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Original Article

Mesenchymal stem cell-derived exosomes do not promote the proliferation of cancer cells *in vitro*

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Abstract: Nowadays, the use of Mesenchymal stem cells (MSCs) in clinical therapies have an increased acceleration, while it constitutes two sides of yin-yang with its ameliorating effects in regenerative medicine and promoting effects in carcinogenesis. It has been shown that the treatment activities of MSCs are mediated by paracrine factors secreted. These paracrine factors are transmitting via exosomes secreted from MSCs. With the understanding of this mechanism, cell-free therapies have begun to create a new path in MSC based therapies. At this point, two sides of the yin-yang have once again become controversial. In addition, there are conflicting study results in the literature. Due to this contradiction, we have designed this study to demonstrate the role of MSCs in the carcinogenesis process and we investigated the proliferation effect of MSC-derived exosomes on cancer cell lines. Two parallel experimental setups were established, as an experimental group, the four-different epithelial cancer cell lines and Wharton's Jelly (WJ)-MSC derived exosomes were directly co-cultured with in 6 different concentrations and simultaneously in the control group cells were cultured respectively. PKH-26 labelling was performed for detection of exosome locations in co-cultures. Each group were evaluated by WST-1 and xCelligence assays for proliferation and confirmed with PCNA staining. The results were analysed with paired t-test and Newman-Keuls comparison. The relative comparison demonstrated a significant increase in the rate of proliferation only in exosome co-cultures with WJ-MSCs and it was supported by PCNA staining. Cancer cell lines in co-cultures have not shown any significant increase neither in proliferation assays nor in PCNA staining. MSCs regulate their secretions according to the microenvironment, they have more dominant regenerative feature rather than triggering cancer proliferation.

Keywords: Mesenchymal stem cell, exosome, cancer proliferation

Introduction

Mesenchymal stem cells (MSCs), which are a group of cells that can be isolated from various tissues, have been thought to have important therapeutic potential due to their self-renewal capacity and multilineage differentiation potency. The studies have shown that the importance of the therapeutic effect of MSCs is paracrine actions [1, 2]. Their known paracrine secretions are including the secretion of immunomodulatory cytokines, tissue repair-inducing growth factors and small membrane vesicles. The exosomes are playing a key role as a message cloud in cellular communication by transferring host cell's components and modulating the extracellular niche [3]. In other words, exosomes which transfer the information to the target cell horizontally via the contain of host cell's mRNA, miRNA and protein repertoire [4]. In a remarkable number of publication have proposed that MSC-derived exomes have therapeutic effects in the treatment of several diseases, including kidney, myocardial and lung injuries and wound healing [5, 6]. Their secretions promote reducing infarct size, enhancing tissue repair [7] and increasing angiogenesis [8] by secreting phosphorylated-Akt and -GSK-3B [9] and transferring miRNA to damaged cells [10, 11] in cardiovascular diseases; regenerating tubuloepithelia [12], reducing tubular cell apoptosis [13] and tubular atrophy [14] by transferring mRNA to damage cell [12] and secreting insulin-like growth factor-1 receptor in acute kidney injury [15]; increasing re-epitheli-

alization, inhibiting apoptosis of skin cells and promoting proliferation of skin cells by activating canonical Wnt signalling by *WNT4* in cutaneous wound healing [16].

Cancer, which is defined as the second leading cause of death globally by the World Health Organism, is a genetic disease that caused by pathogenic variants in tumor suppressor genes, oncogenes and mismatch repair genes [17]. There are various carcinogenesis theories such as; stochastic model and cancer stem cell (CSC) model [18]. In a stochastic model, the clonal evolution model assumes that a normal cell in the organism undergoes a series of mutations to form a cancer cell that clonally expands and forms a large part of the tumor [19]. The other theory is a hierarchical model suggests that the origin of cancer is pluripotent and self-renewing CSC. These cells are highly tumorigenic with the ability to form new tumors. CSCs are divided asymmetrically to create new CSCs and progenitor cells, leading to differentiated cancer cells that make up the majority of the tumor [20]. When carcinogenesis occurred by driver mutations, it may accumulate additional oncogenic passenger mutations, but there is a multistage process primarily organized by growth factors for the proliferation of cells [21]. These growth factors also play a key role in signaling pathways that take part in carcinogenesis. TGF-β, FGF, MAPK are just a few most common examples of these growth factors for regulation in cancer progression.

Once the cancer cell has formed, tumor growth and progression is highly affected by its microenvironment which consists of inflammatory cells, tumor-associated fibroblasts, endothelial cells and MSCs [22, 23]. Within the tumor niche, MSC may interact with cancer cells by its secretions with a huge collection of cytokines and alternative combinations of growth factors [24]. This contribution may cause cancer cell survival, growth, motility, and immune escape. These cytokines and growth factors are delivered to the tumor microenvironment by MSC-derived exosomes which secreted as cargo [1].

Studies on the effects of MSC-derived exosome cargos on cancer cells have been shown that, increasing in cell proliferation by affecting angiogenesis [25] and increasing in the cancer stem cell population and breast cancer population by regulating WNT pathway, *SOX2* and *SOX9* through down regulation of mir-140 [26] in breast cancer and promoting the tumour growth by affecting the VEGF-ERK1/2 pathway in gastric carcinoma [27]. As seen in publications, these secreting factors have a role in the cancer process and also play an antagonist role regenerative effect on the damaged tissue [28]. At this point, MSC, which are shown as war heroes with their emerging use in regenerative medicine treatments, has been declared as a killer with the effects on cancer cells. In contrast to these studies, there are also some publications showing that it does not affect the proliferation of cancer cells. These studies have demonstrated the decreasing in cell proliferation by cell-cell communication in malignant glial tumours [29], suppressing the Multiple Myeloma (MM)-cell growth by the transfer of tumour suppressor microRNA that is mir-15 that from MSC-derived exosomes to MM and [30] the anti-proliferative effect on bladder cancer provided by phosphorylation of Akt proteins [31].

For the controversies on variety effect of MSC-derived exosomes, we aimed to observe the effects of exosomes acquired from Wharton's Jelly (WJ) derived MSCs on different cancer cell lines by means of promoting the proliferation of cancer cells via using different techniques.

