Experimental Cell Research ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Experimental Cell Research

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

Research Article

Wharton's jelly mesenchymal stromal cells have contrasting effects on proliferation and phenotype of cancer stem cells from different subtypes of lung cancer

Francesca Vulcano [a,1], Luisa Milazzo [a,1], Carmela Ciccarelli [b], Adriana Eramo [a], Giovanni Sette [c], Annunziata Mauro [d], Giampiero Macioce [a], Andrea Martinelli [e], Renato La Torre [f], Patrizia Casalbore [g], Hamisa Jane Hassan [a,2], Adele Giampaolo [a,*,2]

[a] Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
[b] Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy
[c] Regina Elena National Cancer Institute, Rome, Italy
[d] Faculty of Veterinary Medicine, University of Teramo, Italy
[e] Experimental Animal Welfare Sector of the Istituto Superiore di Sanità, Rome, Italy
[f] Department of Gynecology, Obstetrics and Urological Sciences, Sapienza University of Rome, Italy
[g] Institute of Cell Biology and Neurobiology, National Research Council, Rome, Italy

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form
31 May 2016
Accepted 8 June 2016

Keywords:
Mesenchymal stromal cells
Wharton's jelly
Lung cancer stem cells
CD133
ALDH
Tumor xenografts

ABSTRACT

Studies on the role of multipotent mesenchymal stromal cells (MSC) on tumor growth have reported both a tumor promoting and a suppressive effect. The aim of the present study was to determine the effect of MSC isolated from Wharton's jelly of umbilical cord (WJMSC) on lung cancer stem cells (LCSC) derived from human lung tumors: two adenocarcinomas (AC) and two squamous cell carcinomas (SCC). LCSC derived from SCC and AC expressed, to varying extents, the more relevant stem cell markers. The effect of WJMSC on LCSC was investigated *in vitro* using conditioned medium (WJ-CM): a proliferation increase in AC-LCSC was observed, with an increase in the ALDH+ and in the CD133+ cell population. By contrast, WJ-CM hampered the growth of SCC-LCSC, with an increase in the pre-G1 phase indicating the induction of apoptosis. Furthermore, the ALDH+ and CD133+ population was also reduced. *In vivo*, subcutaneous co-transplantation of AC-LCSC/WJMSC generated larger tumors than AC-LCSC alone, characterized by an increased percentage of CD133+ and CD166+ cells. By contrast, co-transplantation of WJMSC and SCC-LCSC did not affect the tumor size. Our results strongly suggest that WJMSC exert, both *in vitro* and *in vivo*, contrasting effects on LCSC derived from different lung tumor subtypes.

1. Introduction

Human multipotent mesenchymal stromal cells (MSC) can be isolated from a wide variety of tissues [1]. MSC are characterized by the expression of cell surface markers CD73, CD90 and CD105, and by the absence of the expression of hematopoietic lineage markers, in accordance with criteria set forth by the ICST [2]. MSC derived from Wharton's jelly of the umbilical cord (WJMSC) are easier to isolate and expand than MSC from other fetal and adult tissues, and impact fields such as regenerative medicine and biotechnology [3].

The role of MSC in carcinogenesis is still controversial. While numerous studies have shown that MSC promote tumor progression and metastasis, others have reported that they suppress tumor growth. Although the reason for this discrepancy is unknown, it may be ascribed to the heterogeneity of MSC, different tumor models or differences in experimental designs [4–6].

Abbreviations: AC, adenocarcinoma; ALDH, aldehyde dehydrogenase; CSC, cancer stem cells; DEAB, diethylaminobenzaldehyde; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLDC, glycine decarboxylase; HE, hematoxylin-eosin; LCSC, lung cancer stem cells; MSC, mesenchymal stromal cells; NHDF, normal human dermal fibroblast; NHDF-CM, normal human dermal fibroblast – conditioned medium; PE, phycoerythrin; PI, proliferation index; SCC, squamous cell carcinoma; WJ-CM, Wharton's jelly mesenchymal stromal cell – conditioned medium; WJMSC, Wharton's jelly mesenchymal stromal cells

