

Interaction of adipose-derived mesenchymal stem cells with MCF-7 cells in vitro: a study emphasizing signaling molecule expression and transcriptional changes

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Abstract: Adipose-derived stem cells (ADSCs) promote metastasis of breast cancer cells that can differentiate into carcinoma-associated cells in tumor microenvironments. However, the precise mechanism is poorly understood. This study shows that interaction of ADSCs with breast cancer MCF-7 cells changes the level of metastasis-related functional proteins in MCF-7 cells, as well as that of oncogenes in ADSCs. ADSCs were isolated from adipose tissues of patients. The interaction of ADSCs with MCF-7 cells was performed by coculturing ADSCs (or MCF-7 cells) with exosomes derived from MCF-7 cells (or ADSCs). Exosomes were labeled by DiI. In cocultures, migration-related regulators in MCF-7 cells were significantly enhanced in both protein (Smad, Slug, Snail1/2, Twist1, N-cadherin, vimentin) and mRNA (*SMAD*, *SLUG*, *SNAIL1/2*, *TWIST1*, *N-cadherin*, and *vimentin*) levels. The expression levels of oncogenes (*RAS* and *HER-2*) and an antioncogene gene (*P53*) in ADSCs were upregulated and downregulated, respectively. Interestingly, variation tendencies of the molecules were more conspicuous in inflammatory circumstances than without. In conclusion, interaction of MCF-7 cells with ADSCs increases the levels of a series of metastasis-related functional proteins in MCF-7 cells and enhances the expression of oncogenes in ADSCs.

Key words: Adipose-derived stem cells, metastasis, functional proteins, oncogene

1. Introduction

Adipose-derived mesenchymal stem cells (ADSCs) are a heterogeneous population of adult stem cells derived from adipose tissue. ADSCs functionally differentiate into various cell lineages in a special microenvironment (Uzbas et al., 2015). ADSCs are autologous, available in sufficient quantities in most patients, and are widely used in soft tissue repair, for instance, in breast reconstruction in surgical patients (Rowan et al., 2014). However, recent papers indicate ADSCs potentially induce a tumor microenvironment and affect tumor cell biology. In mammary tissue (Muehlberg et al., 2009), resident stem cells promote breast cancer metastasis, home to the tumor site, and stimulate tumor growth not only when coinjected locally but also when injected intravenously. Kucerova et al. (2011) reported that ADSCs could stimulate proliferation of several breast cancer cells in vitro, such as MDA-MB-361, T47D, and EGFP-MCF-7. Kim et al. (2013) found that breast and abdominal adipose tissues have comparable gene expression profiles and are comparable in supporting breast cancer cell proliferation and metastasis. On the other hand, it has been previously

reported that the morphology and phenotype of ADSCs are altered when cocultured with tumor cells. ADSCs (Jotzu et al., 2011) that received tumor-derived factors differentiated into carcinoma-associated fibroblast-like cells, which functionally contribute to tumor growth, invasion, and metastasis. ADSCs directly cocultured with H358 lung cancer cells differentiated into tumor stroma that play a supportive role during cancer progression (Park et al., 2013).

Of note, except for the diffusible factors (cytokines, chemokine, and growth factors), cell-derived exosomes have been described as a new signal in cell-to-cell communication. Exosomes are membrane vesicles (30–100 nm) from luminal membranes of multivesicular bodies and are constitutively released upon fusion with cell membranes (Tan et al., 2016). It has been previously confirmed that ADSCs secrete exosomes (Cho et al., 2012; Lin et al., 2013), promote MCF-7 cell migration, and that exosomes from breast cancer cells convert ADSCs into myofibroblast-like cells. These results provide evidence that exosomes play a role in crosstalk between ADSCs and MCF-7 cells.

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Epithelial to mesenchymal transition (EMT) is involved in tumor cell metastasis and serves as the first step in the migration of tumor cells (Kumar et al., 2015). During EMT formation, numerous functional intracellular signaling pathways (TGF β -Smad) and functional proteins, such as *SMAD*, *SLUG*, *SNAIL1/2*, *TWIST1*, *N-cadherin*, and *vimentin*, are significantly disturbed (Yamada et al., 2014). *P53*, *HER-2*, and *RAS* are well known for their roles in regulation of cell growth, differentiation, and survival, and are usually considered classical markers of carcinoma cells (Shin et al., 2013). However, the mechanisms of communication of ADSCs and tumor cells are still unclear. The aim of this study was to investigate signal molecular alteration of ADSCs (or MCF-7 cells) in the presence of exosomes derived from MCF-7 cells (or ADSCs).

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS) and polyinosinic: polycytidylic acid (poly (I:C)) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies for Slug (sc-166476), Snail1/2 (sc-393172), Smad (sc-10486), Twist1 (sc-6269), E-cadherin (sc-71008), N-cadherin (sc-53488), vimentin (sc-373717), P53 (sc-47698), HER-2 (sc-33684), and RAS (sc-271436) were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Primary antibodies for CD29 (ab58603), CD90 (ab92574), CD34 (ab8536), and CD45 (ab40763) were purchased from Abcam (Cambridge, MA, USA). Secondary antibodies (goat antimouse/BA1050, goat antirabbit/BA1054) were purchased from Boster, Ltd. (Wuhan, China). All chemicals used in the present study were of analytical grade.

2.2. Cell culture

ADSCs were prepared according to the method described previously (Lin et al., 2013). This study was approved by the ethics committee of the affiliated Zhujiang Hospital of Southern Medical University (Guangzhou, China). Informed consent was obtained from all the participants. Briefly, ADSCs were isolated from adipose tissues of patients undergoing tumescent liposuction. The cells were cultured in 75 cm² culture flasks and incubated in a humidified environment containing 5% CO₂ at 37 °C. Passage 0 ADSCs were cultured using regular growth medium, which contained 58% Dulbecco's modified Eagle's medium/Ham's F-12 medium (Gibco, Grand Island, NY, USA), 40% medium complete with trace elements-201 (Gibco), 2% fetal bovine serum (Gibco), 1 $\times$ 10⁹ mol/L insulin-transferrin-selenium (Gibco), 1 $\times$ 10⁹ mol/L dexamethasone, 1 $\times$ 10⁹ mol/L ascorbic acid-2-phosphate, 20 ng/mL IL-6, 10 ng/mL epidermal growth factor, 10 ng/mL platelet-derived growth factor BB (all from Sigma-Aldrich), 100 U/mL penicillin, and 100 mg/mL streptomycin. Passage 5 cells were used for the following study.

MCF-7 breast cancer cells were purchased from Cancer Institute of Chinese Academy of Medical Sciences (Beijing, China). MCF-7 cells were cultured in complete DMEM containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 U/mL) at 37 °C in air with 5% CO₂.

2.3. Flow cytometric analysis of CD surface markers on ADSCs

For flow cytometric analysis of phenotype in ADSCs, cells in passage 5 were treated with 0.05% trypsin-0.53 mM EDTA, and washed twice with PBS. Cell aliquots (1 $\times$ 10⁵ cells/100 μ L) were stained with primary-conjugated antibodies at room temperature for 30 min. The conjugated antibodies used for these experiments were PE-conjugated antihuman CD29, CD34, CD45, and CD90. Appropriate fluorescein- or phycoerythrin- conjugated isotypes were used as a control for each assay. Flow cytometric analysis used a flow cytometer (FACSCalibur; Becton-Dickinson, San Jose, CA, USA). The results were processed with Cell Quest Software (Becton-Dickinson) for statistical analyses.

