0.25% trypsin (0.02% EDTA) and cultivated in the same medium to generate tumor spheres of the next generation.

Preparation of mouse washed platelets and spleen mononuclear cells

Washed platelets were extracted and prepared following general method described previously.[16-18] latelets were obtained by cardiac puncture. Citrated blood was diluted with 250 μL of modified Tyrodes-HEPES buffer (134 mmol/L NaCl, 0.34 mmol/L Na2HPO4, 2.9 mmol/L KCL, 12 mmol/L NaHCO3, 20 mmol/L HEPES, 5 mmol/L glucose, 1 mmol/L MgCl2, PH 7.3) and centrifuged at 120 × *g* for 20 min. Platelet-rich plasma (PRP) was obtained, and the rest of blood was centrifuged at 800 × *g* for 10 min to remove platelet-poor plasma (PPP). PRP was subsequently cen-