Subjects and method

Study design

In total, four different cancer cell lines were incubated separately for 7 days, in microenvironments supplied with 6 different concentrations (6.25, 12.5, 25, 50, 100 ug) of WJ-MSC derived exosomes and were monitored by using WST-1, Xelligence, and immunostaining with proliferation marker (PCNA). In order to determine if exosomes were uptaken by cells or not, we labelled the supplied exosomes with PKH-26 (Red Fluorescent Cell Linker Kit) and examined the cells under the fluorescence microscope.

Four of these cell lines which are Human Colorectal Adenocarcinoma Cells (ATCC CCL-218), Human Thyroid Carcinoma Cells (ATCC CRL-1803) and Mammary Gland Adenocarcinoma from Metastatic Site (ATCC HTB-26), were purchased from ATCC (Manassas, VA). Malign Breast Stromal Cells (MBSC) were taken

from a patient with written informed consent with the declaration of Helsinki. The study received ethical approval from the Kocaeli University, Faculty of Medicine Ethical Committee (KAEK 2012/35). Additionally, the Wharton's Jelly derived MSC (WJ-MSC) (PCS-500-010) was purchased from ATCC (Manassas, VA).

A comparison and a control group were set with these 5 different cell lines. The comparison group consists of cell lines co-cultured with exosomes derived from WJ-MSCs while the control group consists of cell lines without any additional treatment on cells. Overall methodology workflow was illustrated in **Figure 1**.

Cell lines culture

WJ-MSC cell culture: WJ-MSC cell line was cultured in DMEM-F12 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), %1 streptomycin (100 mg/mL) solution at 37°C in a humidified 5% CO_2 incubator. After reaching 70% to 80% confluency, adherent cells were harvested with trypsinization by 0.05% trypsin-EDTA (Gibco, Germany).

Cancer cell line culture

Four different cell lines were cultured in RPMI 1649 (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and streptomycin (100 mg/mL) solution at 37°C in a humidified 5% CO_2 incubator. After reaching 70% to 80% confluency, adherent cells were harvested with trypsinization by 0.05% trypsin-EDTA (Gibco, Germany).

Exosome isolation, characterization and labelling: Characterized WJ-MSC were cultured with a serum-free medium to produce a concentrated and an adequate amount of exosome. Exosomes were produced in a humidified atmosphere containing 5% CO_2 at 37°C for 24 hours then the serum-free medium was collected and centrifuged at 300 g for 5 minutes for discarding the remaining cells. Until the last step, the pellet is thrown away and the supernatant is used for the following step.

The second and the third step are designed to eliminate dead cells and cell debris by successive centrifugations at increasing speeds. At the second step, the supernatant was centrifuged at 1000 g for 10 minutes and in the third step, the supernatant was centrifuged at 5000 g for 20 minutes. The final supernatant is ultracentrifuged at 100,000 g for 70 minutes and collected pellet that the small vesicles that correspond to exosomes from the pellet.

Quantification of protein in exosomes

By the BCA protein determination method, it is shown in order to show the presence of protein in the isolated exosomes. After the exosomes were isolated, 20 µl of the resulting pellet was prepared by adding 200 µl working solution (196 µl Bicinchoninic acid + 4 µl $CuSO_4$) and incubated at 37°C for one hour. After incubation, the amount of protein was determined by spectrophotometer at 562 nm with respect to the BSA standard.

Co-culture of cancer cell lines and exosomes

Different cancer cell lines were seeded into cell culture flasks for proliferation and after reaching 70% to 80% confluency, WJ-MSC derived exosomes directly co-cultured on these adherent cells for 7 days.

Isolated exosomes were labelled with CD81, CD9 and CD63 and the characterization data were acquired with Flow Cytometry analysis. The obtained exosomes were labelled with PKH26 (PKH26 Red Fluorescent Cell Binding Kit, PKH26GL-1KT, Sigma) in order to observe whether the exosomes were localized in the co-cultured cells or not.

Proliferation tests

The proliferation effect of WJ-MSC exosomes on cancer cells was demonstrated by WST-1 and XCelligence tests. Each proliferation comparison group repeated as triplicate.

WST-1 proliferation assay

Cancer cell lines cultivated with exosomes in 6-well temperature-sensitive culture plates, cells were tested with WST-1 assay at days 1, 3 and 7. The absorbance after the incubation period was read at 480 nm using microplate reader with the monochromatic system (VersaMax, Molecular Device, USA). Comparative analysis of viability and proliferation of the cell types is shown in the graph.