2.4. Exosome extraction and quantification

Exosomes were prepared according to the methods published previously (Singh et al., 2014). ADSC (or MCF-7) culture medium (48 h) was collected and centrifuged at 500 $\times$ g for 10 min and then recentrifuged at 2000 $\times$ g for 20 min to remove cells. The supernatant was subjected to filtration on a 0.1- μ m-pore polyethersulfone membrane filter (Corning, Inc., Corning, NY, USA) to remove cell debris and large vesicles, followed by concentration by a 100,000 Mw cut-off membrane (CentriPlus-70; Millipore, Billerica, MA, USA). The supernatant volume was reduced from approximately 250–500 mL to 25 mL. Subsequently, the supernatant was filtered using a 0.22 μ m filter (Millipore), and condensed fluids were ultracentrifuged at 100,000 $\times$ g for 1 h at 4 °C using a 70Ti rotor (Beckman Coulter, Brea, CA, USA). Finally, the pellets were resuspended in PBS and ultracentrifuged at 100,000 $\times$ g for another 1 h at 4 °C using a 100Ti rotor (Beckman Coulter). Purified exosomes were labeled with 1 M DiI (Invitrogen, Carlsbad, CA, USA) as previously described (Hood et al., 2009). The exosomes were washed to remove unbound DiI and then resuspended in PBS for further use.

The number of exosomes was quantified to determine the protein concentration of exosomes. Exosomal proteins from the supernatant of the cells after exosomal isolation were quantified using the Bradford method. In particular, the actual protein concentrations were calculated using the standard curve equation and the appropriate dilutions.

2.5. Interaction of ADSCs with MCF-7 cells

To study the interaction of ADSCs with MCF-7 cells during tumor progression under inflammatory environments, LPS and poly (I:C), which stimulate cells that produce

proinflammatory cytokines, were used to activate ADSCs and MCF-7 cells. Cocultured groups in vitro were set as following: MCF-7 cells, MCF-7 cells cultured with ADSC-secreted exosomes (100 µg/mL, 200 µg/mL), MCF-7 cells cultured with ADSC exosomes stimulated by 5 µg/mL LPS (100 µg/mL, 200 µg/mL), MCF-7 cells cultured with ADSC exosomes treated with 20 µg/mL poly (I:C) (100 µg/mL, 200 µg/mL), ADSCs, ADSCs cultured with MCF-7-released exosomes (100 µg/mL, 200 µg/mL), ADSCs cultured with exosomes from LPS-stimulated MCF-7 cells (100 µg/mL, 200 µg/mL), ADSCs cultured with exosomes from poly (I:C)-stimulated MCF-7 cells (100 µg/mL, 200 µg/mL). The time for coculture was designated as 48 h.

2.6. Western blotting

Cultured medium was removed and residual cells were washed 3 times with PBS. Subsequently, the cells were mixed with lysates for 30 min, and the lysates were centrifuged at 12,000 rpm for 15 min to separate the supernatant. The protein levels in the supernatant were quantified by BCA Protein Kit (Thermo Scientific, Waltham, MA, USA). Western blotting analysis (Kruger et al., 2014) was performed to evaluate the levels of target proteins. Briefly, the samples were separated in SDS polyacrylamide gels. The membranes (Millipore) were incubated with primary antibodies followed by shaking overnight at 4 °C. The following antibodies and concentrations were used: 1:1000 (Smad), 1:1000 (Slug), 1:1000 (Snail1/2), 1:1000 (Twist1), 1:2500 (E-cadherin), 1:2500 (N-cadherin),

1:1000 (vimentin), 1:1000 (RAS), 1:1000 (P53), and 1:1000 (HER-2). After that, the membranes were incubated with corresponding secondary antibodies (Boster, Ltd.) for another 2 h at room temperature. Finally, immunodetection was visualized by a chemiluminescent ECL reagent (Pierce Biotechnology, Inc., Waltham, MA, USA). Three independent experiments were performed, and average results were recorded. The quantification of proteins was analyzed by IPP software (Media Cybernetics, Rockville, MD, USA).

2.7. Real-time quantitative polymerase chain reaction

Total RNA was extracted from the cells by TRIzol reagent (Invitrogen) according to the instructions. RNA levels were evaluated by real-time reverse-transcription polymerase chain reaction (RT-PCR) with the SYBR Prime Script RT-PCR kit (Takara, Kusatsu, Japan). The PCR primers designed by Sangon Biotech (Shanghai, China) are listed in the Table. β -Actin was used to normalize gene expression in each experiment. PCR efficiency was first determined by β -actin to ensure that the normalizing genes were acceptable and to test primer efficiency. Relative levels of gene expression (amount of target gene normalized to the endogenous control gene) were calculated using the comparative Ct method formula $2^{-\Delta\Delta CT}$ offered by Bio-Rad CFX Manager Software 1.6.

2.8. Statistical analysis

Data are presented as means $\pm$ SE. Comparisons among groups were carried out using one-way analysis of variance

Table. Primer sequences used.

Gene	Gene ID	Primer	Primer sequence
<i>SLUG</i>	NM_003068.4	Forward	agaccccatgccattgaag
		Reverse	cttctccccgtgtgagttc
<i>SNAIL1/2</i>	NM_005985.3	Forward	acccacatccttctcactg
		Reverse	tacaaaaaccacgcagaca
<i>SMAD2</i>	NM_001003652.3	Forward	atccagcagattctgggatg
		Reverse	aggatggcccagagaatctt
<i>TWIST1</i>	NM_000474.3	Forward	gtccgcagtcttacgaggag
		Reverse	ccagcttgagggtctgaatc
<i>E-cadherin</i>	NM_004360.3	Forward	tgcccagaaaatgaaaagg
		Reverse	gtgtatgtggcaatgcgttc
<i>N-cadherin</i>	NM_001308176.1	Forward	gacaatgcccctcaagtgtt
		Reverse	ccattaagccgagtgatggt
<i>Vimentin</i>	NM_003380.3	Forward	gagaactttgccgtgaagc
		Reverse	tcagcagcttctgttaggt
<i>ACTIN</i>	NM_001102.3	Forward	gcacccttcaacaactggat
		Reverse	gatggttgtaggggttgg

(ANOVA) followed by Student's t-test (SPSS version 12; SPSS Software Products, Chicago, IL, USA). A difference was considered to be significant at $P < 0.05$.

3. Results

3.1. Identification and characterization of ADSCs

The phenotype of ADSCs in passage 5 was analyzed for different antibodies typically expressed by mesenchymal stem cells. Flow cytometric analysis (Figure 1) showed that ADSCs were positive for mesenchymal stem cell markers CD29 and CD90 and were negative for CD34 and CD45, which preclude contamination with hematopoietic cells.

