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

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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.

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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].

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2.7. Construction of lentiviral expression vector pLKO.1-NRG-1-shRNA

The pLKO.1-TRC was used for cloning and expressing short hairpin RNA (shRNA) sequences targeting human NRG-1. A 1.9-kb stuffer in pLKO.1-TRC was released by digestion with restriction enzymes *Age* I and *Eco*R I. The synthesized DNA templates of shRNA (forward: 5′-CCGGCCACAGAAGGAGCAAATACTTCTCGA-GAAGTATTTGCT CCTTCTGTGGTTTTTG-3'; reverse: 5′-AATT-CAAAAACCACAGAAGGAGCAAATACT TCTCGA-GAAGTATTTGCTCCTTCTGTGG-3′) were annealed and then subcloned into the space between the *Age* I and *Eco*R I sites to construct pLKO.1-NRG-1-shRNA. The constructed vector was verified by direct DNA sequencing.

2.8. Production of lentivirus

Lentiviral production was performed as described [13]. The virus-containing media were collected, aliquot and stored at −80 °C.

2.9. Specific knockdown of ErbB3 or NRG-1 expression with a lentiviral system

To obtain specific knockdown of ErbB3 expression in human breast cancer cell MDA-MB-453, or NRG-1 expression in human UC-MSC, corresponding shRNA-expressing lentivirus were used. Before infection, the lentivirus-containing media were thawed completely at room temperature and mixed with fresh medium and polybrene (8 mg/ml). Then the culture media of the candidate cells were replaced with the lentivirus-containing media. After 24 h, the virus-infected cells were selected with puromycin (1 mg/ml) for 48 h, and then collected and subjected to required experiments.

2.10. Statistical analysis

All results were confirmed by at least three independent experiments. Data are presented as mean ± SD. Tow-sided Student's *t*-tests were used for comparisons of means of quantitative data between groups. Values of $P < 0.05$ were considered significant.

2.11. Study approval

The two different human umbilical cords were acquired with informed consent following protocols approved by Fuzhou General Hospital of Xiamen University Research Ethics Board.

3. Results

3.1. Mesenchymal stem cells drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cell without affecting its proliferation rate

To investigate the possible role of MSCs in paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cell, the effect of conditioned medium of UC-MSCs_002 (CM_UC-MSCs) on paclitaxel sensitivity of MDA-MB-453 was firstly studied. Our cell viability assay showed that treatment with CM_UC-MSCs significantly decreased paclitaxel-mediated growth inhibition in MDA-MB-453, an effect which could be achieved by rNRG1-β1 (Fig. 1A). At the same time, cocultured with UC-MSCs was also showed to decrease the paclitaxel sensitivity of MDA-MB-453 (Fig. 1B and C). Furthermore, we found that treatment with CM_UC-MSCs could significantly decrease the paclitaxel-induced apoptosis in MDA-MB-453 (Fig. 1D and E). However, whether coculture with UC-MSCs or treatment with CM_UC-MSCs did not show much effect on the proliferation rate of MDA-MB-453 (Fig. 1F). Collectively, these data indicate that MSCs drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cell without altering its proliferation rate.

3.2. Identification of expression of NRG-1 in human mesenchymal stem cells

Two mesenchymal stem cell lines (UC-MSCs_002 and UC-MSCs_016) derived from different human umbilical cords were established and maintained in our own lab. As shown in Fig. 2A, the mRNA expression levels of *NRG-1* in two mesenchymal stem cell lines were relatively high. As compared, the mRNA expression of *NRG-1* cannot even be detected in MDA-MB-453, a human breast cancer cell line whose coexpression of ErbB2 and ErbB3 has been repeatedly reported by many studies including our own [10]. Meanwhile, the protein expression of NRG-1 in both UC-MSCs_002 and UC-MSCs_016 was also shown (Fig. 2B). More importantly, our western blotting analysis indicated that the soluble NRG-1 was present in conditioned medium of both UC-MSCs_002 and UC-MSCs_016 (Fig. 2C).

