Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Mesenchymal stem cells drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells via paracrine of neuregulin 1

Jin Chen [a, b, 1], Qun Ren [b, 1], Yuanming Cai [c], Tingting Lin [b], Weimin Zuo [b, d], Jin Wang [b], Rong Lin [b], Ling Zhu [b], Ping Wang [b], Huiyue Dong [b], Hu Zhao [b], Lianghu Huang [b], Yunfeng Fu [b], Shunliang Yang [b], Jianming Tan [b], Xiaopeng Lan [a, *], Shuiliang Wang [b, **]

[a] Institute of Laboratory Medicine, Fuzhou General Hospital, Second Military Medical University, Fuzhou, 350025, China
[b] Fujian Key Laboratory of Transplant Biology, Fuzhou General Hospital, Xiamen University, Fuzhou, 350025, China
[c] Class 7 of Clinical Medicine, School of Basic Medicine, Xinjiang Medical University, Urumchi, 830011, China
[d] Department of Medical Ultrasound, Guangzhou First Peoples's Hospital, Guangzhou Medical University, Guangzhou, 510180, China

ARTICLE INFO

Article history:
Received 23 April 2018
Accepted 27 April 2018
Available online xxx

Keywords:
Mesenchymal stem cells (MSCs)
Paclitaxel-resistance
ErbB2/ErbB3-coexpressing breast cancer
Tumor microenvironment

ABSTRACT

We had previously demonstrated that increased expression of ErbB3 is required for ErbB2-mediated paclitaxel resistance in breast cancer cells. In the present study, we have explored the possible role of mesenchymal stem cells (MSCs) in regulating the paclitaxel-sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. We show that human umbilical cord-derived MSCs express significantly higher level of neuregulin-1 as compared with ErbB2/ErbB3-coexpressing breast cancer cells themselves. Coculture or treatment with conditioned medium of MSCs not only decreases the anti-proliferation effect of paclitaxel on ErbB2/ErbB3-coexpressing breast cancer cells, but also significantly inhibits paclitaxel-induced apoptosis. We further demonstrate that this MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells could be attributed to upregulation of Survivin via paracrine effect of NRG-1/ErbB3/PI-3K/Akt signaling, as either specific knockdown expression of ErbB3, or blocking of downstream PI-3K/Akt signaling, or specific inhibition of Survivin can completely reverse this effect. Moreover, targeted knockdown of NRG-1 expression in MSCs abrogates theirs effect on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. Taken together, our study indicate that paracrine of NRG-1 by MSCs induces paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells through PI-3K/Akt signaling-dependent upregulation of Survivin. Our findings suggest that simultaneously targeting mesenchymal stem cells in tumor microenvironment may be a novel strategy to overcome paclitaxel resistance in patients with ErbB2/ErbB3-coexpressing breast cancer.

1. Introduction

Breast cancer remains the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide [1]. Surgery in conjunction with adjuvant chemotherapy is the main treatment of choice for patients with locally advanced breast cancer, leading to reduce cancer-related symptoms and prolong survival. Paclitaxel, as a novel microtubule-stabilizing agent, induces apoptosis in cancer cells by activation of the mitotic check points and subsequent mitotic blockage, which is resulted in inhibition of cancer cell proliferation [2]. It is used against a wide range of solid tumors include locally advanced and metastatic breast cancer [3]; however, resistance to paclitaxel remains the greatest obstacle to the successful treatment of breast cancer that attributed to a high mortality rate in women.

Resistance to chemotherapeutics can be intrinsic or acquired. Intrinsic resistance means that resistance-mediating factors pre-exist in the context of tumor cells that make the therapy ineffective while patient receiving initial chemotherapy. Acquired drug resistance can develop during treatment of tumors and be caused

Abbreviations: hUC-MSCs, Human umbilical cord mesenchymal stem cells; NRG-1, neuregulin 1; PI-3K, phosphoinositide 3-kinase; qRT-PCR, real-time quantitative reverse transcriptase PCR; shRNA, short hairpin RNA.

* Corresponding author.
** Corresponding author.
E-mail addresses: Lanxp@sina.com (X. Lan), shuiliang.wang@xmu.edu.cn (S. Wang).

[1] These authors contributed equally to this study.

https://doi.org/10.1016/j.bbrc.2018.04.218
0006-291X/

by mutations arising during treatment, as well as through various other adaptive responses including activation of alternative compensatory signaling pathways [4]. Since the use of paclitaxel against breast cancer, the molecular mechanisms contributing to paclitaxel resistance have been extensively investigated [5]; however, most of the studies focused on the alteration of cancer cells themselves. For the past two decades, the role of tumor microenvironment in the biology of tumor has increasingly been recognized [6]. Mesenchymal stem cells (also mesenchymal stromal cells, MSCs) are connective tissue progenitor cells that contribute to fibrotic reactions during tissue remodeling and repair in places of wounding and inflammation. In response to chemokines from tumor cells, MSCs are continuously recruited to and become integral components of the tumour microenvironment [7]. MSCs in tumor microenvironment have been shown to exert influence on multiple hallmarks of cancer including resistance to chemotherapy [8,9]. Therefore, MSCs seem to act as "co-conspirators" within the tumor microenvironment, protecting cancer cells from chemotherapy. However, little has been done to investigate the detrimental effects of MSCs in chemotherapeutic resistance of breast cancers.