3.2. Effects of ADSC-secreted exosomes on the mRNA levels of functional proteins in MCF-7 cells

To investigate the effects of ADSCs on the metastasis-related functional proteins of MCF-7, 5.0×10^4 ADSCs were cultured in conditioned growth media for 48 h. Exosomes

from ADSC culture media were extracted and used to stimulate MCF-7 cells for 48 h at two levels (exosome L: 100 $\mu\text{g/mL}$; exosome H: 200 $\mu\text{g/mL}$). To characterize the transcriptional changes of MCF-7 cells, the mRNA profiles of functional proteins were analyzed. As seen in Figure 2A, after treatment with ADSC-derived exosomes, *SMAD*, *SLUG*, *SNAIL1/2*, *TWIST1*, *N-cadherin*, and *vimentin* mRNA were obviously upregulated ($P < 0.01$), while levels of *E-cadherin* were observably reduced ($P < 0.01$). LPS is a component of the outer membrane of gram-negative bacteria, and can trigger an immune response. Poly (I:C) is a synthetic analog of double-stranded RNA and is usually used as a vaccine adjuvant to enhance innate and adaptive immune responses (Durai et al., 2015; Reisinger et al., 2015). Inflammatory responses can speed up tumor progression; accordingly, to investigate the interaction of ADSCs with MCF-7 cells in an inflammatory environment, LPS and poly (I:C) (which stimulate cells that produce

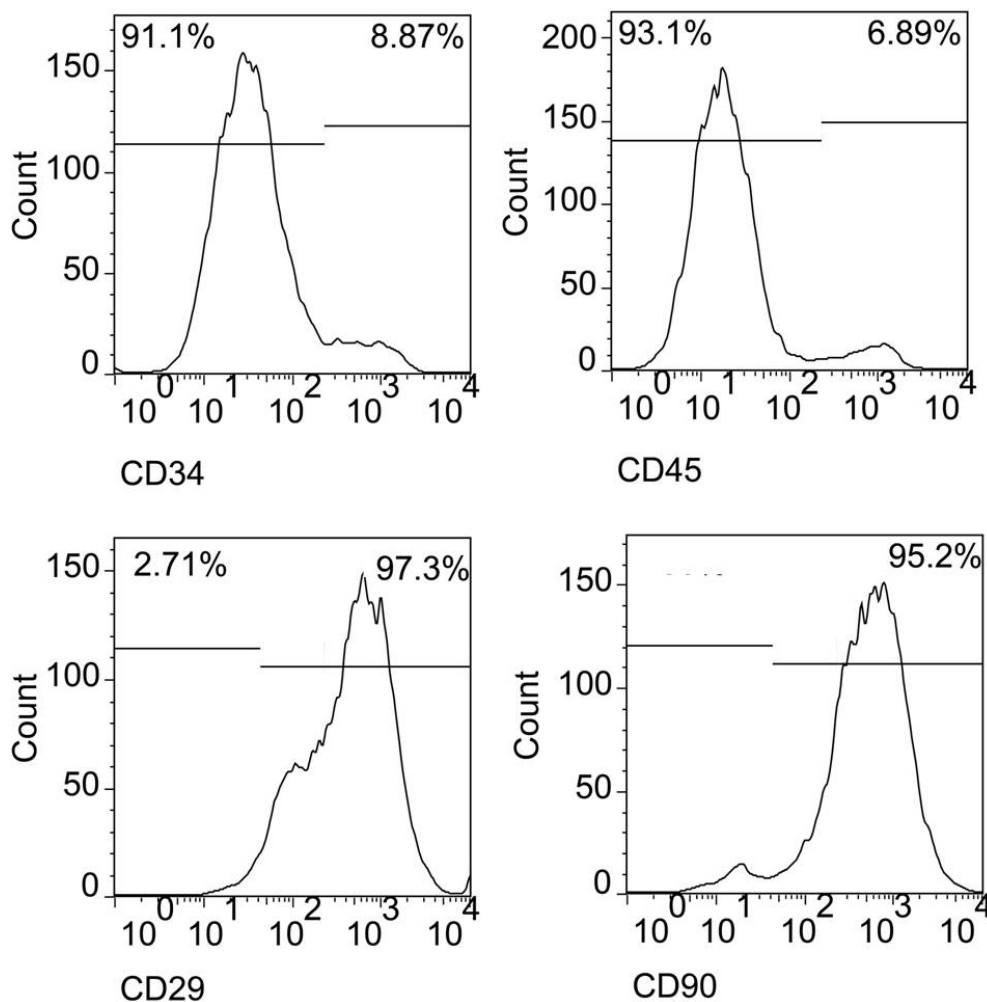


Figure 1. ADSCs express CD surface markers typical for mesenchymal stem cells. Flow cytometric analysis of CD surface markers on ADSCs.