3.3. Treatment with CM_UC-MSCs results in upregulation of Survivin in ErbB2/ErbB3- coexpressing breast cancer cell via activation of PI-3K/Akt signaling without alteration of cell cycle regulators

We have previously reported that elevated expression of ErbB3 results in paclitaxel resistance in ErbB2-overexpressing breast cancer cells via PI-3K/Akt signaling pathway-dependent upregulation of Survivin [10]. Thus, to explore the mechanism by which MSCs drive paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cell, we first examined the effect of CM_UC-MSCs on PI-3K/Akt signaling as well as the expression of Survivin in MDA-MB-453. As anticipated, CM_UC-MSCs did markedly activate the ErbB2/ErbB3 → PI-3K/Akt signaling in MDA-MB-453, which in turn results in upregulation of protein but not mRNA level of Survivin (Fig. 2D and E). Interestingly, this effect could be mimicked or completely abrogated by rNRG1-β1 or antibody against NRG-1 (Anti-NRG1-β1), respectively (Fig. 2D). The MAPK signaling plays a key role in regulating fundamental cellular processes such as proliferation. Consistent with our aforementioned finding that MSCs did not affect the proliferation rate of ErbB2/ErbB3-coexpressing breast cancer cell (Fig. 1F), treatment with either CM_UC-MSCs or rNRG1-β1 did not show any effect on not only the MAPK signaling but also the proteins and mRNAs levels of cell cycle regulators such as Cyclin D1, Cyclin E1, p21, and p27 (Fig. 2D–F). Taken together, our data demonstrate that the paracine of NRG-1 by MSCs elicits a PI-3K/Akt signaling pathway-dependent mechanism to upregulate Survivin, which is essential for MSCs to decrease paclitaxel-mediated cytotoxicity in ErbB2/ErbB3-coexpressing breast cancer cells.

3.4. Specific blocking of ErbB2/ErbB3 → PI-3K/Akt → Survivin signaling reverses MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells

To further clarify the role of ErbB2/ErbB3 → PI-3K/Akt → Survivin signaling in MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells, the knockdown expression of ErbB3 in MDA-MB-453 was first achieved with a lentiviral system (Fig. 3A). While treatment with CM_UC-MSCs or rNRG1-β1 still significantly decreased paclitaxel-mediated growth inhibition in

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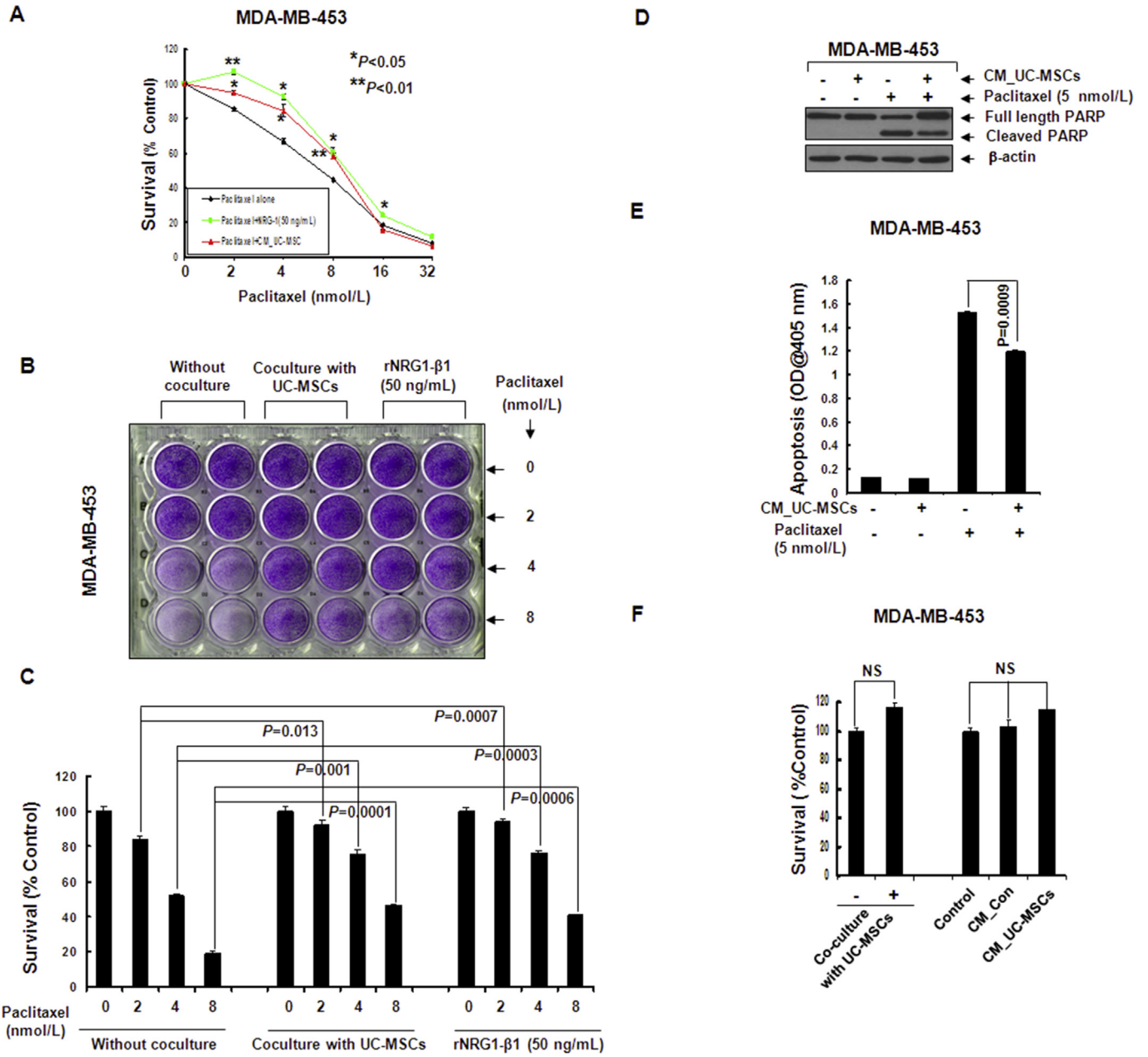