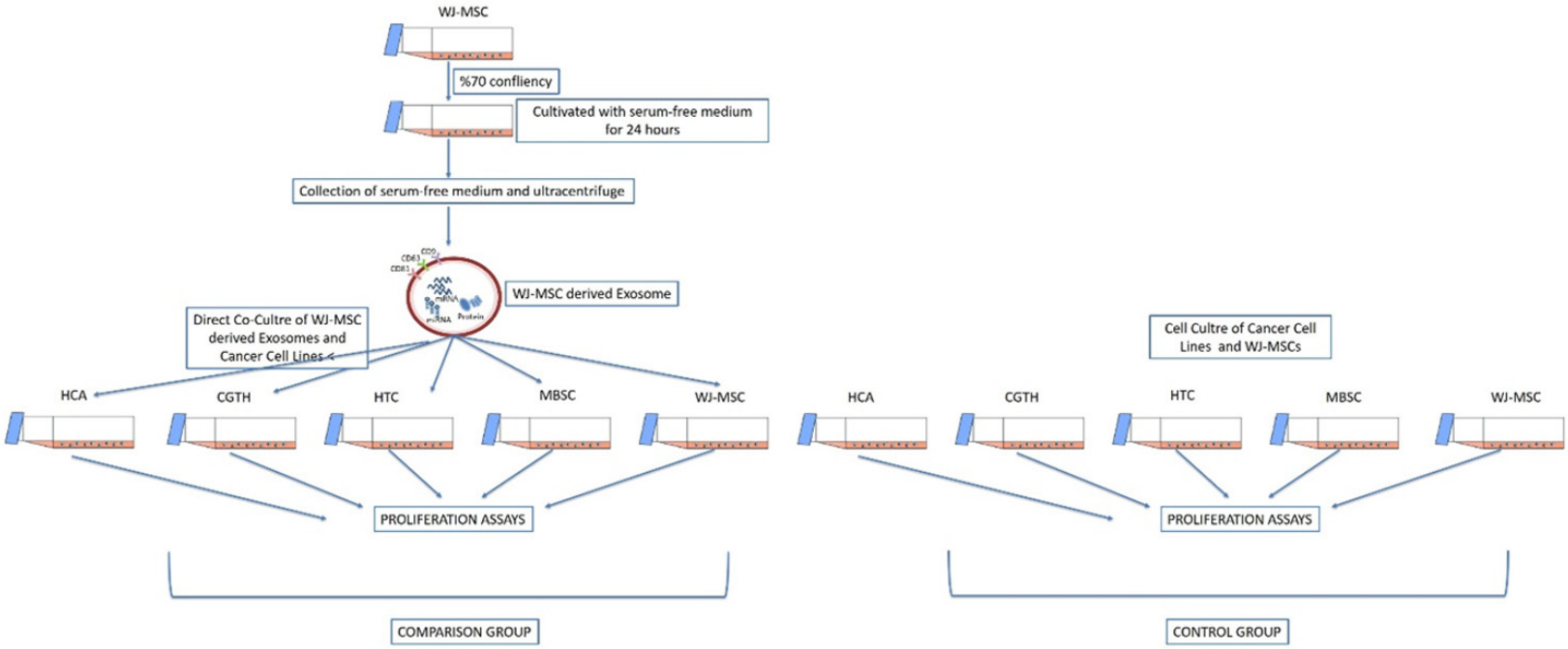

Figure 1. Overall workflow of the study.

XCelligence proliferation assay

To support the WST-1 test, Xelligence was used to monitor the viability and proliferation of the cells during cell culture. Each experiment set was grouped with cancer cell line and co-cultured cell line with exosomes. 50 µL from each set was seeded in E96 xCELLigence plates and plate was equilibrated to 37°C. Simultaneous proliferation curves were determined by density change per time in each well. Density changes were determined with the RTCA (Real-Time Cell Analysis) (ACEA Biosciences) software.

Immunostaining by proliferation marker PCNA

Exosomes were stained by the immunoblotting of PCNA (ab29) in order to show proliferation in the cells. For immunohistochemical staining, cells were fixed with 70% methanol. After incubation with primer antibody for 1 hour, staining was performed by IHC kit protocol. PCNA positive cells counted under the light microscope.

Statistical analysis

SPSS 10.0 (SPSS Inc, Chicago, IL, USA) software was used for statistical analyses. The data were analyzed by paired t-test and for multiple sample analysis Newman-Keuls comparison method was used. Each experiment was repeated at least three times. The level of significance was set at $P<0.05$ and $P<0.01$ it was identified as highly significant for all statistical analyses.

Results

Characterization of WJ-MSC and MSC-derived exosomes

First, MSC characterization was reviewed under the parameters which are cell surface marker staining and differentiation experiments in our previous publication [32].

We isolated exosomes from the serum-free culture supernatants of MSCs to investigate the potential effects of proliferation on cancer cells. To determine whether MSC-derived exosomes were successfully purified, we performed flow cytometry. As shown in **Figure 2**, representative markers of exosomes which are CD9, CD63 and CD81 were detected in the isolated exosomes 80% or higher. This means that MSC-derived exosomes were successfully purified.

Cellular uptake of MSC-derived exosomes into cancer cells

In order to determine cellular internalization of exosomes by cancer cells, we labelled the supplied WJ-MSC derived exosomes with a fluorescent dye, PKH-26. After the incubation of PKH-labelled exosomes with cancer cell lines, exosomes were examined under the fluorescence microscope. As shown in **Figure 3**, PKH26-labeled exosomes were uptaken by cancer cells.

The effect of MSC-derived exosomes on proliferation rate of different cancer cell lines in vitro

To evaluate the role of MSC-derived exosomes in proliferation in vitro, four different cancer cell lines were incubated separately for 7 days, in microenvironments supplied with 5 different concentrations (6.25, 12.5, 25, 50 and 100 ug) of WJ-MSC derived exosomes. The proliferation response to exosomes was assessed on cancer cell lines by using WST-1 and XCelligence. Additionally, the proliferation markers were indicated by immunostaining.

First of all, viability and proliferation amount of HCA, CGTH, MBSC, MDA and WJ-MSCs co-cultured with WJ-exosomes are determined via the WST-1 test on 1st, 3rd and 7th days. A relatively significant proliferation increase was observed only on groups of WJ-MSC which were treated with 100 ug and 50 ug of WJ-MSC derived exosomes ($P<0.05$, P*; $P<0.01$, P**; $P<0.001$, P***) (**Figure 4**). There was no significant change in the rate of proliferation in co-cultures with cancer cell lines compared to control groups.

Following the WST-1, proliferation assay for co-cultured cells and WJ-exosomes was examined via xCELLigence. A significant increase was not observed (**Figure 5**).

Comparing the number of PCNA positive cells between the comparison and control groups, no significant change was observed between the groups. The number of PCNA positive cells are found significantly higher in WJ-exosome treated control group when compared with

