Stem Cell Research 17 (2016) 342–351

Contents lists available at ScienceDirect

Stem Cell Research

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

The adipose tissue of origin influences the biological potential of human adipose stromal cells isolated from mediastinal and subcutaneous fat depots

Camilla Siciliano [a,1], Antonella Bordin [a,1], Mohsen Ibrahim [b,1], Isotta Chimenti [a], Francesco Cassiano [c], Ilenia Gatto [a], Giorgio Mangino [a], Andrea Coccia [a], Selenia Miglietta [c], Daniela Bastianelli [a], Vincenzo Petrozza [a], Antonella Calogero [a], Giacomo Frati [a,d,1], Elena De Falco [a,*,1]

[a] Department of Medical-Surgical Sciences and Biotechnologies, Sapienza University of Rome Polo Pontino, C.so della Repubblica 79, 04100 Latina, Italy
[b] Division of Thoracic Surgery, Department of Medical-Surgical Science and Translational Medicine, Sapienza University of Rome, S. Andrea Hospital, via di Grottarossa 1035, 00189 Rome, Italy
[c] Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, Section of Anatomy, Electron Microscopy Unit, Laboratory "Pietro M. Motta", Sapienza University of Rome, Rome, Italy
[d] Department of AngioCardioNeurology, IRCCS NeuroMed, 86077 Pozzilli, IS, Italy

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 25 July 2016
Accepted 29 July 2016
Available online 30 July 2016

Keywords:
Mesenchymal stem cells
Adipose tissue
Platelet lysate angiogenesis
Transmission electron microscopy

ABSTRACT

Indirect evidence suggests that adipose tissue-derived stromal cells (ASCs) possess different physiological and biological variations related to the anatomical localization of the adipose depots. Accordingly, to investigate the influence of the tissue origin on the intrinsic properties of ASCs and to assess their response to specific stimuli, we compared the biological, functional and ultrastructural properties of two ASC pools derived from mediastinal and subcutaneous depots (thoracic compartment) by means of supplements such as platelet lysate (PL) and FBS. Subcutaneous ASCs exhibited higher proliferative and clonogenic abilities than mediastinal counterpart, as well as increased secreted levels of IL-6 combined with lower amount of VEGF-C. In contrast, mediastinal ASCs displayed enhanced pro-angiogenic and adipogenic differentiation properties, increased cell diameter and early autophagic processes, highlighted by electron microscopy. Our results further support the hypothesis that the origin of adipose tissue significantly defines the biological properties of ASCs, and that a homogeneric function for all ASCs cannot be assumed.

1. Introduction

Adipose tissue is a versatile organ not limited to the lipidic function, but also exerting significant endocrine and inflammatory activity and influencing vascular function (Meissburger et al., 2016; Chudek and Wiecek, 2006). From a physiological standpoint, the development of adipose tissue within the body is not univocal and many factors cooperate to orchestrate its varied distribution, including genetic background and variations due to clinical conditions (Gesta et al., 2007). Furthermore, the adipose tissue is highly heterogeneous and recent clinical evidence indicates that both the compartmentalization and anatomical deposition of fat depots considerably differ in metabolic and physiological profiles (Kwok et al., 2016; Lee et al., 2013). These dissimilarities have been ascribable to a different developmental origin and epigenetic regulation, implying profound differences in depot-specific patterning genes and pathway activity mainly related to the anatomic region of origin (Gesta et al., 2006; Pinnick et al., 2014; Collas, 2010; Karantalis and Hare, 2015). Notably, differences in adipose depots also cause clinical consequences in those pathologies where fat tissue exerts a pathophysiological role such as endocrinological, metabolic and cardiac disorders, obesity, diabetes and cancer (Lapidus et al., 1984; Despres, 2006; Lim and Meigs, 2013). For instance, a clear association between cardiovascular risk and type of fat depot has been clearly elucidated. Visceral rather than subcutaneous adipose tissue has been suggested to be directly proportional to adverse cardiac and cancer risk due to the prevalence of oxidative and inflammatory systemic states (Pou et al., 2007; Misra et al., 1997; Harada et al., 2015; Chau et al., 2014).

Recently, work has been focused on the adipose tissue-derived stromal cell (ASCs) population, since retrieval is straight forward and non-

Abbreviations: ASCs, adipose tissue-derived stromal cells; PL, Platelet lysate; CM, conditioned media.