Fig. 1. Effect of human umbilical cord-derived mesenchymal stem cells (UC-MSCs) on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. (A) Cell viability assay of MDA-MB-453 upon treatment as indicated for 72 h. (B, C) Visualization of surviving MDA-MB-453 cells upon treatment as indicated for 72 h by cell staining with crystal violet (B) following by cell viability assay (C). (D, E) Western blotting analysis of PARP cleavage (D) and quantification of apoptosis with apoptotic-ELISA (E). (F) Cell viability assay of MDA-MB-453 upon treatment as indicated for 72 h. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

MDA-MB-453 infected with control lentivirus, this effect could be reversed by specific knockdown expression of ErbB3 (Fig. 3B). Moreover, knockdown expression of ErbB3 was also showed to abrogate the MSCs-induced decreasing of the paclitaxel sensitivity of MDA-MB-453 (Fig. 3C and D). Since the expression of Survivin in MDA-MB-453 with knockdown expression of ErbB3 could not be upregulated by CM_UC-MSCs or rNRG1-β1 (Fig. 3E), it was rational that treatment with CM_UC-MSCs could no longer decrease the paclitaxel-induced apoptosis (Fig. 3F and G). In addition, specific blocking of downstream PI-3K/Akt→Survivin signaling either with PI-3K inhibitor (LY294002), Akt inhibitor (Akti), or Survivin inhibitor (YM155) was able to antagonizes MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells (Fig. 3H–J). Thus, these data indicate that specific blocking of ErbB2/ErbB3→PI-3K/Akt→ Survivin signaling could reverse MSCs-drived paclitaxel resistance in ErbB2/ErbB3- coexpressing breast cancer cells.

3.5. Targeted knockdown of NRG-1 expression in UC-MSCs abrogates theirs effect on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cell

Given our findings here that paracrine of NRG-1 by MSCs induces resistance of paclitaxel in ErbB2/ErbB3-coexpressing breast cancer cells through activation of ErbB2/ErbB3 →PI-3K/Akt signaling, to explore whether simultaneously targeting mesenchymal stem cells might overcome paclitaxel resistance in breast cancer cells, lentiviral system was also used to achieve specific