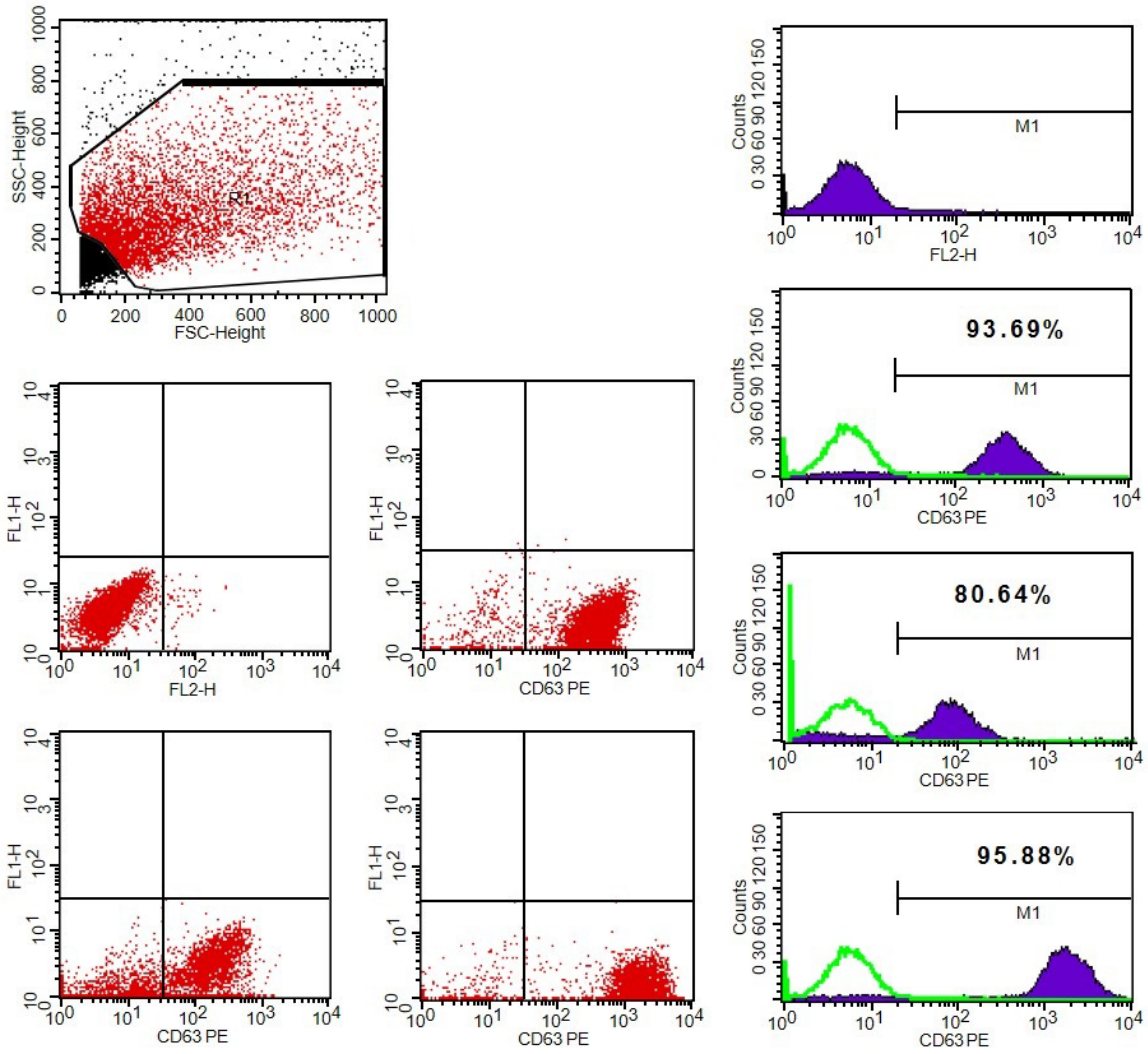

Figure 2. Characterization of WJ-MSC derived exomes by flow cytometry.

other experimental groups (P<0.05, P*; P<0.01, P**; P<0.001, P***) (**Figure 6**).

Discussion

MSCs are a group of progenitor cells capable of differentiating into several mesenchymal lineages [33] and an attractive cell source for cellular therapies [34]. The certain mechanism of cellular therapy action of MSCs is still doubtful [35]. The widely accepted concept is a secretory or an expressed factor that reaches neighbouring parenchymal cells via either a paracrine effect or a direct cell-to-cell interaction promoting functional activity, survival and proliferation of the parenchymal cells.

Exosomes exhibit certain different characteristics and secreted factors according to the source from which they are derived that are potentially associated with their biogenesis, targeting and putative immunological function [36]. That is why the sources of exosomes evaluated for microenvironment formation are important. They provide a potential pathway short- or long-range for the cell to cell communication, which may be likely to lie mechanism underlying MSCs' tumour-suppressive properties [31]. The secreted factors from the exosomes are known to be the roles of the exosomes in establishing and modifying tumour microenvironments [37]. Some studies have shown that MSC-derived exosomes are one of the key elements in carcinogenesis process through cellular communication, cell growth and cell migration. These triggering paths have been elucidated by demonstrating that adi-