We have previously showed that elevated expression of ErbB3 results in paclitaxel resistance, and therapeutic targeting of ErbB3 enhances antitumor activity of paclitaxel against ErbB2-overexpressing breast cancer [10,11]. Our current study aimed to explore the role of MSCs in paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells and its underlying molecular mechanism. We found that neuregulin 1 (NRG-1, also Heregulin-β1), a direct ligand for ErbB3 tyrosine kinase receptor that could be produced by MSCs, acts in a paracrine manner to induce resistance to paclitaxel through PI3K/Akt signaling-dependent upregulation of survivin in ErbB2/ErbB3-coexpressing breast cancer cells. Our findings suggested that targeting MSCs in tumor microenvironment may be a novel strategy to overcome paclitaxel resistance.

2. Materials and methods

2.1. Reagents and antibodies

Paclitaxel was obtained from Fuzhou General Hospital pharmacy. MISSION® Non-target shRNA, which does not target human and mouse genes, control vector (pLKO.1-ConshRNA), pLKO.1-TRC without any insert of shRNA template and pLKO.1 containing human *ErbB3* shRNA (pLKO.1-ErbB3shRNA) were purchased from Sigma (St. Louis, MO, USA). The packaging plasmids psPAX2 and pMD2. G for lentiviral expression vector were from Addgene Inc. (Cambridge, MA, USA). The CellTiter96AQ cell proliferation kit was product of Promega (Madison, WI, USA). Specific inhibitors of PI-3K (LY294002) and Akt (Akt1/2 inhibitor VIII) were products from Sigma. Oligonucleotides were synthesized in Sangon (Shanghai, China).

Antibodies were obtained as follows: ErbB2, ErbB3, PARP, P-MAPK (E10), MAPK, P-Akt (Ser473), Akt, Cyclin D1, Cyclin E1, p21, p27 (Cell Signaling Technology, Inc., Beverly, MA, USA); Survivin (Epitomics, Burlingame, CA, USA); Recombinant human NRG1 protein (rNRG1-β1) and Anti-NRG1 (Abcam, Cambridge, MA, USA); β-actin (AC-75) (Sigma). All other reagents were purchased from Sigma unless otherwise specified.

2.2. Cells and cell culture

Human breast cancer cell line MDA-MB-453 and embryonic kidney cell line HEK293T were obtained from American Type Culture Collection (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium: Nutrient Mix F-12 (DMEM/F-12 1:1) supplemented with 10% fetal bovine serum (FBS). Two human umbilical cord mesenchymal stem cell lines (UC-MSCs_002 and UC-MSCs_016) were established in our own lab as described previously and maintained in DMEM/Low glucose supplemented with 10% FBS [12]. For most of the experiments in our current study, the UC-MSCs_002 was used unless otherwise specified. All cell lines were cultured in a 37 °C humidified atmosphere containing 95% air and 5% CO_2 and were split twice a week.

2.3. Harvest of conditioned medium

For harvest of conditioned medium, human UC-MSCs were plated onto 100-mm dishes with full culture medium. Cells were refreshed with DMEM/Low glucose alone without FBS and cultured for additional 48 h. Supernate was harvested and centrifuged brifely for removing of cell debris. The supernate were then transferred to Amicon® Ultra-15 3K (Merk Millipore Ltd., Darmstadt, Germany) and centrifuged at 5000 rpm for 30 min at 4 °C. Conditioned medium of hUC-MSCs were stored at −80 °C.

2.4. Cell viability assay

The CellTiter96AQ cell proliferation kit was used to determine cell viability as we previously described [13]. For co-culture assays, a transwell system with permeable membrane inserts with 0.4-μm pores (Corning, NY, USA) was used. MDA-MB-453 cells were plated onto 24-well plates with 0.7 ml medium with 10% FBS at a density of 1.5×10^4 cells/well. After 24 h, cells were refreshed with either 0.7 ml medium with 0.5% FBS as control, or 0.7 ml of the same medium containing a series doses of paclitaxel alone, or 0.7 ml of the same medium containing a series doses of paclitaxel in combination with NRG-1 (50 ng/mL). For co-culture experiment, 1×10^4 of human UC-MSCs were suspended in 0.3 ml of DMEM/Low glucose with 0.5% FBS containing a series doses of paclitaxel and added in the upper culture chambers. Cells were incubated in a 37 °C humidified atmosphere containing 95% air and 5% CO_2 for 72 h. The percentages of surviving cells from each group relative to controls, defined as 100% survival, was determined by reduction of MTS following by staining with 0.5% crystal violet for visualization of viable cells.

2.5. Analysis of mRNA expression with conventional regular or real-time quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA was prepared using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was generated using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's instructions. The analysis of human *NRG-1* mRNA expression was examined by conventional RT-PCR as we had described previously [14]. The qRT-PCR was performed to quantify the mRNA expression levels of *Cyclin D1*, *Cyclin E1*, *p21*, *p27* and *Survivin* as we described previously [14]. Sequences of specific primers used are listed in Supplementary Table S1.

2.6. Western blotting analysis and quantification of apoptosis

Protein expression and activation were determined by western blotting analysis as previously described [10]. An apoptosis enzyme-linked immunosorbent assay kit (Roche Diagnostics Corp., Indianapolis, IN, USA) was used for quantification of apoptosis as previously reported [10].

Please cite this article in press as: J. Chen, et al., Mesenchymal stem cells drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells via paracrine of neuregulin 1, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.218