ORIGINAL ARTICLE

Implication of stem cells from adipose tissue in wound healing in obese and cancer patients

Implicación de las células madre derivadas del tejido adiposo en la cicatrización de heridas de pacientes obesos y pacientes oncológicos

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Abstract

Objective: *Certain diseases such as obesity and cancer can cause impaired wound healing. Adipose tissue derived stem cells (ASCs) are a novel field of research. Many studies have evidenced their high degree of safety and potential for wound repair due to their immunomodulatory and tissue-regeneration properties. The purpose of this study is to determine the impact of obesity and cancer on the therapeutic potential of ASCs.* **Materials and methods:** *We isolated and characterized the phenotype, differentiation capacities, secretome, and in vitro migration capacities of ASCs. Furthermore, we analyze their capacity of in vitro migration associated with the plasma of the different patients.* **Results:** *We observed that ASCs isolated from obese and cancer patients have the same phenotype, cell proliferation, and migration capacities as ASCs derived from healthy donors. However, they do not have the same differentiation potential and exhibit distinct profiles of both pro-inflammatory and regulatory secreted cytokines, which, together with the signals received from the bloodstream, could account for the impaired healing in patients with these diseases.* **Conclusions:** *We consider the ASCs from patients with either obesity or cancer are slightly altered, and this may be the cause of worse wound healing in these patients.*

Keywords: *Wound healing. Obesity. Cancer. Mesenchymal stem cells.*

Resumen

Objetivo: *Enfermedades como la obesidad y el cáncer pueden alterar la cicatrización de las heridas. Las células madre derivadas del tejido adiposo (ASC) abren un nuevo campo de investigación ya que muchos estudios han demostrado su utilidad y alto grado de seguridad para la reparación de heridas debido a sus propiedades inmunomoduladoras y de regeneración tisular. El propósito de este estudio es determinar el impacto de la obesidad y el cáncer en el potencial terapéutico de las ASCs.* **Material y métodos:** *Aislamos y caracterizamos el fenotipo, la capacidad de diferenciación, el secretoma y la capacidad de migración in vitro de las ASC. Asimismo, analizamos la capacidad de migración in vitro asociada al plasma de los diferentes pacientes.* **Resultados:** *Observamos que las ASC aisladas de pacientes obesos y con cáncer tienen el mismo fenotipo, proliferación celular y capacidades de migración que las ASCaisladas de donantes sanos. Sin embargo, no tienen el mismo potencial de diferenciación y exhiben perfiles distintos de citoquinas secretadas tanto proinflamatorias como*

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reguladoras. **Conclusiones:** *Consideramos que las ASC de pacientes con obesidad o cáncer están levemente alteradas. Esta puede ser la causa de una peor cicatrización de las heridas en este tipo de pacientes.*

Palabras clave: *Cicatrización de heridas. Obesidad. Cancer. Células madre mesenquimales.*

Introduction

Chronic wounds currently affect more than 6 million people in the United States, resulting in billions of dollars in health-care costs each year[1]. In Europe, meanwhile, nearly 2 million people suffer from acute or chronic wounds[2]. This generates a pressing worldwide health problem causing a substantial burden on global health both economically and socially.

The process by which wounds heal has traditionally been divided into three main stages: inflammation, cell proliferation, and remodeling. Nowadays, however, this is understood to be much more complex than a simple three-stage phenomenon[3-5]. Eicosanoids, cytokines, growth factors, and nitric oxide are the inflammatory mediators implicated in wound repair, and the cell response involves platelets, neutrophils, macrophages, monocytes, fibroblasts, keratinocytes, endothelial cells, T lymphocytes, and possibly resident stem cells[6,7]. It is possible that both the signals and the cells above mentioned are altered by different mechanisms associated with some pathologies.

Wounds that fail to heal usually become arrested in the chronic inflammation phase, where both systemic factors and cell migration could be altered, rendering them unable to undergo the normal healing process[8]. In recent years, many studies have sought to further the understanding of the biology and pathology of wound healing, though much remains to be learned about the treatment of chronic wounds[9]. To address this issue, emerging cell therapies, including those that employ adipose-derived stem cells (ASCs), have become therapeutic alternatives for tissue regeneration[10,11].