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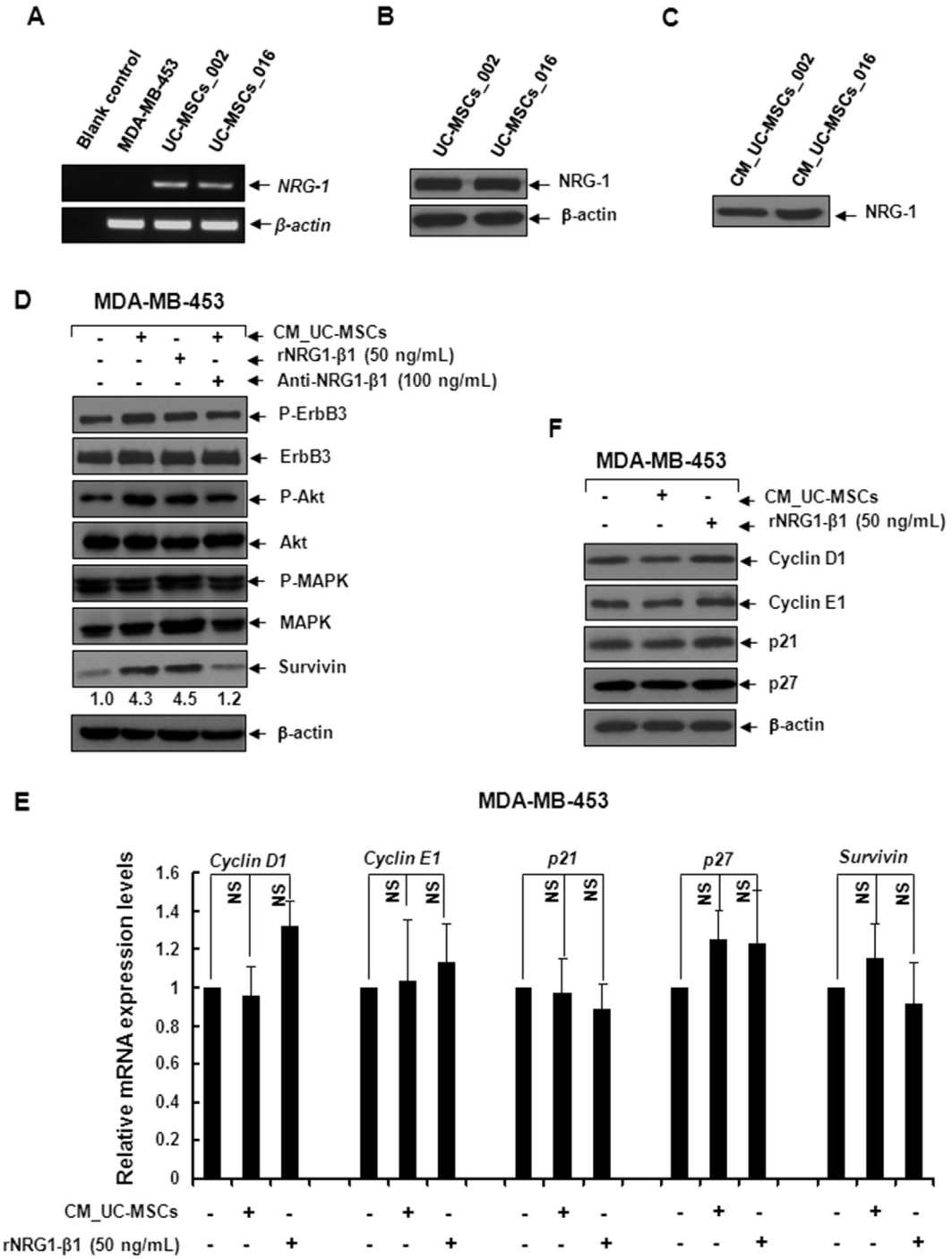

Fig. 2. Upregulation of Survivin in ErbB2/ErbB3-coexpressing breast cancer cells by CM_UC-MSCs via activation of ErbB2/ErbB3 signaling without alteration of cell cycle regulators. (A) Analysis of *NRG-1* mRNA expression by conventional RT-PCR. (B) Analysis of NRG-1 protein expression in human UC-MSCs by western blotting. (C) The present of NRG-1 in CM_UC-MSCs analyzed with western blotting. (D) Western blot analysis of relevant proteins in MDA-MB-453 cells upon treatment as indicated for 24 h. The densitometry analyses of Survivin signals were shown underneath, and the arbitrary numbers indicate the intensities of each cell line relative to controls, defined as 1.0. (E) qRT-PCR analysis of mRNAs levels of *Cyclin D1*, *Cyclin E1*, *p21*, *p27*, and *Survivin* in MDA-MB-453 cells upon treatment as indicated for 24 h. (F) Western blot analysis of the proteins levels of Cyclin D1, Cyclin E1, p21, and p27 in MDA-MB-453 cells upon treatment as indicated for 24 h.

knockdown of NRG-1 expression in UC-MSCs (Fig. 4A). Importantly, decreased paclitaxel sensitivity of MDA-MB-453 induced by coculture with UC-MSCs was completely overcome by targeted knockdown of NRG-1 expression (Fig. 4B and 4C). These preliminary data suggest that MSCs in tumor microenvironment may act as a candidate target in developing combinatorial treatment against ErbB2/ErbB3-coexpressing breast cancer.