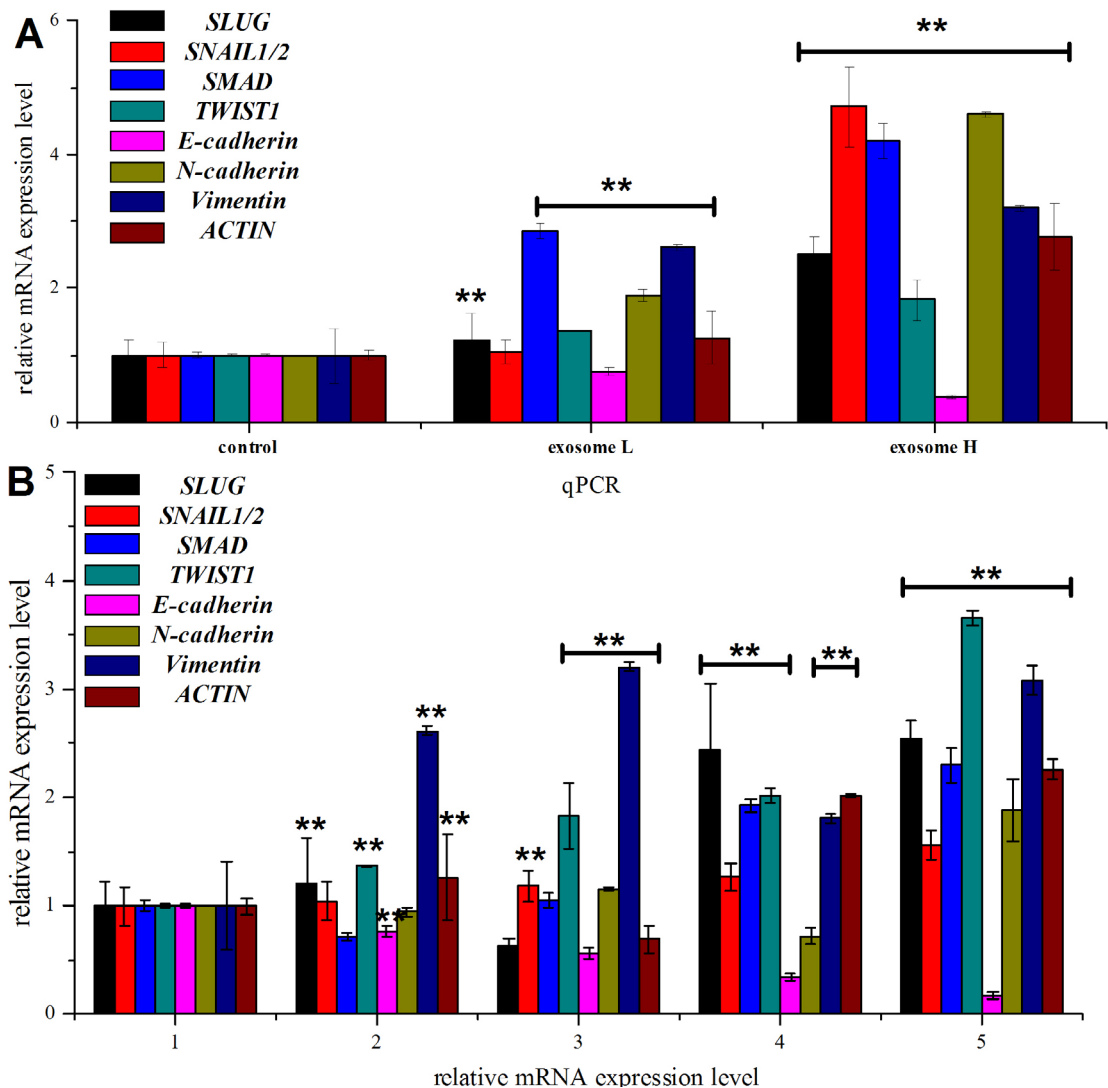


Figure 2. ADSC-secreted exosomes changed the mRNA levels of functional proteins in MCF-7 cells. A: Compared with that of control group, ADSC-secreted exosomes increased mRNA levels of *SMAD*, *SLUG*, *SNAIL1/2*, *TWIST1*, *N-cadherin*, and *vimentin* and meanwhile reduced the levels of *E-cadherin* in MCF-7 cells; exosome L: 100 µg/mL; exosome H: 200 µg/mL. B: Compared with the control group, exosomes from LPS- or poly (I:C)-treated ADSCs significantly increased mRNA levels of *SMAD*, *SLUG*, *SNAIL1/2*, *TWIST1*, *N-cadherin*, *vimentin*, and reduced the levels of *E-cadherin* in MCF-7 cells. 1: Control group (MCF-7 cells without exosome treatment); 2: MCF-7 cells treated with 100 µg/mL exosomes from LPS-stimulated ADSCs; 3: MCF-7 cells treated with 200 µg/mL exosomes from LPS-stimulated ADSCs; 4: MCF-7 cells treated with 100 µg/mL exosomes from poly (I:C)-stimulated ADSCs; 5: MCF-7 cells treated with 200 µg/mL exosomes from poly (I:C)-stimulated ADSCs. ***P* < 0.01 compared to group 1. Data are shown as the means of three parallel experiments.

proinflammatory cytokines) were used to activate ADSCs or MCF-7 cells. As expected, the exosomes of LPS- or poly (I:C)-treated ADSCs altered these regulators in the same manner as exosomes from the LPS- or poly (I:C)-untreated ADSC group (*P* < 0.01, Figure 2B).

3.3. Effects of ADSC-secreted exosomes on the expression of functional proteins in MCF-7 cells

As shown in Figures 3A and 3B, treatment with ADSC-derived exosomes significantly increased expression of

Smad, Slug, Snail1/2, Twist1, N-cadherin, and vimentin, and reduced the levels of E-cadherin in MCF-7 cells compared with those in untreated MCF-7 cells (*P* < 0.01). In addition to studying the prometastasizing activity of ADSCs under inflammatory environments, the levels of the functional proteins in MCF-7 cells stimulated by ADSC exosomes in the presence of LPS or poly (I:C) were investigated. The results in Figures 3C and 3D showed that LPS- or poly (I:C)-treated ADSC exosomes changed the

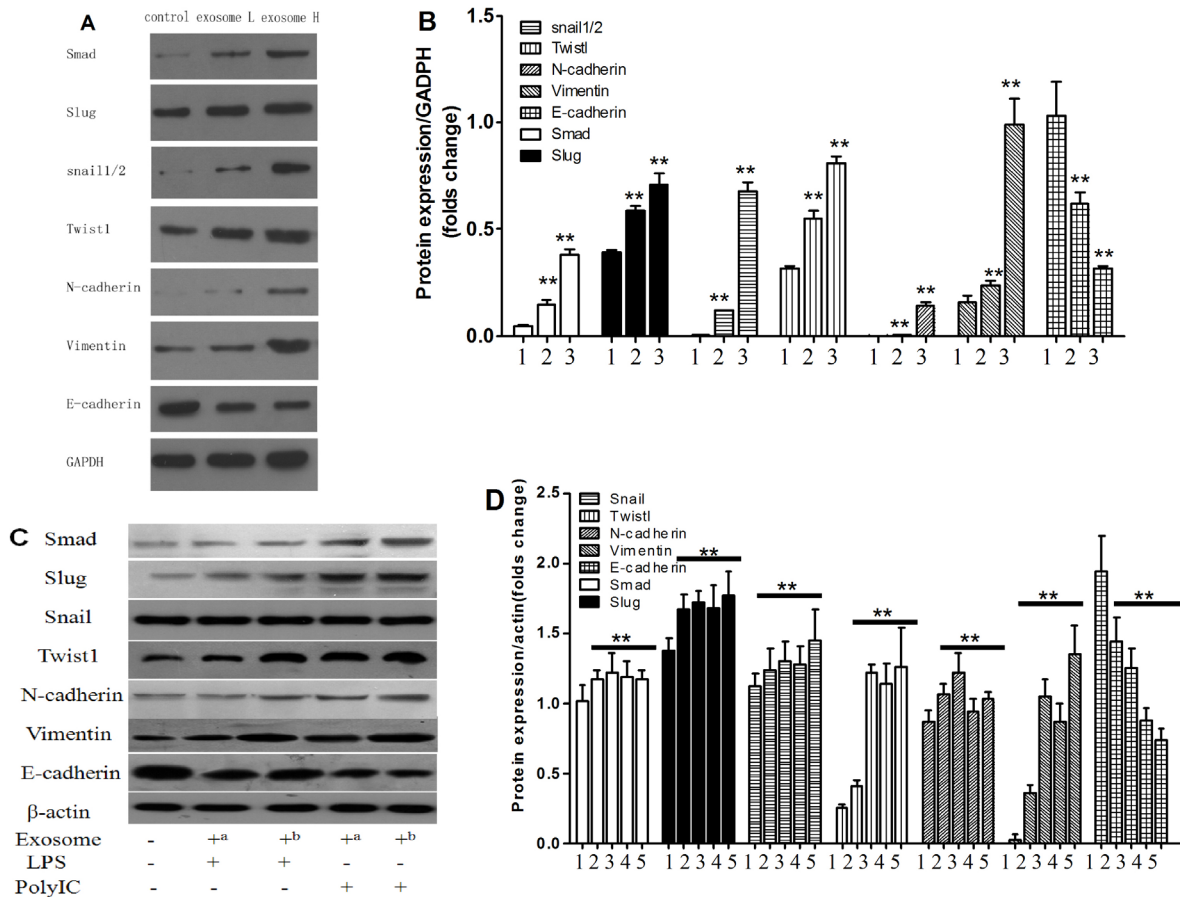


Figure 3. ADSC-secreted exosomes altered the expression of functional proteins in MCF-7 cells. A: Representative protein bands, exosome L: 100 µg/mL; exosome H: 200 µg/mL. B: Densitometric analysis of Figure 3A. ADSC-secreted exosomes significantly increased the levels of Smad, Slug, Snail1/2, Twist1, N-cadherin, and vimentin and reduced the level of E-cadherin in MCF-7 cells compared with the control group. 1: Control group (MCF-7 cells without exosomes treatment), 2: MCF-7 cells treated with 100 µg/mL exosomes, 3: MCF-7 cells treated with 200 µg/mL exosomes. ***P* < 0.01 compared to group 1. Data are shown as the means of three parallel experiments. C: representative protein bands, +^a: exosomes with a concentration of 100 µg/mL; +^b: exosomes with a concentration of 200 µg/mL. D: Densitometric analysis of Figure 3C. Exosomes significantly increased levels of Smad, Slug, Snail1/2, Twist1, N-cadherin, and vimentin and reduced the levels of E-cadherin in MCF-7 cells compared to the control group. 1: Control group (MCF-7 cells without exosomes treatment), 2: MCF-7 cells treated with 100 µg/mL exosomes from LPS-stimulated ADSCs, 3: MCF-7 cells treated with 200 µg/mL exosomes from LPS-stimulated ADSCs, 4: MCF-7 cells treated with 100 µg/mL exosomes from poly (I:C)-stimulated ADSCs; 5: MCF-7 cells treated with 200 µg/mL exosomes from poly (I:C)-stimulated ADSCs. ***P* < 0.01 compared to group 1. Data are shown as the means of three parallel experiments.

expressions of Smad, Slug, Snail1/2, Twist1, N-cadherin, vimentin, and E-cadherin in the same manner as exosomes from the LPS- or poly (I:C)-untreated ADSC group (*P* < 0.01).