Adipose tissue is a source of mesenchymal stem cells, which are widely distributed throughout the body. These cells are capable of secreting several factors that stimulate the wound-healing process, such as TGFα, VEGF, KGF, FGF2, PDGF, HGF, fibronectin, and collagen, among others[12]; in addition, ASCs also secrete cytokines and chemokines, which can regulate the inflammatory process during healing[13-15]. Many studies suggest that ASCs can improve tissue regeneration through paracrine mechanisms and have the capacity to stimulate other cells such as keratinocytes and fibroblasts[16,17]. They exhibit substantial anti-inflammatory and regeneration activity and are easy to obtain, expand, and culture[18], and many ongoing clinical trials are using human adipose-derived stem cells to treat damaged tissues in several diseases.

To explore the viability of ASCs to benefit wound healing, the previous studies by our group has analyzed the characteristics of ASCs derived from either obese or cancer patients whose risk of poor healing discouraged use of these cells as therapeutic tools[19-22]. Obesity, defined as abnormal or excessive accumulation of fat, is associated with low-grade chronic inflammation of adipose tissue[19], possibly impairing health[20]. In cancer patients, tumors activate molecular and cellular mechanisms linked to impaired wound healing[21,22]. This impairment is also affected by the systemic toxic effect of the chemotherapy drugs and radiation therapies these patients frequently receive[23,24]. In light of these difficulties, we analyzed ASCs from different patients, studying their phenotype, proliferation, migration activity, differentiation potential, and cytokine secretion to determine the ways in which these cells could influence the healing process[25,26]. In addition to comparing the differences between the cells of the different pathological groups, at a functional level the migration capacity of the different cell groups, using a protocol described by Grada et al.[27] also known as the “in vitro scratch assay;” It was analyzed using in all cases the same plasma enriching the cell culture medium, plasma from a donor without associated pathologies, it could be considered to be equivalent to an allogeneic graft (allogeneic use), since histocompatibility studies were not analyzed between the donor and the patients from whom the cells came.

Materials and methods

Patients samples

The study protocol is adhered to the ethical guidelines of the 1975 Declaration of Helsinki, a prerequisite for the approval granted by the Institutional Ethics Committee of the Fundación Jiménez Díaz University Hospital, Madrid, Spain (PIC number 23/2015_FJD). All patients provided written informed consent.

ASCs were obtained from three different donor groups undergoing elective surgery in the Fundación Jiménez Díaz University Hospital located in Madrid, Spain. These groups included healthy donors (n = 4), obese patients (n = 7), and cancer patients (n = 5). Obese patients were subjects having either a body mass index (BMI) ≥ 35 with associated comorbidities or a BMI ≥ 40 who had undergone elective laparoscopic gastric bypass surgery. Cancer patients were subjects diagnosed with gastric and esophageal cancer. These patients had undergone elective total gastrectomy or esophagectomy (n = 1), transhiatal esophagectomy (n = 1), or three-field minimally invasive esophagectomy (n = 3). A very homogeneous group of patients have been selected in terms of location and tumor stage. Considering that all samples (blood and adipose tissue) are collected before and at the beginning of the surgery, respectively, we can limit the variability between the collected samples. Hematological and biochemical parameters were collected for all (Table 1).

ASC isolation and cell culture

Cells were isolated and cultured according to the protocol previously published by our laboratory[28], though with slight modifications for adipose tissue biopsy. Briefly, adipose tissue samples were thoroughly washed with phosphate buffered saline (PBS) and digested mechanically with scissors and enzymatically using 0.075% collagenase type I (Gibco®, Invitrogen™ Life Technologies™, San Diego, CA, USA) for 1 h at 37°C under constant agitation. These digested samples were then inhibited using fetal bovine serum (FBS) and centrifuged, and the cell pellet was resuspended in Dulbecco's modified Eagle's medium high-glucose (DMEM) plus 10% FBS and 1% penicillin/streptomycin. Cells were incubated at 37°C in 5% CO_2. Each experiment was performed at least in triplicate, using cells in passages 2-7.

Flow cytometry analysis

The analysis was performed using monoclonal antibodies against CD90, CD73, CD29, CD45, CD34, and HLA-DR (Merck-Millipore, Billerica, MA, USA) according to the minimal criteria established by the International Society of Cell Therapy to verify their status as mesenchymal stem cells[29]. Briefly, cells in passage 2 were trypsinized, washed, and resuspended with PBS and incubated with the specific antibodies at 4°C for 30 min and analyzed using a BD FACSCanto II flow cytometer (Becton Dickinson, San Jose, CA, USA). At least 10 000 events were obtained in each case.