4. Discussion

ErbB2 is one of the most well characterized ErbB receptor tyrosine kinase in breast carcinogenesis. Its overexpression has been implicated in the genesis and progression of a subset of breast and ovarian tumors [15]. ErbB3 has been shown to serve as a critical co-receptor of ErbB2, and its expression is a rate-limiting factor for

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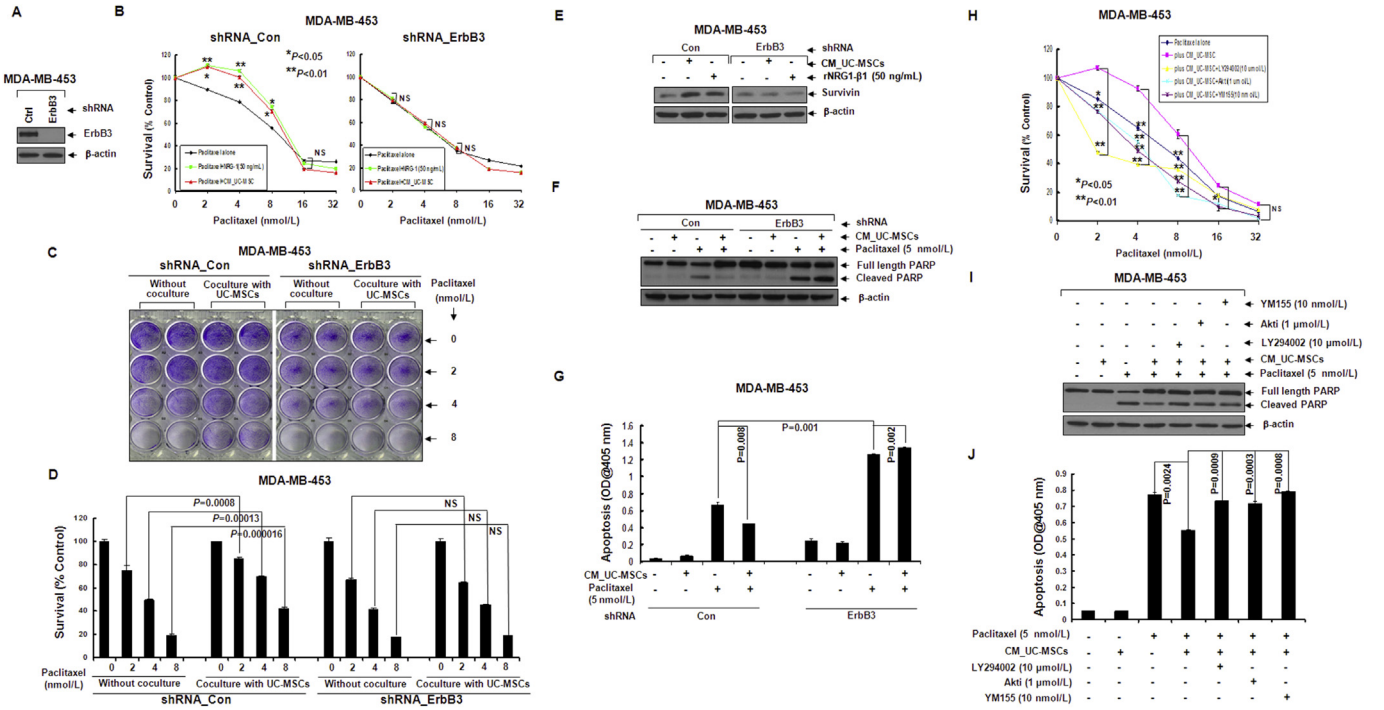

Fig. 3. Effects of blocking of ErbB2/ErbB3→PI-3K/Akt→Survivin signaling on MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells. (A) Western blot analysis of knockdown of ErbB3 expression in MDA-MB-453 cells. (B) Cell viability assay of MDA-MB-453 without or with knockdown of ErbB3 expression upon treatment as indicated for 72 h. (C, D) Visualization of surviving MDA-MB-453 cells without or with knockdown of ErbB3 expression upon treatment as indicated for 72 h by cell staining with crystal violet (C) following by cell viability assay (D). (E) Western blotting analysis of Survivin expression in MDA-MB-453 cells without or with knockdown of ErbB3 expression upon treatment as indicated for 24 h. (F, G) Western blotting analysis of PARP cleavage (F) and quantification of apoptosis with apoptotic-ELISA (G). (H) Cell viability assay of MDA-MB-453 upon treatment as indicated for 72 h. (I, J) Western blotting analysis of PARP cleavage (I) and quantification of apoptosis with apoptotic-ELISA (J). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ErbB2-mediated breast cancer cell survival and proliferation [16]. In fact, the ErbB2/ErbB3 heterodimer has been identified as the most potent form of all ErbB receptor complexes to activate the oncogenic signalings in breast cancers, such as PI-3K/Akt cascades, a key pathway that induces resistance to ErbB2-targeted therapy [17]. We have previously demonstrated that elevated expression of ErbB3, mainly through activation of PI-3K/Akt signaling, plays a critical role in ErbB2/ErbB3-mediated therapeutic resistance to paclitaxel [10]. In this study, our data further indicate that paracrine of NRG-1 by MSCs may play important role in activation of ErbB2/ErbB3→PI-3K/Akt signaling and is required for upregulation of Survivin-mediated resistance to paclitaxel in ErbB2/EerbB3-coexpressing breast cancer cells.