* Corresponding author.
E-mail addresses: francesca.vulcano@iss.it (F. Vulcano), luisa.milazzo@iss.it (L. Milazzo), carmela.ciccarelli@univaq.it (C. Ciccarelli), adriana.eramo@iss.it (A. Eramo), giovanni.sette@gmail.com (G. Sette), amauro@unite.it (A. Mauro), giampiero.macioce@iss.it (G. Macioce), andrea.martinelli@iss.it (A. Martinelli), renato.latorre@uniroma1.it (R. La Torre), patrizia.casalbore@cnr.it (P. Casalbore), jane.hassan@iss.it (H.J. Hassan), adele.giampaolo@iss.it (A. Giampaolo).

[1] Equal first authorship.
[2] Equal senior authorship.

http://dx.doi.org/10.1016/j.yexcr.2016.06.003
0014-4827/

Several studies have documented that fetal MSC (*i.e.* from amniotic fluid, umbilical cord blood and umbilical cord) harbor therapeutic potential in cancer treatment, as they can target the tumor site and reduce tumor burden [7]. In particular, WJMSC have been reported to reduce the growth of human breast carcinoma [8], prostate cancer [9], osteosarcoma and ovarian carcinoma cells [10].

The interaction between MSC and lung cancer has yet to be fully understood [11]. One study on the interaction between bone marrow MSC and lung adenocarcinoma cell lines (CL1-5 and A549) reported that MSC have an inhibitory effect on cell proliferation [12], whereas another reported that they enhance tumor formation [13]. In yet another study, bone marrow MSC induced a specific increase in cell proliferation in the stem cell fraction sorted from the A549 cell line [14]. Cancer stem cells (CSC), which are reported to play a major role in tumor origin, are also considered to be responsible for tumor progression as well as for metastasis and drug resistance [15]. Lung CSC (LCSC) from primary tumors were first identified and characterized by Eramo et al. [16]. Cells derived from lung tumor tissues and cultured in stem cell medium form spheres that express stem cell markers CD133 [16], aldehyde dehydrogenase (ALDH) [17], CD44 [18], CD166 and glycine decarboxylase (GLDC), a subset of the CD166-positive cells [19], and display a high tumorigenic potential and capacity to recapitulate the histology of the specific tumor subtype from which CSC are derived [20].

The aim of the present study was to determine the effect of WJMSC on cancer stem cells derived from adenocarcinoma (AC-LCSC) and squamous cell carcinoma (SCC-LCSC) *in vitro* and on tumor formation in a NOD/SCID mouse xenograft model.

2. Material and methods

2.1. Isolation and culture of WJMSC

Fragments of umbilical cords were obtained from the "Azienda Policlinico Umberto I" of Rome, upon written parental informed consent, in accordance with the Declaration of Helsinki. WJMSC were obtained from human umbilical cord deprived of vessels, according to the enzymatic method, as described previously [21]. The cells were resuspended in medium containing Dulbecco's Modified Eagle Medium low glucose (EuroClone, Pero, MI, Italy) supplemented with 10% Fetal Bovine Serum (Hyclone by Thermo Scientific, South Logan, UT, USA), 1% Non-essential Amino Acids (Sigma, Saint Louis, MO, USA), 1% Antibiotics-Antimycotics (Gibco by Life Technologies, Grand Island, NY, USA) and 2 mM L-Glutamine (EuroClone), were plated into 25 cm^2 tissue-culture flasks and were incubated at 37 °C in 5% CO_2. Medium was replaced every three days. WJMSC from passages 7–10 were used for all the experiments.

2.2. LCSC culture

The AC-LCSC-229 and −223 and SCC-LCSC-136 and −36 used in this study have been described previously [16,22]. Briefly, LCSC were isolated from surgically resected human lung tumor samples (two AC and two SCC) through selective culture in serum-free stem cell medium (50 μg/ml insulin, 100 μg/ml apo-transferrin, 10 μg/ml putrescine, 0.03 μM sodium selenite, 2 μM progesterone, 0.6% glucose, 5 mM HEPES, 0.1% sodium bicarbonate, 0.4% BSA, 2 mM L-Glutamine and 1% antibiotics, dissolved in DMEM-F12 medium (Gibco-Invitrogen), supplemented with 20 ng/ml EGF and 10 ng/ml basic FGF (PeproTech, London, UK)) [16]. The resulting undifferentiated tumor spheres were grown in non-treated flasks to reduce cell adherence.