3.4. Effects of MCF-7 cells produced exosomes on the mRNA levels of *RAS*, *P53*, and *HER-2* in ADSCs

To further characterize the oncogenes in ADSCs, the mRNA levels of *RAS*, *P53*, and *HER-2* in ADSCs were analyzed. The results in Figure 4A show that MCF-7-derived exosomes caused obvious upregulation in the expression of *RAS* and *HER-2* and a decrease in the levels

of *P53* in ADSCs (*P* < 0.01). Similarly, MCF-7-produced exosomes in the presence of LPS or poly (I:C) increased *RAS* and *HER-2* mRNA levels and lowered *P53* level in ADSCs as well (Figure 4B; *P* < 0.01).

3.5. Effects of MCF-7 cells produced exosomes on the protein levels of *RAS*, *P53*, and *HER-2* in ADSCs

As presented in Figures 5A and 5B, treatment with MCF-7-derived exosomes significantly increased expression of *RAS* and *HER-2*, and decreased the level of *P53* in ADSCs (*P* < 0.01). Moreover, a similar change in levels of *RAS*, *HER-2*, and *P53* was observed in ADSCs cocultured with

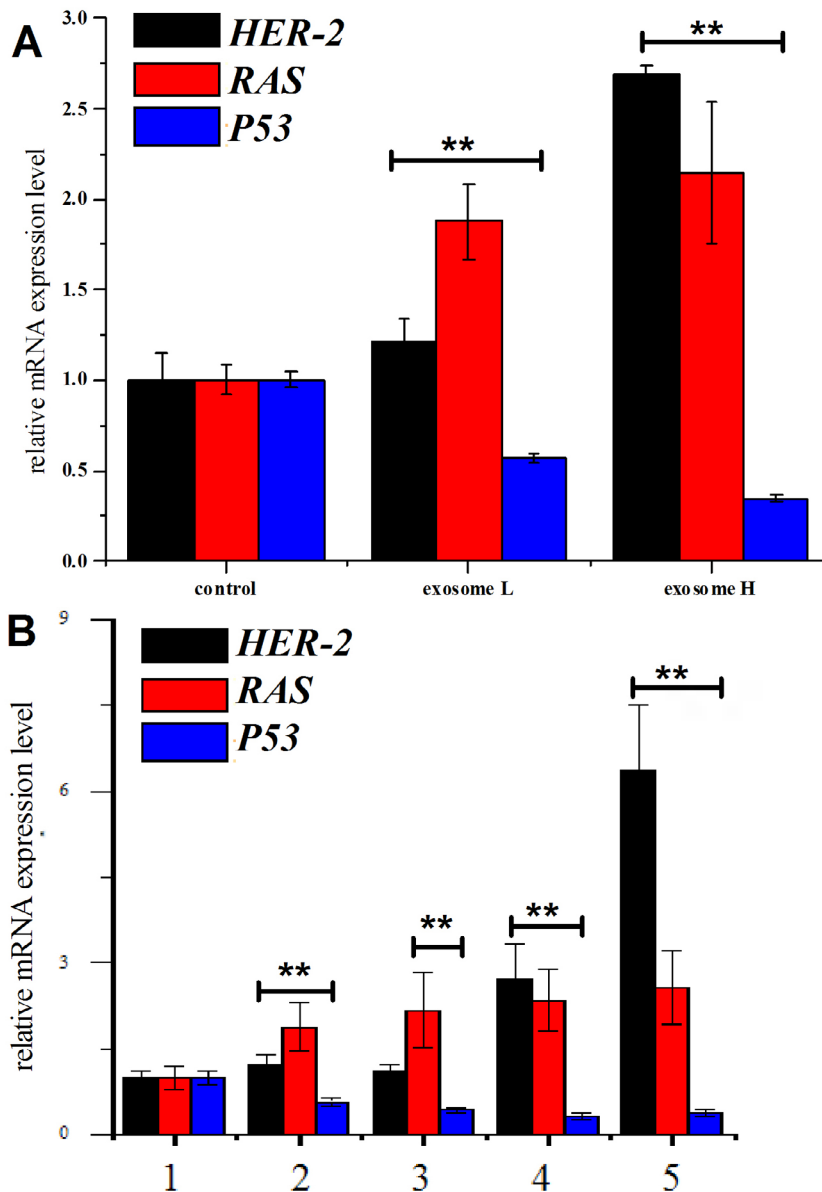


Figure 4. MCF-7-secreted exosomes changed mRNA levels of *RAS*, *P53*, and *HER-2* in ADSCs. A: Compared with the control group, MCF-7-secreted exosomes increased mRNA levels of *RAS* and *HER-2* while reducing the level of *P53* in ADSCs; exosome L: 100 µg/mL; exosome H: 200 µg/mL. B: Compared with the control group, exosomes from LPS- or poly (I:C)-treated MCF-7 cells significantly increased mRNA levels of *RAS* and *HER-2*, and reduced the level of *P53* in ADSCs. 1: Control group (ADSCs without exosome treatment); 2: ADSCs treated with 100 µg/mL exosomes from LPS-stimulated MCF-7 cells; 3: ADSCs treated with 200 µg/mL exosomes from LPS-stimulated MCF-7 cells; 4: ADSCs treated with 100 µg/mL exosomes from poly (I:C)-stimulated MCF-7 cells; 5: ADSCs treated with 200 µg/mL exosomes from poly (I:C)-stimulated MCF-7 cells. ***P* < 0.01 compared to group 1. Data are shown as the means of three parallel experiments.

exosomes from LPS- or poly (I:C)-treated MCF-7 cells (*P* < 0.01, Figures 5C and 5D).

4. Discussion

Several recent studies (Muehlberg et al., 2009; Kucerova et al., 2011; Kim et al., 2013) confirm that ADSCs stimulate breast cancer cell growth and migration in

vitro, and coinjection of ADSCs with breast cancer cells promotes the growth of xenograft tumors in mice. On the other hand, it has been previously demonstrated ADSCs are prone to differentiating into carcinoma-associated fibroblast-like cells in the presence of tumor-derived factors, and change into tumor stroma when treated with lung cancer cells (Jotzu et al., 2011; Park et al., 2013).