Cell differentiation

The cells were differentiated following the manufacturer's instructions (StemPro® Adipogenesis Differentiation Kit and StemPro® Osteogenesis Differentiation Kit, Gibco®). Briefly, we seeded the cells in a 12-well plate at a density of 10 000 cells/cm^2 for adipogenic differentiation and 5000 cells/cm^2 for osteogenic differentiation, and these cells were incubated for 14 or 28 days, respectively. The specific differentiation medium was replaced every 3-4 days. After specific periods of culture, differentiated cells were detected with Oil Red O staining for adipogenic differentiation and Alizarin Red S staining for osteogenic differentiation. Finally, staining intensity was measured by spectrophotometry: Oil Red O was extracted with 100% isopropanol and measured at 510 nm, and Alizarin Red S was extracted with cetylpyridinium chloride in 10 mM Na2HPO4 (pH7) and measured at 540 nm.

Cell proliferation assay

Cell proliferation was analyzed by AlamarBlue® (AbDSerotec, Oxford, United Kingdom) assay according to the manufacturer's protocol. Briefly, cells were seeded in a multiwell plate containing 48 wells at a density of 10 000 cells/cm^2. After 24 h, AlamarBlue® was added at a concentration of 10% to each well and was incubated for 4 h. Fluorescence was measured in a microplate reader (EnSpire® ultimo de Plate Reader, Perkin Elmer, Waltham MA, USA) at days 1, 4, and 7. The samples were analyzed in triplicate in all cases.

Wound healing scratch assay

Wound healing was assessed by scratch assay performed in triplicate using multiwell culture dishes containing 12 wells each. Cells were seeded at confluence and allowed to attach and form a cell monolayer. After 24 h, a scratch was performed in the cell culture using a 100-μl sterile pin tool to form a cell-free zone to allow cells migration[30]. Cells were treated with DMEM plus low concentrations of healthy donor plasma at

Table 1. Patients' clinical features

	Healthy	Obese	Cancer
Age (years)	43.75 (± 7.93)	39.43 (± 6.97)	56.60 (± 10.88)
Gender (Male/Female)	75%/25%	14.3%/85.7%	100%/0
BMI (kg/m^2)	26.25 ± 4.03	44.14 ± 5.46	23.6 ± 4.04
ASA score*	ASA ≤ II: 4 (100%)	ASA ≤ II: 4 (57.1%) ASA > II: 3 (42.9%)	ASA ≤ II: 4 (80%) ASA > II: 1 (20%)
Comorbidities			
Smoking	25%	14,3%	40%
Alcohol consumption	0	0	20%
Hypertension	0	14.3%	20%
Hematological and biochemical parameters			
Hemoglobin (g/dl)	14.73 ± 1.54	14.11 ± 1.12	13.10 ± 2.67
Absolute lymphocyte count (×10^3 µl)	2.45 ± 0.82	2.16 ± 0.99	2.14 ± 0.64
Glucose (mg/dl)	88 ± 6.78	95 ± 15.58	90 ± 4.42
Urea (mg/dl)	32 ± 8.29	28.20 ± 6.87	25.25 ± 7.22
Creatinine (mg/dl)	0.9 ± 0.08	0.74 ± 0.15	0.84 ± 0.11
Plasma protein (g/dl)	7.2 ± 0.14	7.17 ± 0.55	6.3 ± 0.95
Albumin (g/dl)	4.35 ± 0.24	4.3 ± 0.35	3.82 ± 0.31
AST (UI/l)e	25 ± 8.72	20.67 ± 8.17	28.4 ± 10.36
ALT (UI/l)[c]	24 ± 9.54	28.86 ± 14.61	37.25 ± 20.42
Total cholesterol (mg/dl)	180 ± 17.01	193.43 ± 21.31	178.67 ± 19.09
Triglyceride (mg/dl)	**97 ± 26.51**	141 ± 78.48	119.33 ± 68.66
HDL cholesterol[b]	49.50 ± 9.19	39.71 ± 10.18	**60.33 ± 6.51**
LDL cholesterol[ò]	128.5 ± 12.02	122.14 ± 19.54	**97.33 ± 20.43**
Cholesterol/HDL	3.88 ± 0.59	**5.38 ± 1.55**	2.98 ± 0.42
Iron (µg/dl)	93.33 ± 40	78.43 ± 18.17	88.67 ± 84.3
Transferrin (mg/dl)	282.33 ± 60.29	278.83 ± 45.46	251.67 ± 71.57
Glycoside hemoglobin -HbAc1 (%)	5.25 ± 0.07	6.2 ± 1.72	NA