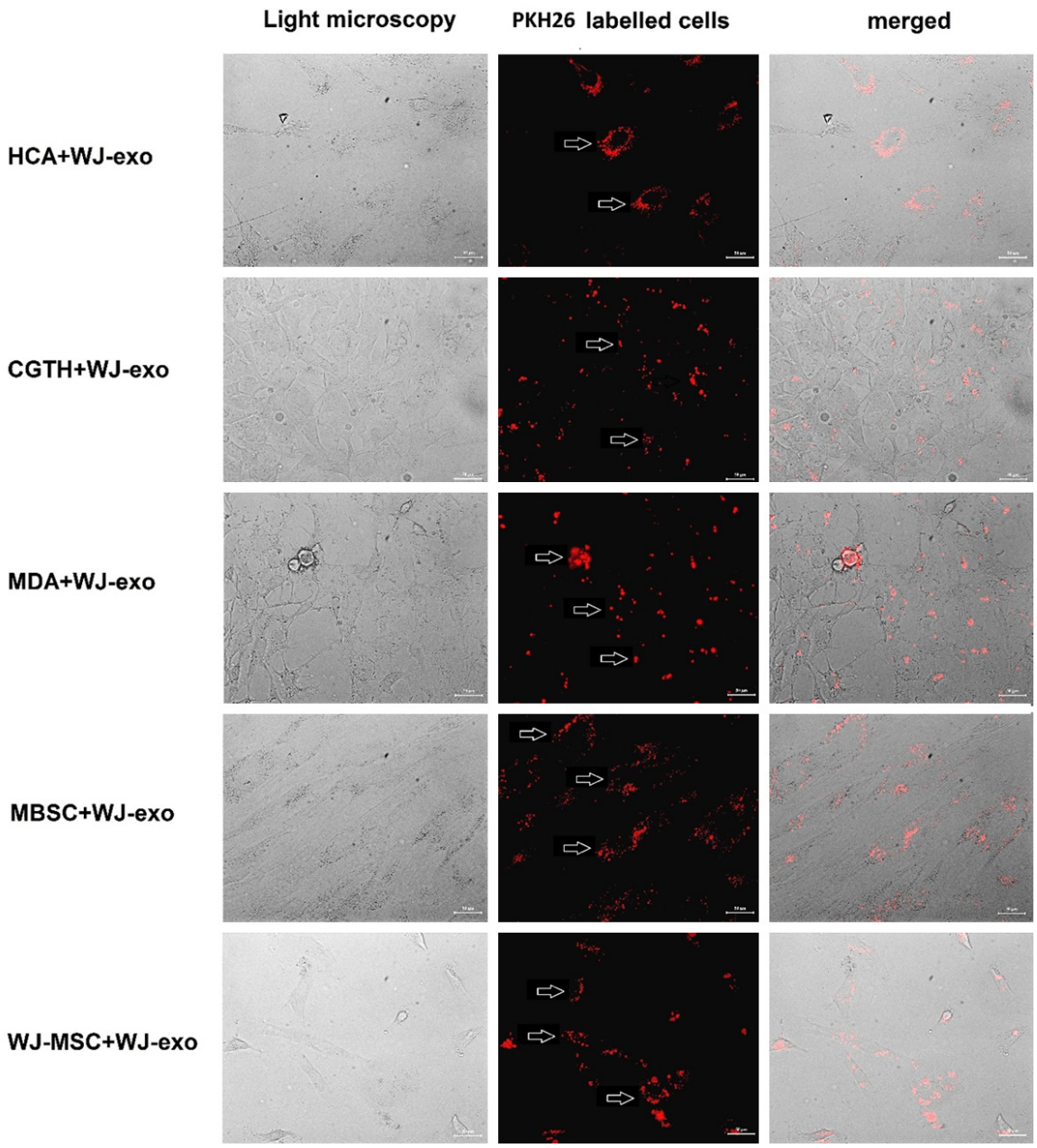

Figure 3. Fluorescence microscope images of HCA, CGTH, MBSC, MDA and WJ-MSC cells co-cultured with PKH26 labeled WJ-exosomes (red) up taking exosomes. PKH26 labeled exosomes can be seen in cytoplasm's of all cells used in this experiment.

pose-MCS derived exosomes increase tumour growth in glioblastoma cells by cellular communication [29], and again Adipose-MCS derived exosomes stimulate cell migration and cell proliferation by inducing Wnt signalling in breast cancer cells [38]. When we had examined the other studies that put forward exosomes trigger cancer, we realized that cancer stem cells derived exosomes are the main character in the carcinogenesis process. As reported by these studies, the expression of mir15a was decreased in the MM patients' bone marrow-MSC derived exosomes rather than exosomes derived from healthy bone marrow-MSC this diminish triggered tumour growth [30]; cell growth in glioblastoma is triggered by the *CLIC1* which is the cargo of the GBM-CSC-derived exosomes [39] and angiogenesis in renal cancer is

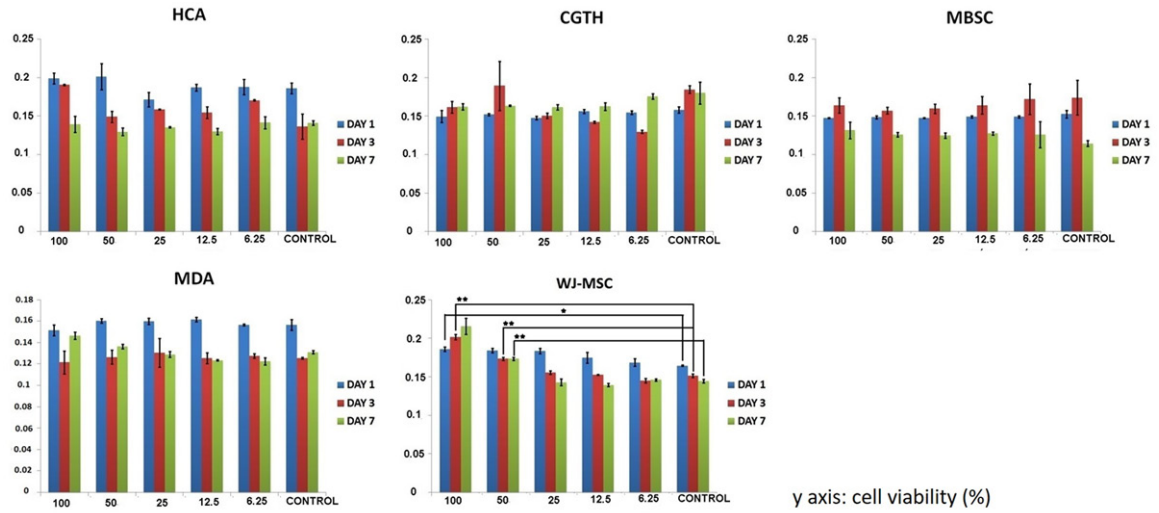

Figure 4. Viability and proliferation amounts of HCA, CGTH, MBSC, MDA and WJ-MSCs co-cultured with different quantities of WJ-exosomes (6.25, 12.5, 25, 50, 100 ug) are determined via WST-1 test on 1st, 3rd and 7th days. A significant increase was seen on groups of WJ-MSC which were treated with 100 mg and 50 mg WJ-exosomes (P<0.05, P*; P<0.01, P**).

induced by transferring of proangiogenic factors of CD105 positive-CSC-derived exosomes [40]. This is obvious that the roles of exosomes in the carcinogenesis process are determining by their sources.

Our previous study suggested that WJ-MSC is an alternative source for clinical use [41]. Consequently, in this study we co-cultured WJ-MSC derived exosomes and 4 different cancer cell lines then measured the proliferation amounts with WST-1 and xCelligence assays and measured the common proliferation markers PCNA. We have tried to understand whether WJ-MSC derived exosomes promote proliferation of different epithelial originated cancer cells or not. In conclusion, we observed that WJ-MSC derived exosomes did not cause an additional proliferation on cancer cell lines. There are other studies that supporting our result. Wu s. et. al. showed that WJ-MSC derived exosomes inhibit the cancer cell viability of bladder tumour T24 cells by cell cycle arrest and induce apoptosis in T24 cells in vitro and in vivo by downregulating phosphorylation of Akt protein kinase and upregulating cleaved caspase-3 exosomes play anti-proliferative role on T24 cells [31]. As same as previous study, WJ- and BM-MSC derived exosomes also induced apoptosis by inducing sub-G1 phase of malignant glioblastoma U87MG cells [29]. In addition to the apoptosis, WJ- and Bone Marrow-MSC derived exosomes reduced the proliferation rate both in vitro and in vivo.

miRNAs are key modulators that can regulate the gene expression both during the mRNA translation and during the post translationally [42]. MSC derived exosomes are demonstrated anti-tumour [43, 44] functions by releasing mRNA and miRNA cargos for cellular communication by cellular uptake [45, 46]. A few studies have shown that, MSC-derived exosomes have shuttled anti-tumorigenic miRNAs. One of them has shown that miR-124 is downregulated in glioma cells than normal tissues [47, 48]. WJ-MSC derived exosomes delivered miR-124 and decreases the migration of U87 glioblastoma multiform (GBM) cells [49]. Also, MSC-derived exosomes inhibited overexpressed mir-146b and reduced glioma growth in rats [50].