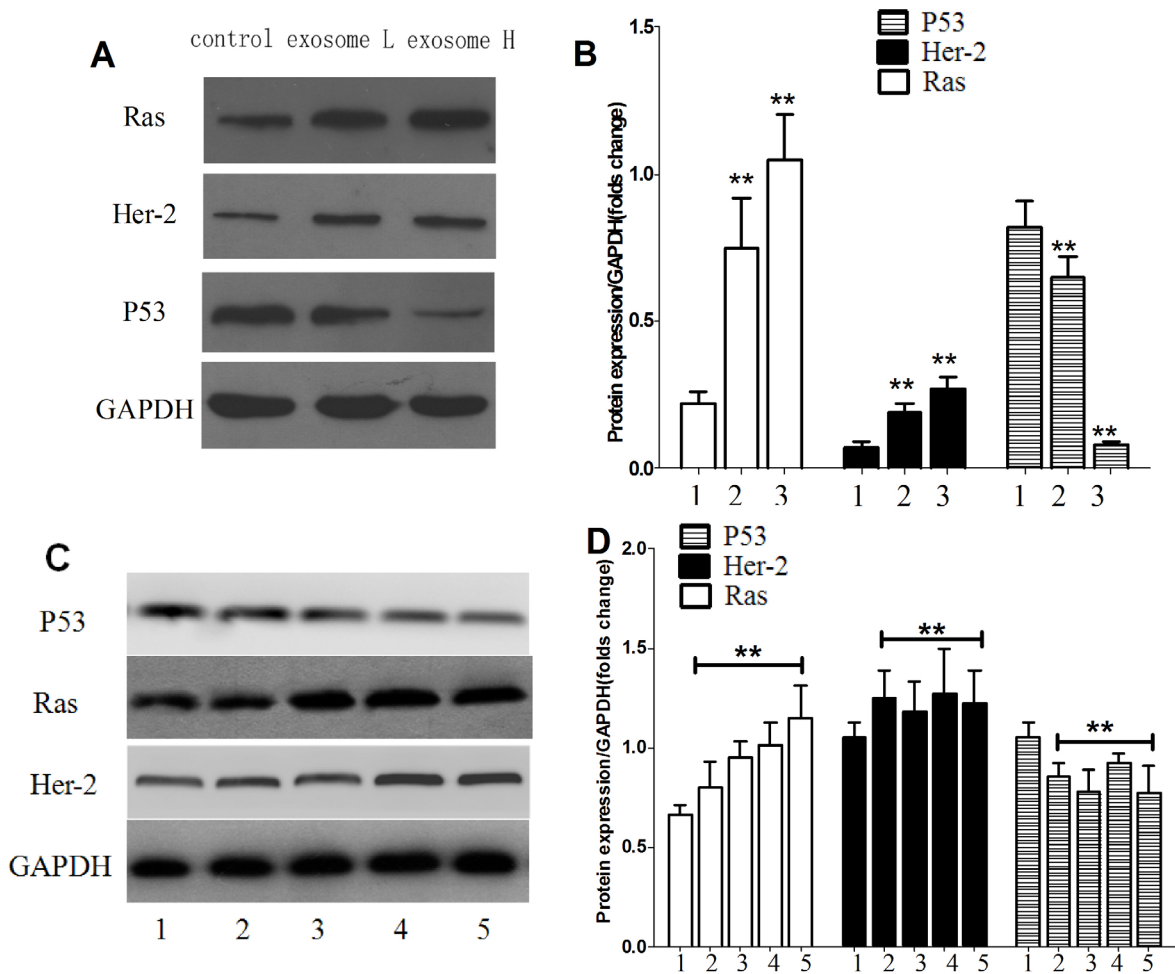


Figure 5. MCF-7-secreted exosomes altered the expression of RAS, P53, and HER-2 in ADSCs. A: Representative protein bands, exosome L: 100 μ g/mL; exosome H: 200 μ g/mL. B: MCF-7-secreted exosomes significantly increased levels of RAS and HER-2, and reduced the level of P53 in ADSCs compared with the control group. 1: Control group (ADSCs without exosome treatment), 2: ADSCs treated with 100 μ g/mL exosomes, 3: ADSCs treated with 200 μ g/mL exosomes. ** $P < 0.01$ compared to group 1. Data are shown as the means of three parallel experiments. C: Representative protein bands. D: Exosomes significantly increased levels of RAS and HER-2, and reduced the level of P53 in ADSCs compared with that of control group. 1: Control group (ADSCs without exosomes treatment), 2: ADSCs treated with 100 μ g/mL exosomes from LPS-stimulated MCF-7 cells. 3: ADSCs treated with 200 μ g/mL exosomes from LPS-stimulated MCF-7 cells. 4: ADSCs treated with 100 μ g/mL exosomes from poly (I:C)-stimulated MCF-7 cells. 5: ADSCs treated with 200 μ g/mL exosomes from poly (I:C)-stimulated MCF-7 cells. ** $P < 0.01$ compared to group 1. Data are shown as the means of three parallel experiments.

These findings indicate that the interaction of ADSCs with tumor cells could induce significant phenotype and functional alterations. Other papers suggest inflammation is involved in the interaction of ADSCs and tumor cells. For example, both ADSCs and breast cancer cells could release proinflammatory cytokines such as IL-6 and IL-8 (Kilroy et al., 2007; Senst et al., 2013). ADSCs (Razmkhah et al., 2010) protect breast cancer cells from host immune responses by tumor-promoting factors like insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and IL-8. In

breast cancer tissues, *IGF-1* and *IL-8* mRNAs are 28.6- and 56-fold more expressed in high-stage compared to low-stage patients; in ADSCs, relative quantifications of *VEGF*, *IL-8*, *HGF*, and *IGF-1* were approximately 2-fold higher in patients than in controls. Recent research (Walter et al., 2009; Welte et al., 2012) further notes that stromal-cell-derived IL-8 promotes invasion of cancer cells, and depletion of IL-6 in ADSCs abrogates its stimulatory effect on migration and invasion of breast tumor cells. However, no more detailed mechanisms of the interaction of ADSCs with breast cancer cells are discussed.

To acquire the traits for performing multiple steps of metastasis, cancer cells usually undergo EMT. EMT is an imperative process in cancer metastasis and is characterized by a loss of E-cadherin expression and increased levels of mesenchymal markers, including N-cadherin and vimentin (Kumar et al., 2015). TGF β 1-Smad is a ubiquitous signaling pathway that participates in the pathological process of numerous diseases. A recent work proves overactive TGF β 1-Smad accelerates the formation of EMT (Jiang et al., 2015). The binding of TGF β 1 to its receptors induces activated serine/threonine kinases and phosphorylated Smad2/Smad3 (heteromeric complexes). The complexes translocate into the nucleus and promote transcription of several EMT-associated genes, including *SNAIL*, *SLUG*, and *TWIST1* (Javelaud et al., 2011). Kallergi et al. (2011) found that Vimentin, *TWIST1*, *SNAIL*, and *SLUG* are expressed in circulating tumor cells of early metastatic breast cancer patients. In an in vitro study, Snail-depleted cancer-associated fibroblasts (CAF) failed to obtain myofibroblastic traits in response to TGF β 1, and human breast cancers with Snail1(+) CAF tended to exhibit desmoplastic areas with anisotropic fibers, lymph node involvement, and poorer outcomes (Stanisavljevic et al., 2015). Accordingly, Twist knockdown in WT tumor cells increases E-cadherin and reduces cell invasion and metastasis, while Twist overexpression in KO cells decreases E-cadherin and increases cell invasion (Qin et al., 2009).