Neuregulins represent the largest subclass of ErbB/HER receptor ligands and bind specifically to ErbB3 and ErbB4. The neuregulins are produced as transmembrane ligands that can be released as soluble factors by the action of cell surface proteases [18]. NRG-1 and neuregulin 2 (NRG-2) are both ErbB3 ligands. NRG-1 has a higher binding affinity and preferentially acts through ErbB2-dependent recruitment of PI-3K [19]. Increasing evidence indicates that neuregulins may play a relevant role in breast cancer [20]. Expression of transmembrane neuregulins in breast cancer cells activates ErbB2 and favors their proliferation *in vitro* [21], while blockage of heregulin expression inhibits tumorigenicity and metastasis of breast cancer [22]. Although many studies suggested that cancer cells themselves exhibit autocrine production of neuregulin, there is also study showed that bone marrow-derived MSCs promote invasion, survival and tumorigenesis of colorectal cancer cells through paracrine NRG-1/ErbB3 signaling [23]. Interestingly, we found that the expression of NRG-1 in human MSCs is relatively high as compared with ErbB2/EerbB3- coexpressing breast cancer cells (Fig. 2A), which suggesting that paracrine effect of NRG-1 may play a more important role in causing activation of ErbB3 signaling than autocrine.

The tumour microenvironment is emerging as important player in regulating the tumor's response to therapies. Several studies have shown that MSCs can induce tumour cell resistance to chemotherapy [24]. While MSCs derived from multiple myeloma patients were showed to protect against chemotherapy through autophagy-dependent activation of NF-κB signaling by Yang et al. [25], targeting autophagy was also showed to overcome chemoresistance in acute myeloid leukemia [26]. In our current study, we found that 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 (Fig. 1). 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 (Fig. 2), as either specific knockdown expression of ErbB3 (Fig. 3A–G), or blocking of downstream PI-3K/Akt signaling, or specific inhibition of Survivin can completely reverse this effect (Fig. 3H–J). Moreover, targeted knockdown of NRG-1 expression in MSCs abrogates theirs effect on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells (Fig. 4A–C). Since exosomes are major players in inter- and intra-cellular communication [27], whether the induced paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells was caused by MSCs-derived exosome would be of particular interest and await further investigation.

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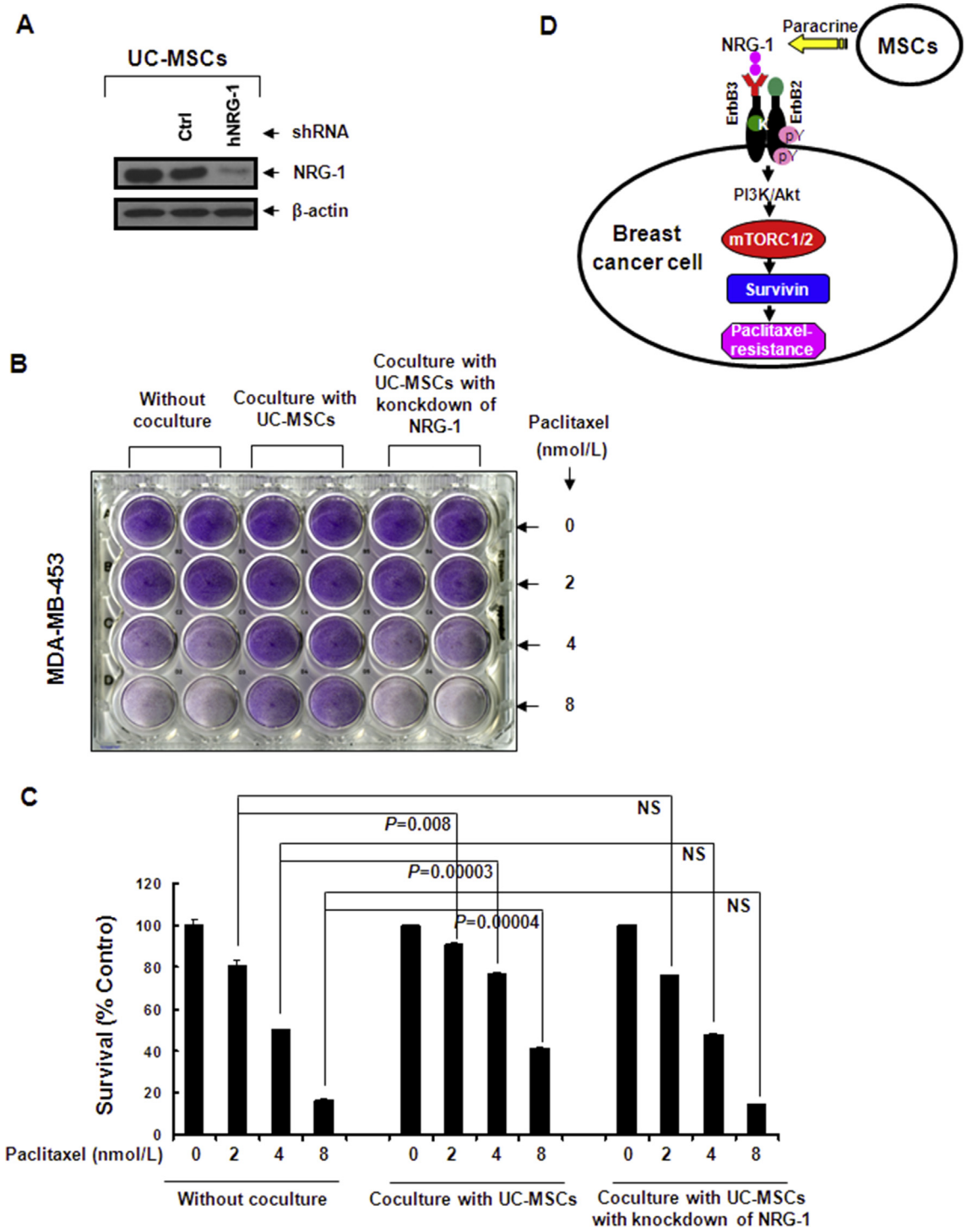