*ASA: The American Society of Anaesthesiologists (ASA) Physical Status Classification System is used to establish a person's functional capacity. ASA grades are a simple **scale** describing a person's fitness to be given an anesthetic for a procedure:
ASA I A normal healthy patient. ASA II A patient with mild systemic disease. ASA III A patient with severe systemic disease. ASA IV A patient with severe systemic disease that is a constant threat to life. ASA V A moribund patient who is not expected to survive without the operation. [e]AST: aspartate aminotransferase [c]ALT: alanine aminotransferase [b]HDL Cholesterol: High-density lipoprotein cholesterol. [ò]LDL Cholesterol: Low-density lipoprotein cholesterol. NA: not applicable.
Clinical, hematological, and biochemical features of different patients used in the study. All values are shown as mean ± standard deviation. The most relevant values appear in bold type.

1% without FBS[31], and photographs of the cell cultures were taken at 0, 24, and 48 h. These photographs were analyzed by ImageJ software to measure the wound area (denuded area) at each time point in accordance with Grada et al.[27] Denuded areas in control cultures treated with DMEM plus 10% FBS were considered positive controls.

Cytokine analysis

To obtain the cell secretome, cells were seeded in a 48-well plate. At 80% confluence, they were washed 3 times and the medium was replaced with serum-free DMEM. After 48 h, the medium was collected and centrifuged at 1000× *g* for 10 min. The supernatant was stored at −80°C until use.

To analyze the levels of cytokines present in the secretome released by the cells, the supernatants were tested using MILLIPLEX® MAP Human High Sensitivity T Cell Magnetic Bead Panel (EMD Millipore Corp). The cytokines and chemokines analyzed were ITAC(CXCL11), GM-CSF, Fractalkine, IFNγ, IL-10, MIP-3α, IL-12, IL-13, IL-17A, IL-1β, IL-2, IL-21, IL-4, IL-23, IL-5, IL-6, IL-7, IL-8, MIP-1α, MIP-1β, and TNF-α. The experiments were carried out following the manufacturer's instructions and the levels of cytokines were detected by MAGPIX® technology (Millipore, MA, USA), with all samples analyzed in triplicate.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software. Results are expressed as median values ± SD. Normality was analyzed using the Shapiro–Wilk test and statistical significance was assessed by the Kruskal–Wallis test. Values of $p < 0.05$ were considered statistically significant.

Results

Patient data

A total of 16 patients were included in the study, and their demographic features are shown in table 1. Obese and cancer patients had a higher rate of associated comorbidities. However, there were no significant differences between the three groups in terms of comorbidities or ASA score.

As concerns hematological and biochemical parameters, the obese patients had higher levels of glucose and glycoside hemoglobin (HbAc1). They also had higher levels of cholesterol, triglycerides, and total cholesterol/HDL ratio, but no statistically significant differences were observed.

The cancer patients showed lower iron and transferrin levels, as well as lower levels of plasma protein. Their levels of albumin were significantly lower ($p < 0.05$), lending further support to their status as malnourished patients.

Cell morphology

Cells were expanded in tissue-culture flasks and their morphology was examined under a light microscope. Human ASCs derived from different patient groups showed the typical spindle-shaped and fibroblast-like morphology of mesenchymal stem cells (not shown). In all cases remained undifferentiated over time, that is, up to 1 month.

Characterization of ASCs: surface-marker analysis and differentiation potential

A surface-marker analysis of all cells by flow cytometry revealed that ASCs were positive for the expression of CD90, CD73, and CD29, and negative for CD34, CD45, and HLA-DR (Fig. 1A-C). This phenotype remained constant in all groups and passages (between 2 and 7), though obese and cancer patients showed lower proportions of CD29 and CD73 positive cells as compared to healthy donor cells.

Human ASCs from patients were differentiated into adipocytes using commercial differentiation media after 14 days of induction. At that time, the cells were stained with Oil Red O and measured by spectrophotometry at 510 nm.