Another published study has shown the anti-tumour effects of exosomes can be examined by the effects of proapoptotic role, epithelium-mesenchymal transition (EMT) induction and angiogenesis modulation by miRNAs. EMT has a crucial role in cancer development and metastasis [51]. Not surprisingly, some paracrine signals have a role in cancer also play role in EMT such as WNT/β-catenin signalling, FGF and TGF-β [52]. It was reported that, bone marrow-MSC derived exosomes induced cell death in the ovarian tumour, Kaposi's sarcoma and

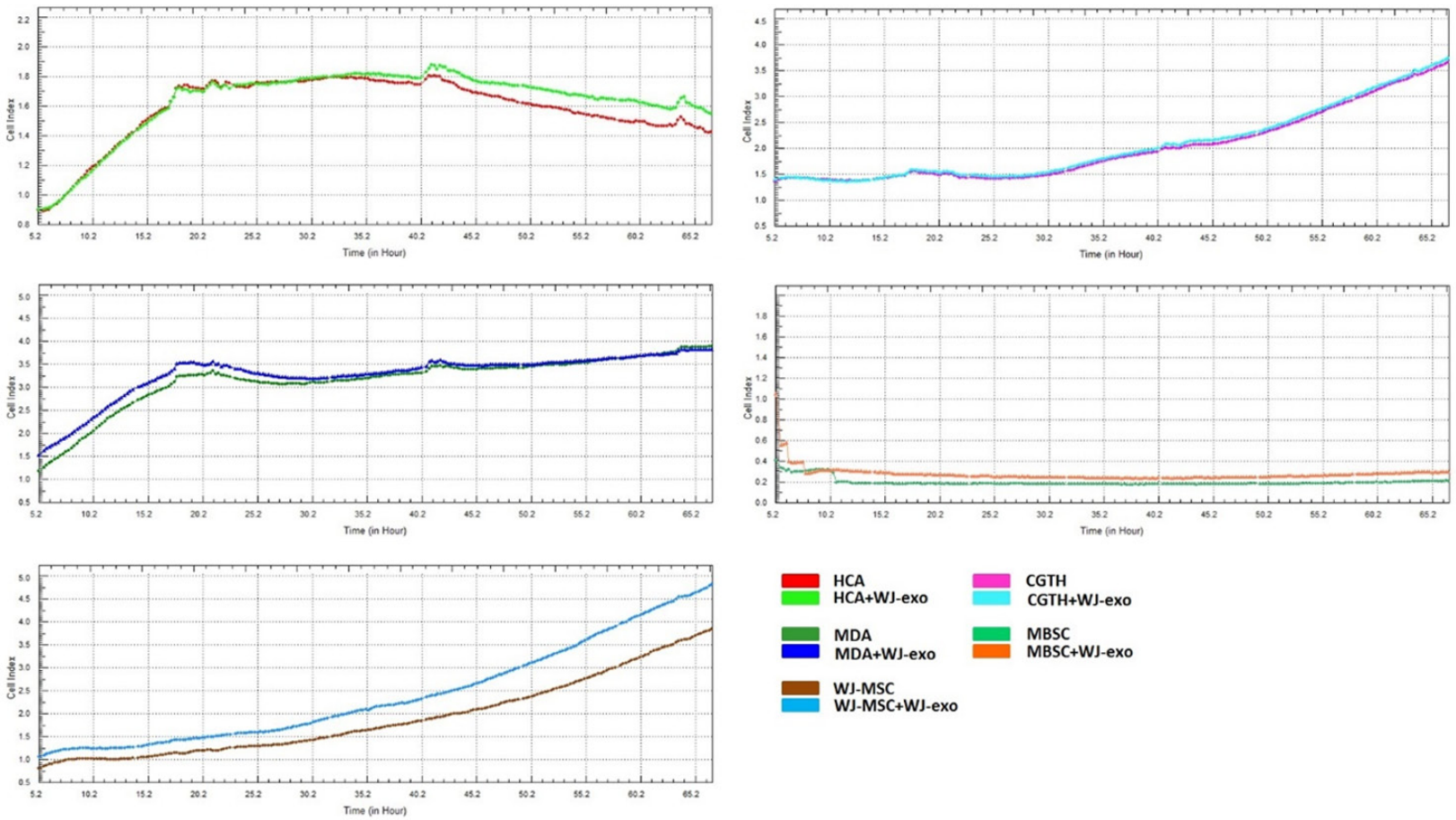

Figure 5. Proliferation assay for co-cultured cells and WJ-exosomes via xCELLigence (RTCA-DP, ACEA Biosciences).