Fig. 4. Specific knockdown of NRG-1 expression in UC-MSCs abrogates theirs effect on paclitaxel sensitivity of ErbB2/ErbB3-coexpressing breast cancer cells. (A) Western blot analysis of knockdown of NRG-1 expression in UC-MSCs. (B, C) Visualization of surviving MDA-MB-453 cells upon treatment as indicated for 72 h by cell staining with crystal violet (B) following by cell viability assay (C). (D) Diagram of proposed model underlying the mechanism of MSCs-drived paclitaxel resistance in ErbB2/ErbB3-coexpressing breast cancer cells. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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 (Fig. 4D). 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.

Acknowledgements

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Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.04.218.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.04.218.

References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA A Cancer J. Clin. 65 (2015) 87–108.
[2] P.G. Morris, M.N. Fornier, Novel anti-tubulin cytotoxic agents for breast cancer, Expert Rev. Anticancer Ther. 9 (2009) 175–185.
[3] A. Eniu, F.M. Palmieri, E.A. Perez, Weekly administration of docetaxel and paclitaxel in metastatic or advanced breast cancer, Oncol. 10 (2005) 665–685.
[4] C. Holohan, S. Van Schaeybroeck, D.B. Longley, P.G. Johnston, Cancer drug resistance: an evolving paradigm, Nat. Rev. Canc. 13 (2013) 714–726.
[5] G.A. Orr, P. Verdier-Pinard, H. McDaid, S.B. Horwitz, Mechanisms of Taxol resistance related to microtubules, Oncogene 22 (2003) 7280–7295.
[6] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[7] J. Stagg, Mesenchymal stem cells in cancer, Stem Cell Rev. 4 (2008) 119–124.
[8] D.W. McMillin, J.M. Negri, C.S. Mitsiades, The role of tumour-stromal interactions in modifying drug response: challenges and opportunities, Nat. Rev. Drug Discov. 12 (2013) 217–228.
[9] Y. Shi, L. Du, L. Lin, Y. Wang, Tumour-associated mesenchymal stem/stromal cells: emerging therapeutic targets, Nat. Rev. Drug Discov. 16 (2017) 35–52.
[10] S. Wang, X. Huang, C.K. Lee, B. Liu, Elevated expression of erbB3 confers paclitaxel resistance in erbB2-overexpressing breast cancer cells via upregulation of Survivin, Oocogene 29 (2010) 4225–4236.
[11] S. Wang, J. Huang, H. Lyu, B. Cai, X. Yang, F. Li, J. Tan, S.M. Edgerton, A.D. Thor, C.K. Lee, B. Liu, Therapeutic targeting of erbB3 with MM-121/SAR256212 enhances antitumor activity of paclitaxel against erbB2-overexpressing breast cancer, Breast Canc. Res. 15 (2013). R101.
[12] J. Cai, Z. Wu, X. Xu, L. Liao, J. Chen, L. Huang, W. Wu, F. Luo, C. Wu, A. Pugliese, A. Pileggi, C. Ricordi, J. Tan, Umbilical cord mesenchymal stromal cell with autologous bone marrow cell transplantation in established type 1 diabetes: a pilot randomized controlled open-label clinical study to assess safety and impact on insulin secretion, Diabetes Care 39 (2016) 149–157.