P53 is a tumor suppressor that detects oncogenic events in cancer cells and eliminates them through apoptosis, while the *HER-2* gene is a protooncogene that usually has the strongest prognostic value in breast cancer (Khurshid et al., 2014). RAS is well known for its function as a molecular switch by cycling between GTP-bound active and GDP-bound inactive forms in intracellular signaling pathways that control cell growth, differentiation, and survival (Shima et al., 2015). Previous studies (Lo et al., 2013; Rubio et al., 2013) proved increased adipogenic differentiation of ADSCs was correlated with downregulation of *P53* in a time-dependent manner, and deficiency of *P53* alone in ADSCs promoted leiomyosarcoma-like tumors in vivo. Moreover, RAS has been demonstrated to be involved in reciprocal signaling between ADSCs and tumor cells. RAS-transfected ADSCs lose their ability to differentiate into adipocytes and instead differentiate into cells characterized by cytoplasmic granules, extensive nonspecific esterase activity, and anchorage-independent growth (Tzen et al., 1990). It has been previously confirmed (Abd Elmageed et al., 2014) that prostate-cancer-derived exosomes containing H-ras and K-ras transcripts, as well as the RAS superfamily of GTPases, induce the tumorigenic reprogramming of ADSCs.

LPS, a component of the outer membrane in gram-negative bacteria, triggers a Toll-like receptor-4 (TLR4)

immune response and plays a significant role in innate and adaptive immunity. Poly (I:C), a synthetic analogue of double-stranded RNA, is the agonist of TLR3 and is usually used as a vaccine adjuvant to enhance innate and adaptive immune responses (Durai et al., 2015; Reisinger et al., 2015). To date, these reagents have been widely used to stimulate various cells to produce pro-inflammatory cytokines that imitate an inflammation environment in vivo. In the present work, we first investigated the effects of ADSC-derived exosomes on EMT-relevant proteins of MCF-7 in vitro, and the influence of MCF-7-derived exosomes on tumor-cell-associated markers in ADSCs. The results showed that ADSC-derived exosomes significantly increase EMT-relevant regulators both at the protein and mRNA levels, while MCF-7-released exosomes observably alter oncogene expression at both protein and mRNA levels, leading to increased levels of RAS and *HER-2* and decreased levels of *P53*. Finally, these molecules showed similar tendencies in variation in cells that received exosome treatment in the presence of LPS or poly (I:C) compared to cells treated with exosomes in the absence of LPS or poly (I:C). However, in consideration of human experimental ethics, we were unable to obtain the direct effects of autogenous ADSCs (or MCF-7) on MCF-7 (or ADSC) properties in vivo; the effect may be measured in animal models for our next study. Interestingly, Mukherjee et al. (2014) found that primary breast cancer stem cells showed an increased cell migration ability with the development of tumor progression, and a gradual decrease in the expression levels of E-cadherin protein with increasing stages of breast cancer in vivo. These results are consistent with our present data. Several recent published studies investigated ADSCs–MCF-7 communication in vitro. For example, Tang et al. (2015) showed that mesenchymal stem cells (MSCs) are recruited to malignant fluids by a tumor-cell-derived chemokine-mediated signaling pathway on the MSC. TGF- β can alter tight junction formation in the mammary epithelium and induce a number of embryonic signaling pathways, including the Wnt, Notch, and Hh pathways. *SNAIL* and *SNAIL2/Slug* (Snail) association with *SMAD3* and *SMAD4* in the TGF- β signaling pathway initiates cancer growth and metastasis during EMT by inhibiting or suppressing transcription of *E-cadherin* (Takebe et al., 2011). McAndrews et al. (2015) observed that MSCs induce directional migration of invasive breast cancer cells through TGF- β . Accordingly, these findings suggest the communication of ADSCs with MCF-7 is, at least in part, via metastasis-related signaling pathways.

It should be noted that there are conflicting reports on ADSCs that describe both the promotion and inhibition of cancer progression, which may be closely related to immunoregulatory activity, subtype of ADSCs, and tumor

microenvironment in vivo. A previous study (Torsvik et al., 2010) concludes that ADSCs inhibit tumor growth by increasing inflammatory infiltration, while another concludes that some cancers may originate from normal stem cells (Magee et al., 2012). To date, it is apparent that stem cells play multiple roles in immunoregulation, which is evidenced by their ability to act as immune suppressors or stimulators, and their expression of MHC-II can either increase or decrease following IFN-gamma stimulation (Chen et al., 2007). Moreover, ADSCs are a heterogeneous cell population that may contain stromal cells, MSCs, and endothelial progenitors. It is possible that only a subset of MSCs within a population possesses multipotent differentiation potential and promotes tumorigenesis, while another subset inhibits tumorigenesis. For example, increased tumor-homing properties were found in a

specific MSC subpopulation that exhibited increased expression of integrins (Bolontrade et al., 2012). Additionally, the tumor microenvironment may influence immunosuppressive properties of MSCs, as differentiated MSCs in the tumor microenvironment may show different allogeneic or xenogeneic responses (Zhang et al., 2012).

Based on the results, it can be concluded that in vitro interaction of MCF-7 cells with ADSCs results in increased levels of a series of metastasis-related functional proteins in MCF-7 cells, as well as enhanced expression of oncogenes in ADSCs.

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References

- Abd Elmageed ZY, Yang Y, Thomas R, Ranjan M, Mondal D, Moroz K, Fang Z, Rezk BM, Moparty K, Sikka SC et al. (2014). Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. *Stem Cells* 32: 983-997.
- Bolontrade ME, Sganga L, Piaggio E, Viale DL, Sorrentino MA, Robinson A, Sevlever G, García MG, Mazzolini G, Podhajcer OL (2012). A specific subpopulation of mesenchymal stromal cell carriers overrides melanoma resistance to an oncolytic adenovirus. *Stem Cells Dev* 21: 2689-2702.
- Chen X, McClurg A, Zhou GQ, McCaigue M, Armstrong MA, Li G (2007). Chondrogenic differentiation alters the immunosuppressive property of bone marrow- derived mesenchymal stem cells, and the effect is partially due to the upregulated expression of B7 molecules. *Stem Cells* 25: 364-370.
- Cho JA, Park H, Lim EH, Lee KW (2012). Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. *Int J Oncol* 40: 130-138.
- Durai P, Batool M, Choi S (2015). Structure and effects of cyanobacterial lipopolysaccharides. *Mar Drugs* 13: 4217-4230.
- Hood JL, Pan H, Lanza GM, Wickline SA; Consortium for Translational Research in Advanced Imaging and Nanomedicine (C-TRAIN) (2009). Paracrine induction of endothelium by tumor exosomes. *Lab Invest* 89: 1317-1328.
- Javelaud D, Alexaki VI, Dennler S, Mohammad KS, Guise TA, Mauviel A (2011). TGF- β /SMAD/GLI2 signaling axis in cancer progression and metastasis. *Cancer Res* 71: 5606-5510.
- Jiang HL, Sun HF, Gao SP, Li LD, Hu X, Wu J, Jin W (2015). Loss of RAB1B promotes triple-negative breast cancer metastasis by activating TGF- β /SMAD signaling. *Oncotarget* 6: 16352-16365.
- Jotzu C, Alt E, Welte G, Li J, Hennessy BT, Devarajan E, Krishnappa S, Pinilla S, Droll L, Song YH (2011). Adipose tissue derived stem cells differentiate into carcinoma- associated fibroblast-like cells under the influence of tumor derived factors. *Cell Oncol (Dordr)* 34: 55-67.
- Kallergi G, Papadaki MA, Politaki E, Mavroudis D, Georgoulas V, Agelaki S (2011). Epithelial to mesenchymal transition markers expressed in circulating tumour cells of early and metastatic breast cancer patients. *Breast Cancer Res* 13: R59.
- Khurshid R, Saleem M, Gul-e-Raana, Akhthar MS (2014). Phosphorylation sites of HER2/c-erbB-2: role in cell growth and in disease. *Acta Biochim Pol* 61: 699-703.
- Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, Ludlow JW, Stricker DM, Potiny S, Green P et al. (2007). Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. *J Cell Physiol* 212: 702-709.
- Kim J, Escalante LE, Dollar BA, Hanson SE, Hematti P (2013). Comparison of breast and abdominal adipose tissue mesenchymal stromal/stem cells in support of proliferation of breast cancer cells. *Cancer Invest* 31: 550-554.
- Kruger S, Abd Elmageed ZY, Hawke DH, Wörner PM, Jansen DA, Abdel-Mageed AB, Alt EU, Izadpanah R (2014). Molecular characterization of exosome-like vesicles from breast cancer cells. *BMC Cancer* 14:44.
- Kuceroval L, Kovacicova M, Polak S, Bohac M, Fedeles J, Palencar D, Matuskova M (2011). Interaction of human adipose tissue-derived mesenchymal stromal cells with breast cancer cells. *Neoplasma* 58: 361-370.
- Kumar KJ, Vani MG, Chueh PJ, Mau JL, Wang SY (2015). Antrodin C inhibits epithelial-to-mesenchymal transition and metastasis of breast cancer cells via suppression of Smad2/3 and β -catenin signaling pathways. *PLoS One* 10:e0117111.