All analyzed human ASCs showed adipogenic differentiation by absorbance at 510 nm, but there were no statistically significant differences between samples from healthy donors (0.12 ± 0.02), cancer patients (0.13 ± 0.03), and obese patients. (0.091 ± 0.02), apart from a lower differentiation capacity observed in the cells of obese patients (Fig. 1D). Accordingly, the number of cells with lipid droplet count per field (10 fields/plates were analyzed at random) showed a similar pattern in the different ASCs studied (Supplementary Fig. 1A-D).

The efficiency of osteogenesis was evaluated after 28 days of culture with differentiation culture media after ASC staining with Alizarin Red S and analysis by spectrophotometry at 540 nm. All analyzed human ASCs were positive for osteogenesis and values were significantly higher in cells isolated from both healthy donors (1.22 ± 0.54) and obese patients (1.25 ± 0.40) than cancer patients (0.87 ± 0.28) (Fig. 1E), according to microscopy count (Supplementary Fig. 1E-H).

In vitro migration capacity

We did not observe any significant differences between study groups when analyzing the migration and healing potential of ASCs by means of a scratch wound assay at passage 3-4. The percentage of cell-free wound area at 24 h was 49.5 ± 8.89 for healthy cells, 58.4 ± 8.40 for those derived from obese patients, and 49 ± 18.57 for cancer-patient samples, whereas at 48 h, this percentage was 22.25 ± 14.12, 29 ± 15.12 ($p = 0.5267$), and 28.52 ± 14.77, respectively (Fig. 2).

Cell proliferation

We examined cell proliferation for 1 week, measuring this at a fluorescence intensity of 590 nm at days 1, 4, and 7 by alamarBlue® assay. We found that ASCs derived from obese and cancer patients proliferated less than those of healthy controls at day 7, although the differences were not statistically significant at any time point (Fig. 3).

Secretome profiles

To study the secretome of ASCs, we used a magnetic bead panel with which we detected 21 cytokines and chemokines (Table 1, Supplementary Table 1). In table 2, we only show those that showed statistically significant variations, We found low expression of pro-inflammatory (MIP3a, IL8, and TNFα) and regulatory (GM-CSF, Fractalkine, IL6, IL7, and IL21)