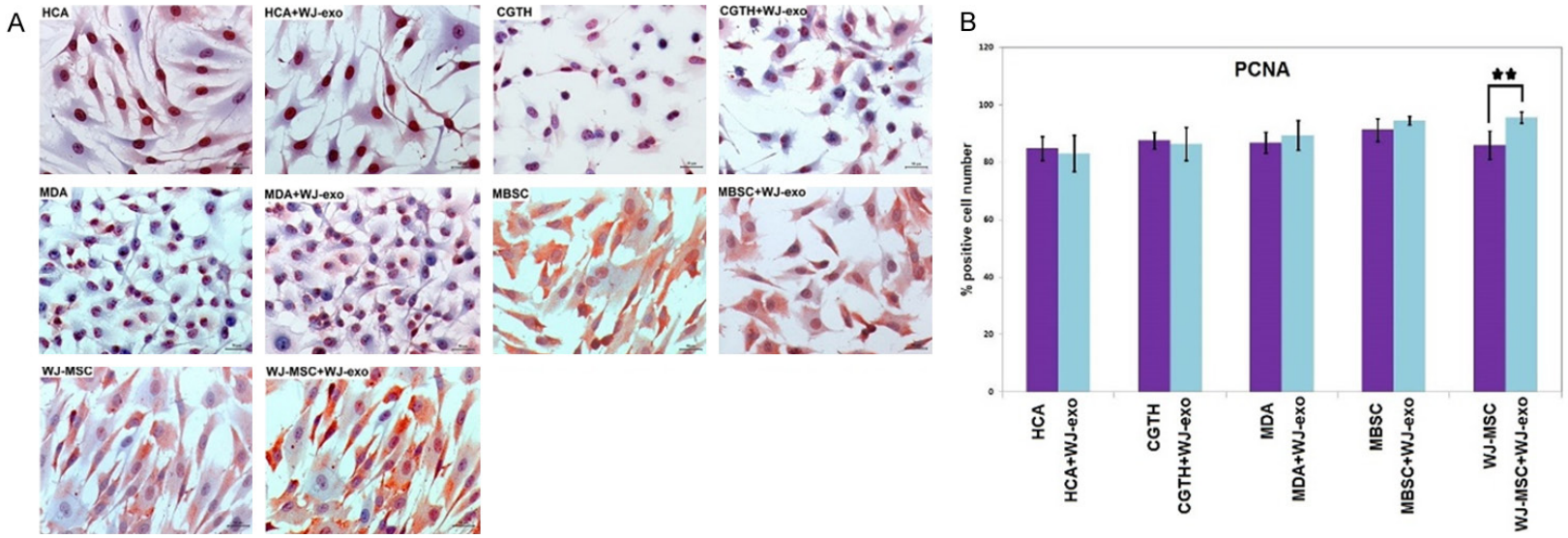

Figure 6. A. Immunohistochemical PCNA staining of HCA, CGTH, MBSC, MDA and WJ-MSC cells co-cultured with exosomes. The number of PCNA positive cells are higher in WJ-exosome treated group when compared with other experimental groups. B. The statistical graph of PCNA positive cells in HCA, CGTH, MBSC, MDA and WJ-MSC cells which are co-cultured with exosomes. Significant difference was observed only in WJ-exosomes treated WJ-MSC group ($P<0.01$, P**).

hepatoma cell lines in vitro [53]. In EMT aspect, they induced EMT markers of *FGF19-FGFR* in carcinomas [54]. Moreover, mir-16 is overexpressed in breast cancer by MSC-derived exosomes and the angiogenesis marker of VEGF is downregulated which is the target of mir-16 [55]. When we discussed all these outcomes, MSC-derived exosomes are inhibiting angiogenesis, tumour growth and inducing the proliferation.

Thus, it is well known that the tumour microenvironment is correlated with tumorigenesis and cancer progression. In this niche, also MSCs are present and they may undergo malignant transformation. However, we cannot say that MSCs have a role in tumorigenesis. On parallel to this information, exosomes are the key components of intercellular communication with the ability to manipulate the local and systemic extracellular niche and tumour microenvironment [25, 56, 57]. Exosomes modulate extracellular niches either immune-stimulatory or inhibitory functions or both [58].

Disclosure of conflict of interest

None.

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References

[1] Gnecchi M, Danieli P, Malpasso G and Ciuffreda MC. Paracrine mechanisms of mesenchymal stem cells in tissue repair. Methods Mol Biol 2016; 1416: 123-146.

[2] Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS and Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005; 11: 367-368.

[3] Raposo G and Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200: 373-383.

[4] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ and Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654.

[5] Fujita Y, Kadota T, Araya J, Ochiya T and Kuwano K. Clinical application of mesenchymal stem cell-derived extracellular vesicle-based therapeutics for inflammatory lung diseases. J Clin Med 2018; 7.

[6] Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W and Xu W. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev 2013; 22: 845-854.

[7] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP and Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010; 4: 214-222.

[8] Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M and Rice GE. Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. PLoS One 2013; 8: e68451.

[9] Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK and de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 2013; 10: 301-312.

[10] Feng Y, Huang W, Wani M, Yu X and Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. PLoS One 2014; 9: e88685-e88685.

[11] Yu B, Gong M, Wang Y, Millard RW, Pasha Z, Yang Y, Ashraf M and Xu M. Cardiomyocyte protection by GATA-4 gene engineered mesenchymal stem cells is partially mediated by translocation of miR-221 in microvesicles. PLoS One 2013; 8: e73304-e73304.

[12] Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C and Camussi G. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol 2009; 20: 1053-67.

[13] Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C and Camussi G. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. Nephrol Dial Transplant 2011; 26: 1474-83.

[14] He J, Wang Y, Sun S, Yu M, Wang C, Pei X, Zhu B, Wu J, Zhao W. Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model. Nephrology 2012; 17: 493-500.

[15] Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti E, Capelli C, Introna M, Remuzzi G and Benigni A. Transfer of growth factor receptor mRNA via exosomes unravels the re-