[13] S. Wang, L. Zhu, W. Zuo, Z. Zeng, L. Huang, F. Lin, R. Lin, J. Wang, J. Lu, Q. Wang, L. Lin, H. Dong, W. Wu, K. Zheng, J. Cai, S. Yang, Y. Ma, S. Ye, W. Liu, Y. Yu, J. Tan, B. Liu, MicroRNA-mediated epigenetic targeting of Survivin significantly enhances the antitumor activity of paclitaxel against non-small cell lung cancer, Oncotarget 7 (2016) 37693–37713.
[14] S. Wang, J. Huang, H. Lyu, C.K. Lee, J. Tan, J. Wang, B. Liu, Functional cooperation of miR-125a, miR-125b, and miR-205 in entinostat-induced downregulation of erbB2/erbB3 and apoptosis in breast cancer cells, Cell Death Dis. 4 (2013) e556.
[15] D.J. Slamon, G.M. Clark, S.G. Wong, W.J. Levin, A. Ullrich, W.L. McGuire, Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, Science 235 (1987) 177–182.
[16] T. Holbro, R.R. Beerli, F. Maurer, M. Koziczak, C.F. Barbas, N.E. Hynes, The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation, Proc. Natl. Acad. Sci. Unit. States Am. 100 (2003) 8933–8938.
[17] D. Yu, M.C. Hung, Overexpression of ErbB2 in cancer and ErbB2-targeting strategies, Oncogene 19 (2000) 6115–6121.
[18] J.C. Montero, L. Yuste, E. Diaz-Rodriguez, A. Esparís-Ogando, A. Pandiella, Differential shedding of transmembrane neuregulin isoforms by the tumor necrosis factor-alpha-converting enzyme, Mol. Cell. Neurosci. 16 (2000) 631–648.
[19] C.S. Crovello, C. Lai, L.C. Cantley, K.L. Carraway, Differential signaling by the epidermal growth factor-like growth factors neuregulin-1 and neuregulin-2, J. Biol. Chem. 273 (1998) 26954–26961.
[20] D.N. Amin, D. Tuck, D.F. Stern, Neuregulin-regulated gene expression in mammary carcinoma cells, Exp. Cell Res. 309 (2005) 12–23.
[21] E.J. Weinstein, P. Leder, The extracellular region of heregulin is sufficient to promote mammary gland proliferation and tumorigenesis but not apoptosis, Cancer Res. 60 (2000) 3856–3861.
[22] E. Atlas, M. Cardillo, I. Mehmi, H. Zahedkargaran, C. Tang, R. Lupu, Heregulin is sufficient for the promotion of tumorigenicity and metastasis of breast cancer cells in vivo, Mol. Canc. Res. 1 (2003) 165–175.
[23] A. De Boeck, P. Pauwels, K. Hensen, J.L. Rummens, W. Westbroek, A. Hendrix, D. Maynard, H. Denys, K. Lambein, G. Braems, C. Gespach, M. Bracke, O. De Wever, Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression through paracrine neuregulin 1/HER3 signalling, Gut 62 (2013) 550–560.
[24] F. Klemm, J.A. Joyce, Microenvironmental regulation of therapeutic response in cancer, Trends Cell Biol. 25 (2015) 198–213.
[25] H. Yang, Y. Zheng, Y. Zhang, Z. Cao, Y. Jiang, Mesenchymal stem cells derived from multiple myeloma patients protect against chemotherapy through autophagy-dependent activation of NF-kappaB signaling, Leuk. Res. 60 (2017) 82–88.
[26] S. Piya, M. Andreeff, G. Borthakur, Targeting autophagy to overcome chemoresistance in acute myleogenous leukemia, Autophagy 13 (2017) 214–215.
[27] R. Ji, B. Zhang, X. Zhang, J. Xue, X. Yuan, Y. Yan, M. Wang, W. Zhu, H. Qian, W. Xu, Exosomes derived from human mesenchymal stem cells confer drug resistance in gastric cancer, Cell Cycle 14 (2015) 2473–2483.

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