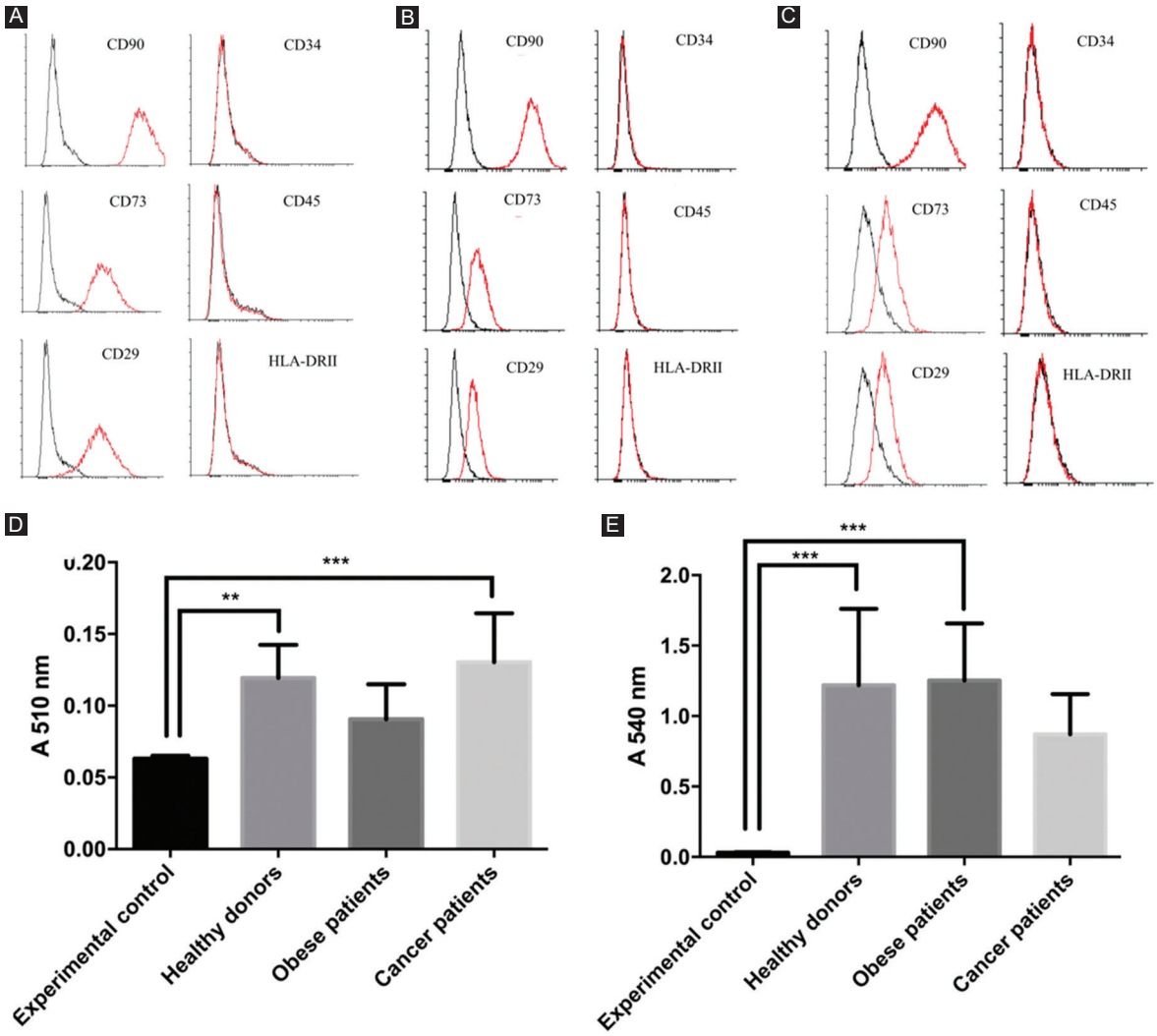

Figure 1. *Phenotypical characterization. Characterization of cultured cells by flow cytometry using 3 cell markers positive for MCS (CD90, CD73, and CD29) and 3 cell markers negative for MSC (CD34, CD45, and HLA-DR). A, Cells from healthy donors; B, from obese patients; C, from cancer patients. D, mean level of adipogenic differentiation of ASCs from different patients (absorbance of Oil Red O). E, mean level of osteogenic differentiation of ASC from different donors (absorbance of Alizarin Red S).*

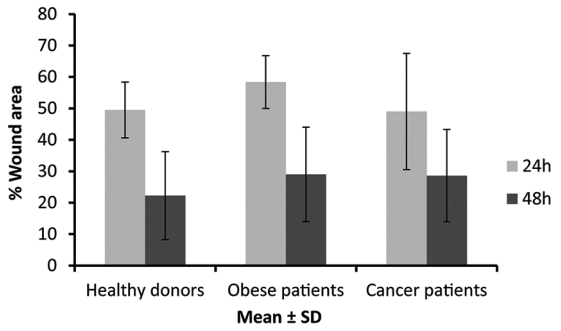

Figure 2. *Wound healing scratch assay. Graphic representation of in vitro wound healing scratch assay using plasm of healthy (allogenic grafts) at 24 (grey) and 48 h (white) of ASCs from different donors (healthy, obese, and cancer). In all cases, measures (mean ± SD) of the wound area (denuded area) at each time point are represented.*

cytokines and chemokines in ASCs derived from obese patients, observing significant differences ($p < 0.01$) in all these values as compared to those of ASCs derived from healthy donors (Table 2). In ASCs derived from cancer patients, the values of GM-CSF, MIP-3a, TNF-α, and IL8 showed significant differences ($p < 0.01$) against those observed in healthy cells (Table 2).

Discussion

Numerous studies have provided evidence of the involvement of ASCs in the wound-healing process owing to the capacity of these cells to secrete a high

Table 2. Secretome analysis

	Healthy		Obese			Cancer		
	Mean	**SD**	**Mean**	**SD**	**p value**	**Mean**	**SD**	**p value**
GM-CSF	53.26	10.4	3.68	0.01	**0.027**	183.69	92.6	> 0.99
Fractalkine	43.74	NA	0	NA	> 0.99	45.18	13.13	0.37
MIP-3α	9.86	1.95	3.40	1.15	> 0.99	22.97	0.25	0.20
IL-21	4.77	0.85	0.77	0.02	0.363	4.72	0.22	> 0.99
IL-6	1356.40	396.27	292.15	90.10	**0.024**	1235.42	874.20	> 0.99
IL-7	11.67	1.65	3.37	0.39	0.176	14.03	2.91	> 0.99
IL-8	5990.13	28	44.75	12.09	0.522	7513.04	941	> 0.99
TNFα	2.01	1. 90	0	NA	**0.004**	0	NA	**0.0009**

Proteins of the secretome revealing differences between cultured cells derived from either healthy individuals or patient groups (obese and cancer). Mean is expressed in pg/mL. ND: not detected, NA: not available. Statistically significant values appear in bold type.