generative effect of mesenchymal stem cells. Stem Cells Dev 2013; 22: 772-80.
[16] Zhang B, Wang M, Gong A, Zhang X, Wu X, Zhu Y, Shi H, Wu L, Zhu W, Qian H and Xu W. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. Stem Cells 2015; 33: 2158-2168.
[17] Payne SR and Kemp CJ. Tumor suppressor genetics. Carcinogenesis 2005; 26: 2031-2045.
[18] Plaks V, Kong N and Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell 2015; 16: 225-38.
[19] Greaves M and Maley CC. Clonal evolution in cancer. Nature 2012; 481: 306.
[20] Ponnusamy MP and Batra SK. Ovarian cancer: emerging concept on cancer stem cells. J Ovarian Res 2008; 1: 4.
[21] Witsch E, Sela M and Yarden Y. Roles for growth factors in cancer progression. Physiology (Bethesda) 2010; 25: 85-101.
[22] Hall B, Andreeff M and Marini F. The participation of mesenchymal stem cells in tumor stroma formation and their application as targeted-gene delivery vehicles. In: Kauser K, Zeiher AM, editors. Bone Marrow-Derived Progenitors. Berlin; Heidelberg: Springer Berlin Heidelberg; 2007. pp. 263-283.
[23] Ribeiro AL and Okamoto OK. Combined effects of pericytes in the tumor microenvironment. Stem Cells International 2015; 2015: 8.
[24] Bergfeld SA and DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev 2010; 29: 249-61.
[25] Vallabhaneni KC, Penfornis P, Dhule S, Guillonneau F, Adams KV, Mo YY, Xu R, Liu Y, Watabe K, Vemuri MC and Pochampally R. Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. Oncotarget 2015; 6: 4953-4967.
[26] Wolfson B, Eades G and Zhou Q. Roles of microRNA-140 in stem cell-associated early stage breast cancer. World J Stem Cells 2014; 6: 591-7.
[27] Zhu W, Huang L, Li Y, Zhang X, Gu J, Yan Y, Xu X, Wang M, Qian H and Xu W. Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo. Cancer Lett 2012; 315: 28-37.
[28] Oviedo NJ and Beane WS. Regeneration: the origin of cancer or a possible cure? Semin Cell Dev Biol 2009; 20: 557-564.
[29] Del Fattore A, Luciano R, Saracino R, Battafarano G, Rizzo C, Pascucci L, Alessandri G, Pessina A, Perrotta A, Fierabracci A and Muraca M. Differential effects of extracellular vesicles secreted by mesenchymal stem cells from different sources on glioblastoma cells. Expert Opin Biol Ther 2015; 15: 495-504.
[30] Roccaro AM, Sacco A, Maiso P, Azab AK, Tai YT, Reagan M, Azab F, Flores LM, Campigotto F, Weller E, Anderson KC, Scadden DT and Ghobrial IM. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J Clin Invest 2013; 123: 1542-1555.
[31] Wu S, Ju GQ, Du T, Zhu YJ and Liu GH. Microvesicles derived from human umbilical cord Wharton's jelly mesenchymal stem cells attenuate bladder tumor cell growth in vitro and in vivo. PLoS One 2013; 8: e61366.
[32] Dai A, Baspinar O, Yesilyurt A, Sun E, Aydemir CI, Oztel ON, Capkan DU, Pinarli F, Agar A and Karaoz E. Efficacy of stem cell therapy in ambulatory and nonambulatory children with Duchenne muscular dystrophy - Phase I-II. Degener Neurol Neuromuscul Dis 2018; 8: 63-77.
[33] Caplan AI. Mesenchymal stem cells. J Orthop Res 1991; 9: 641-650.
[34] Conrad C and Huss R. Adult stem cell lines in regenerative medicine and reconstructive surgery. J Surg Res 2005; 124: 201-208.
[35] Gnecchi M, Zhang Z, Ni A and Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008; 103: 1204-1219.
[36] Kumar D, Gupta D, Shankar S and Srivastava RK. Biomolecular characterization of exosomes released from cancer stem cells: possible implications for biomarker and treatment of cancer. Oncotarget 2015; 6: 3280-3291.
[37] Maia J, Caja S, Strano Moraes MC, Couto N and Costa-Silva B. Exosome-based cell-cell communication in the tumor microenvironment. Front Cell Dev Biol 2018; 6: 18.
[38] Lin R, Wang S and Zhao RC. Exosomes from human adipose-derived mesenchymal stem cells promote migration through Wnt signaling pathway in a breast cancer cell model. Mol Cell Biochem 2013; 383: 13-20.
[39] Setti M, Osti D, Richichi C, Ortensi B, Del Bene M, Fornasari L, Beznoussenko G, Mironov A, Rappa G, Cuomo A, Faretta M, Bonaldi T, Lorico A and Pelicci G. Extracellular vesicle-mediated transfer of CLIC1 protein is a novel mechanism for the regulation of glioblastoma growth. Oncotarget 2015; 6: 31413-31427.
[40] Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B and Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res 2011; 71: 5346-56.
[41] Karaöz E, Çetinalp Demircan P, Erman G, Güngörürler E and Eker Sarıboyacı A. Comparative analyses of immunosuppressive characteristics of bone-marrow, wharton's jelly, and adipose tissue-derived human mesenchymal

stem cells. Turk J Haematol 2017; 34: 213-225.
[42] Fabian MR, Sonenberg N and Filipowicz W. Regulation of mRNA Translation and Stability by microRNAs. Annu Rev Biochem 2010; 79: 351-79.
[43] Du T, Ju G, Wu S, Cheng Z, Cheng J, Zou X, Zhang G, Miao S, Liu G and Zhu Y. Microvesicles derived from human Wharton's jelly mesenchymal stem cells promote human renal cancer cell growth and aggressiveness through induction of hepatocyte growth factor. PLoS One 2014; 9: e96836-e96836.
[44] Fonsato V, Collino F, Herrera MB, Cavallari C, Deregibus MC, Cisterna B, Bruno S, Romagnoli R, Salizzoni M, Tetta C and Camussi G. Human liver stem cell-derived microvesicles inhibit hepatoma growth in SCID mice by delivering antitumor microRNAs. Stem Cells 2012; 30: 1985-98.
[45] Camussi G, Deregibus MC, Bruno S, Cantaluppi V and Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 2010; 78: 838-48.
[46] Christianson HC, Svensson KJ, van Kuppevelt TH, Li JP and Belting M. Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity. Proc Natl Acad Sci U S A 2013; 110: 17380-5.
[47] An L, Liu Y, Wu A and Guan Y. microRNA-124 inhibits migration and invasion by down-regulating ROCK1 in glioma. PLoS One 2013; 8: e69478-e69478.
[48] Wei J, Wang F, Kong LY, Xu S, Doucette T, Ferguson SD, Yang Y, McEnery K, Jethwa K, Gjyshi O, Qiao W, Levine NB, Lang FF, Rao G, Fuller GN, Calin GA and Heimberger AB. miR-124 inhibits STAT3 signaling to enhance T cell-mediated immune clearance of glioma. Cancer Res 2013; 73: 3913-26.
[49] Sharif S, Ghahremani MH and Soleimani M. Delivery of exogenous miR-124 to glioblastoma multiform cells by Wharton's Jelly mesenchymal stem cells decreases cell proliferation and migration, and confers chemosensitivity. Stem Cell Rev 2018; 14: 236-246.
[50] Katakowski M, Buller B, Zheng X, Lu Y, Rogers T, Osobamiro O, Shu W, Jiang F and Chopp M. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett 2013; 335: 201-4.
[51] Heerboth S, Housman G, Leary M, Longacre M, Byler S, Lapinska K, Willbanks A and Sarkar S. EMT and tumor metastasis. Clin Transl Med 2015; 4: 6.
[52] Micalizzi DS, Farabaugh SM and Ford HL. Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia 2010; 15: 117-134.
[53] Bruno S, Collino F, Deregibus MC, Grange C, Tetta C and Camussi G. Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth. Stem Cells Dev 2013; 22: 758-771.
[54] Shi S, Zhang Q, Xia Y, You B, Shan Y, Bao L, Li L, You Y and Gu Z. Mesenchymal stem cell-derived exosomes facilitate nasopharyngeal carcinoma progression. Am J Cancer Res 2016; 6: 459-472.
[55] Lee JK, Park SR, Jung BK, Jeon YK, Lee YS, Kim MK, Kim YG, Jang JY and Kim CW. Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. PLoS One 2013; 8: e84256-e84256.
[56] Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, Bruno S, Bussolati B and Camussi G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. Blood 2007; 110: 2440-2448.
[57] Deregibus MC, Tetta C and Camussi G. The dynamic stem cell microenvironment is orchestrated by microvesicle-mediated transfer of genetic information. Histol Histopathol 2010; 25: 397-404.
[58] Kahlert C and Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. J Mol Med (Berl) 2013; 91: 431-437.