2.3. Preparation of WJMSC- and Normal Human Dermal Fibroblast (NHDF)-conditioned medium (WJ-CM and NHDF-CM)

NHDF were purchased from Lonza (Lonza, Walkersville, MD, USA). Sub-confluent WJMSC and NHDF were grown for 48 h in stem cell medium deprived of hormones and growth factors. WJ-CM and NHDF-CM were then collected, filtered and stored at −80 °C.

Stem cell medium deprived of hormones and growth factors was used as a mock medium. In each experiment, the same preparation of conditioned medium has been used to compare the effect of the different LCSC lines.

2.4. Flow cytometry analysis

Human anti-CD45, anti-CD31 and anti-CD34 (BD Biosciences, Buccinasco, Italy) monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) and human anti-CD90, anti-CD105 (BD Biosciences), anti-CD73 (Dako, Glostrup, Denmark), anti-CD44 (BioLegend, San Diego, CA, USA) anti-CD133/1 (Miltenyi Biotec, Bergisch Gladbach, Germany) and anti-CD166 (RD Systems, Minneapolis, USA) conjugated with phycoerythrin (PE) were used as described [23]. Cells were analyzed using a flow cytometer (FACSCalibur, BD Biosciences) by CellQuest software (BD Biosciences). A minimum of 50,000 events were acquired for each sample.

2.5. Cell cycle analysis

A DNAcon3 kit (Dako) was used for DNA staining. Briefly, 1 ml propidium iodide solution was added into each test tube containing a dehydrated buffer mixture and, after 10 min, cells were added to each tube and incubated at 4 °C for 1 h. The analysis was performed using a flow cytometer (FACSCalibur), and the cell-cycle distribution was analyzed using the Mod-Fit LT software (Verity Software House, Topsham, ME, USA).

2.6. Aldefluor assay

The stem cell population expressing ALDH enzymatic activity was assessed by means of the Aldefluor™ kit (StemCell Technologies, Vancouver, BC, Canada), according to the manufacturer's instructions. Briefly, 1×10^5 cells were resuspended in aldefluor assay buffer containing an ALDH-substrate, and incubated for 45 min at 37 °C; a set of cells was stained using identical conditions with a specific ALDH inhibitor, diethylaminobenzaldehyde (DEAB), as a negative control. Samples were analyzed by FACSCalibur and the resulting fluorescence profiles were compared.

2.7. Western blot analysis

Cells were lysed in 2% SDS containing phosphatase and protease inhibitors (Roche, Mannheim, Germany). Proteins were separated by 5% or 10% SDS-PAGE, and transferred to nitrocellulose membranes (Schleicher & Schüell, Dassel, Germany). Immunoblotting was performed with the following rabbit antibodies: anti-GLDC at a dilution of 1:500 (Abcam, Inc., Cambridge, UK), anti-Cyclin A and anti-Cyclin B at a dilution of 1:250, anti-Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) at a dilution of 1:2000 (SantaCruz Biotechnology, Santa Cruz, CA, USA). Peroxidase-conjugated anti-rabbit IgG (Bethyl, Laboratories Inc., Montgomery, TX, USA) was used to enhance chemiluminescence detection. Protein bands were visualized by means of Amersham ECL detection reagent (GE Healthcare UK) and detected by means of the Chemidoc XRS system (Biorad). The densitometric analysis was performed using Image Lab software (version 4.0, Biorad). Relative

Please cite this article as: F. Vulcano, et al., Wharton's jelly mesenchymal stromal cells have contrasting effects on proliferation and phenotype of cancer stem cells from different subtypes of lung cancer, Exp Cell Res (2016), http://dx.doi.org/10.1016/j.yexcr.2016.06.003