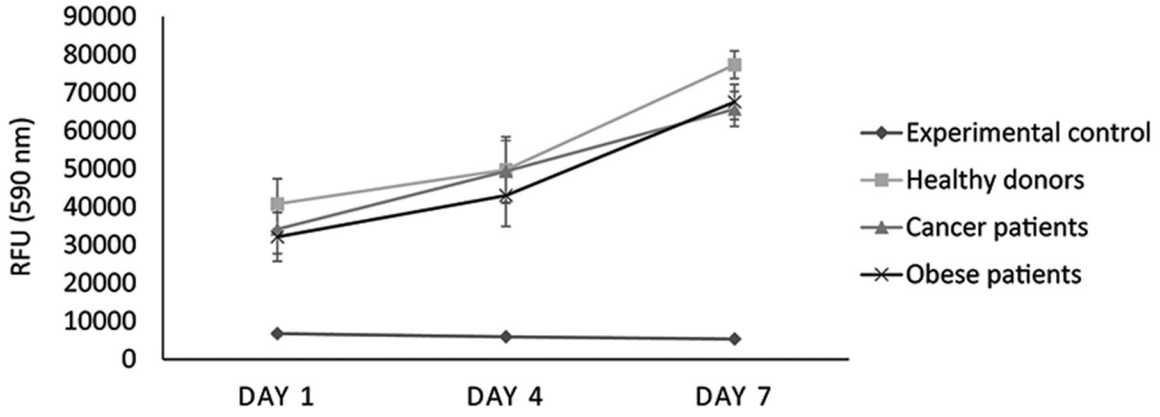

Figure 3. *Proliferation assay. Mean ± SD of the fluorescence measurement at 590 nm on day 1, 4, and 7 as observed following an alamarBlue assay of cells from the different patients.*

number of factors that enhance tissue regeneration[11,13,14,32,33]. Many clinical trials have shown the therapeutic potential of these cells[34,35]. Overall, the ease with which ASCs may be isolated and expanded in vitro, their abundance in adipose tissue, and their regenerative potential make them an exceptionally good cell type for this kind of therapy. However, several factors may impair their qualities, and little is known about the effect of obesity and cancer on the behavior of ASCs[36-38].

In the present study, we have phenotypically and functionally characterized ASCs derived from either obese or cancer patients, comparing these cells to the behavior of healthy ASCs in an attempt to evaluate their potential for therapeutic use for wound repair.

In general, greater similarity is seen between the behavior of ASCs derived from cancer patients and healthy cells than with ASCs isolated from obese patients. However, although the obese patients studied had hyperglycemia as well as higher HbAc1, total cholesterol, triglycerides, LDL, and cholesterol/HDL levels, and lower HDL levels, no statistically significant differences were found when compared to healthy donors. This may be because to enter the bariatric surgery program, obese patients undergo very strict monitoring by an endocrinologist, causing them to lose 5%-10% of their baseline weight and it could has triggered the ASCs and equated their status to the ASCs of the healthy controls. These measures lead to a slight improvement in their metabolic status,

partially normalizing their capacity for wound healing[39,40]. Comparatively, cancer patients had lower BMI and tended to be malnourished, as evidenced by significantly lower levels of albumin, which affected their metabolic status[41]. Indeed, the sample included patients with esophageal and gastric cancer who often experience difficulties with oral intake.