- Lin R, Wang S, Zhao RC (2013). Exosomes from human adipose-derived mesenchymal stem cells promote migration through Wnt signaling pathway in a breast cancer cell model. *Mol Cell Biochem* 383: 13-20.
- Lo Furno D, Graziano AC, Caggia S, Perrotta RE, Tarico MS, Giuffrida R, Cardile V (2013). Decrease of apoptosis markers during adipogenic differentiation of mesenchymal stem cells from human adipose tissue. *Apoptosis* 18: 578-588.
- Magee JA, Piskounova E, Morrison SJ (2012). Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 21: 283-296.
- McAndrews KM, McGrail DJ, Ravikumar N, Dawson MR (2015). Mesenchymal stem cells induce directional migration of invasive breast cancer cells through TGF- β . *Sci Rep* 5: 16941.
- Muehlberg FL, Song YH, Krohn A, Pinilla SP, Droll LH, Leng X, Seidensticker M, Ricke J, Altman AM, Devarajan E et al. (2009). Tissue-resident stem cells promote breast cancer growth and metastasis. *Carcinogenesis* 30: 589-597.
- Mukherjee S, Mazumdar M, Chakraborty S, Manna A, Saha S, Khan P, Bhattacharjee P, Guha D, Adhikary A, Mukherjee S et al. (2014). Curcumin inhibits breast cancer stem cell migration by amplifying the E-cadherin/ β -catenin negative feedback loop. *Stem Cell Res Ther* 5: 116.
- Park YM, Yoo SH, Kim SH (2013). Adipose-derived stem cells induced EMT-like changes in H358 lung cancer cells. *Anticancer Res* 33: 4421-4430.
- Qin L, Liu Z, Chen H, Xu J (2009). The steroid receptor coactivator-1 regulates twist expression and promotes breast cancer metastasis. *Cancer Res* 69: 3819-3827.
- Razmkhah M, Jaberipour M, Hosseini A, Safaei A, Khalatbari B, Ghaderi A (2010). Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma. *Cell Immunol* 265: 80-85.
- Reisinger S, Khan D, Kong E, Berger A, Pollak A, Pollak DD (2015). The poly (I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol Ther* 149: 213-226.
- Rowan BG, Gimble JM, Sheng M, Anbalagan M, Jones RK, Frazier TP, Asher M, Lacayo EA, Friedlander PL, Kutner R et al. (2014). Human adipose tissue-derived stromal/stem cells promote migration and early metastasis of triple negative breast cancer xenografts. *PLoS One* 9:e89595.
- Rubio R, Gutierrez-Aranda I, Sáez-Castillo AI, Labarga A, Rosu-Myles M, Gonzalez- Garcia S, Toribio ML, Menendez P, Rodriguez R (2013). The differentiation stage of p53-Rb-deficient bone marrow mesenchymal stem cells imposes the phenotype of in vivo sarcoma development. *Oncogene* 32: 4970-4980.
- Senst C, Nazari-Shafti T, Kruger S, Höner Zu Bentrup K, Dupin CL, Chaffin AE, Srivastav SK, Wörner PM, Abdel-Mageed AB, Alt EU et al. (2013). Prospective dual role of mesenchymal stem cells in breast tumor microenvironment. *Breast Cancer Res Treat* 137: 69-79.
- Shima F, Matsumoto S, Yoshikawa Y, Kawamura T, Isa M, Kataoka T (2015). Current status of the development of Ras inhibitors. *J Biochem* 158: 91-99.
- Shin SH, Kim SC, Hong SM, Kim YH, Song KB, Park KM, Lee YJ (2013). Genetic alterations of K-ras, p53, c-erbB-2, and DPC4 in pancreatic ductal adenocarcinoma and their correlation with patient survival. *Pancreas* 42: 216-222.
- Singh R, Pochampally R, Watabe K, Lu Z, Mo YY (2014). Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. *Mol Cancer* 13: 256.
- Stanisavljevic J, Loubat-Casanovas J, Herrera M, Luque T, Peña R, Lluch A, Albanell J, Bonilla F, Rovira A, Peña C et al. (2015). Snail1-expressing fibroblasts in the tumor microenvironment display mechanical properties that support metastasis. *Cancer Res* 75: 284-295.
- Takebe N, Warren RQ, Ivy SP (2011). Breast cancer growth and metastasis: interplay between cancer stem cells, embryonic signaling pathways and epithelial-to- mesenchymal transition. *Breast Cancer Res* 13: 211.
- Tang K, Hu L, Ma J, Zhang H, Zhang Y, Li Y, Ma R, Luo S, Liu D, Long G et al. (2015). Brief report: Human mesenchymal stem-like cells facilitate floating tumorigenic cell growth via glutamine-ammonium cycle. *Stem Cells* 33: 2877-2884.
- Tan L, Wu H, Liu Y, Zhao M, Li D, Lu Q (2016). Recent advances of exosomes in immune modulation and autoimmune diseases. *Autoimmunity* 49: 357-365.
- Torsvik A, Røslund GV, Svendsen A, Molven A, Immervoll H, McCormack E, Lønning PE, Primon M, Sobala E, Tonn JC et al. (2010). Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track-letter. *Cancer Res* 70: 6393-6396.
- Tzen CY, Filipak M, Scott RE (1990). Metaplastic change in mesenchymal stem cells induced by activated ras oncogene. *Am J Pathol* 137: 1091-1102.
- Uzbas F, May ID, Parisi AM, Thompson SK, Kaya A, Perkins AD, Memili E (2015). Molecular physiognomies and applications of adipose-derived stem cells. *Stem Cell Rev* 11: 298-308.
- Walter M, Liang S, Ghosh S, Hornsby PJ, Li R (2009). Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. *Oncogene* 28: 2745-2755.
- Welte G, Alt E, Devarajan E, Krishnappa S, Jotzu C, Song YH (2012). Interleukin-8 derived from local tissue-resident stromal cells promotes tumor cell invasion. *Mol Carcinog* 51: 861-868.
- Yamada S, Okumura N, Wei L, Fuchs BC, Fujii T, Sugimoto H, Nomoto S, Takeda S, Tanabe KK, Kodera Y (2014). Epithelial to mesenchymal transition is associated with shorter disease-free survival in hepatocellular carcinoma. *Ann Surg Oncol* 21: 3882- 3890.