Attempts to establish correlations between phenotypical changes observed in ASCs derived from patients and their capacity for wound healing are met with challenges[42]. It is known that CD73 regulates purinergic signaling through the hydrolysis of ATP/ADP to adenosine, modifying the microenvironment[42], marking a key factor for wound healing in which cell migration plays an essential role[43]. Thus, it was relevant to analyze the migration capacity of the ASCs of these patients[38]. It is also known that CD29 (a β1 integrin chain) plays an important role in mediating the cell-matrix adhesive properties of epithelial cells implicated in wound healing, a topic not analyzed in the current study. However, our results do not demonstrate changes in the capabilities of in vitro wound repair[44]. Despite this observed trend, our data cannot confirm these differences in the migration capacities of cells from different patients; we consider that a larger sample size could clarify this trend in the future.

ASCs derived from cancer patients and healthy ASCs showed no differences in their morphology, proliferative capacity, and results of wound healing scratch assays. The only differences concerned the expression of two molecules used as MSC markers, that is, CD29 and CD73, and a decreased capacity to differentiate to bone cells, the latter finding would be consistent with the non-prevalence of bone metastasis in patients with esophageal cancer[45]. Finally a significative higher values in the production of Fractalkine and MIP-3alfa, both chemokines are involved in inflammatory processes of the epithelium, on the one hand, attracting T cells and monocytes and, on the other hand, activating Th17 and therefore attracting B cells and T cells for recoil and inflammatory processes, the increase in both chemokines has already been observed in inflammatory processes associated with tumors[46].

On the other hand, ASCs derived from obese patients were similar to healthy ASCs, except for the proportions of cells expressing CD29 or CD73, a lower capacity to differentiate into adipocytes, as previously reported[42,47], and significantly lower values in the production of GM-CSF, IL6, and TNFα. Despite these changes, cell migration and repair evaluated *in vitro* in wound healing scratch assays remain unchanged, thus suggesting that these cells are efficient for wound healing.

Explanations remain elusive as regards the increased production of certain cytokines, including GM-CSF, IL6, and TNFα, in ASCs isolated from obese patients, as adipose tissue is an active organ that secretes a large variety of factors, such as leptin, adiponectin, TNF, IL-6, MCP-1, and CCL2, among others[48]. Moreover, in obese individuals, adipose tissue is associated with low-grade chronic inflammation and is characterized by macrophage infiltration, a source of the pro-inflammatory factors that enhance the secretory activity of adipocytes[49]. This low-grade of chronic inflammation in obese samples could be a consequence of the treatment patients undergo prior to the collecting of samples (i.e., diet and monitoring of physical activity) or the effect of the culture medium. However, we cannot rule out the possibility that the cells of patients with cancer or obesity actually have different secretory profiles, although their functional significance resists conclusive understanding. We consider that there are two factors that must be analyzed before reaching any conclusion: The microenvironment in which the cells are found in the body, and the signals that the different pathologies "throw" into the bloodstream.

Conclusions

We demonstrate that ASC differentiation potential and cytokine secretion are slightly modified when cells are derived from either obesity or cancer patients, but their proliferation and *in vitro* wound-healing capabilities are unchanged. Taken together, these findings suggest that, although the ASCs could themselves be involved in the decreased healing capacity seen in these pathologies, the influence of the microenvironment and the signals received through the plasma could be the main cause of the reported defects in healing. New studies to confirm these aspects are necessaries. In addition, this altered behavior of ASCs derived from obese patients or cancer patients might be considered when these cells are used as therapeutic agents. Nevertheless, larger-scale studies and *in vivo* studies are needed to reach a definitive conclusion as to the real influence of cancer and obesity on this treatment approach.

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Ethical approval

The study protocol is adhered to the ethical guidelines of the 1975 Declaration of Helsinki, a prerequisite for the approval granted by the Institutional Ethics Committee of the Fundación Jiménez Díaz University Hospital, Madrid, Spain (PIC number 23/2015_FJD). All patients provided written informed consent.

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Conflicts of interest

Prof. García-Olmo and Dr. Garcia-Arranz have applied for two patents related to this study; these patents are entitled "Identification and isolation of multipotent cells from nonosteochondral mesenchymal tissue" (WO 2006/057649) and "Use of adipose tissue-derived stromal stem cells in treating fistula" (WO 2006/136244). Prof. García-Olmo and Dr. Garcia-Arranz are shareholders of Biosurgery, an educational company providing services to Takeda. Rest of the author declared no potential conflicts of interest with respect to the research and authorship.

Ethical responsibilities

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of the data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

SUPPLEMENTARY DATA

Supplementary data are available at *Cirugía y Cirujanos* online (http://10.24875/CIRU.21000110). These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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