* Corresponding author at: University of Rome Sapienza, Faculty of Pharmacy and Medicine, Department of Medical-Surgical Sciences and Biotechnologies, C.so della Repubblica 79, 04100 Latina, Italy.
E-mail addresses: cami.siciliano@gmail.com (C. Siciliano), anto.bordin@libero.it (A. Bordin), mohsen.ibrahim@uniroma1.it (M. Ibrahim), isotta.chimenti@uniroma1.it (I. Chimenti), fra.cassiano@gmail.com (F. Cassiano), ilenia.gatto@uniroma1.it (I. Gatto), gmangino@libero.it (G. Mangino), andrea.coccia@uniroma1.it (A. Coccia), selenia.miglietta@uniroma1.it (S. Miglietta), daniela.bastianelli@uniroma1.it (D. Bastianelli), vincenzo.petrozza@uniroma1.it (V. Petrozza), antonella.calogero@uniroma1.it (A. Calogero), fraticello@inwind.it (G. Frati), elena.defalco@uniroma1.it (E. De Falco).
[1] Equal contribution to the study.

http://dx.doi.org/10.1016/j.scr.2016.07.010

harmful coupled to low-risk surgical procedures. However, biological properties of ASCs have only been assessed by indiscriminate isolation from mostly white fat depots. Little has been done to evaluate potential intrinsic differences between anatomic depot regions. By only considering all ASC populations as functionally and biologically equivalent, this has consequently led to incomparable and sometimes contradictory results. Yet, several reports, while supporting intrinsic differences between fat depots, indirectly suggest divergences in ASCs. Abdominal ASCs demonstrate diminished susceptibility to apoptosis (Toyoda et al., 2009), whereas those derived from pericardium, omentum and groin display different phenotype (Russo et al., 2014). According to the anatomical location of the fat, ASCs could differently exhibit surface epitopes as well as cell yield, ability to differentiate and morphological properties. This may have important consequence on altered response to pharmaceutical treatments (Russo et al., 2014; Perrini et al., 2008; Tchkonia et al., 2002; Gibellini et al., 2015; Schweizer et al., 2015; Elahi et al., 2016; Luna et al., 2014).

Differences between fat depots grown under different conditions are not well known. We have investigated the potential of human mediastinal depot as a novel ASC pool (Siciliano et al., 2015a,b,c), including the treatment with hemoderivate agents, such as platelet lysate (PL) (Siciliano et al., 2015a,b,c). In this study, we aimed to compare ASCs derived from the mediastinal region (fat inside the rib cage) to ASCs derived from the subcutaneous adipose tissue (fat outside the rib cage). Two different supplements, PL and FBS were used to unveil their biological features. Our study revealed functional differences in ASCs derived from the mediastinal region respect to the subcutaneous, showing enhanced pro-angiogenic features, whereas subcutaneous displayed higher proliferative and clonogenic abilities and a greater degree of autophagy associated structures.

2. Materials and methods

2.1. Surgical specimen collection

Surgical procedures were conducted at "Sant'Andrea" Hospital, Rome. The study was approved by the Ethical Committee of the hospital (reference 49_2013/28.01.2016). Written informed consent was obtained from patients, before starting all the surgical and laboratory procedures. The methodology described in this study has been conducted in compliance with the tenets of the Declaration of Helsinki for experiments involving human tissues. Medical records of each patient were collected in a clinical database. All data were de-identified and analysed anonymously. Previous malignancy, chemotherapy, inflammatory states, diabetes or obesity were considered as exclusion criteria. Bioptical samples of mediastinal and subcutaneous adipose tissues were collected from patients (12 male and 10 female patients, mean age of 65.13 ± 9.82) undergoing thoracic surgery (pulmonary amartochondroma), as we previously described (Siciliano et al., 2015a,b,c). Briefly, subcutaneous fat biopsies were obtained just after skin incision, whereas mediastinal samples from the anterior mediastinum outside the pericardium. The identification of both fat depots was performed by eye and biopsies (a minimal amount of 10 g) were always withdrawn from non-necrotic areas, respecting the esthetical anatomical fat distribution of patients. The samples were collected by using an electrocautery, in order to avoid an excessive amount of erythrocytes in the specimen, known to influence the quality of the *in vitro* culture and cell yield (Siciliano et al., 2015c).

2.2. Isolation, characterization and cell growth of subcutaneous and mediastinal ASCs

Both subcutaneous and mediastinal ASCs were isolated as we previously described (Siciliano et al., 2015a,b,c). Briefly, tissue specimens were enzymatically digested using 0.05% Trypsin/0.02% EDTA (Biowest, Nuaillé, France, Cat. N. X0930-100)/1 mg/ml collagenase type-I solution (Gibco, Monza, Italy, Cat. N. 17100) for 45 min at 37 °C. The pellet was resuspended in complete growth medium (low and high glucose DMEM, respectively/1% penicillin–streptomycin/1% glutamine/1% non-essential amino acids (all Biowest, Nuaillé, France) supplemented with either 10% FBS or the patented GMP-compliant Platelet Lysate (PL, Mesengen MES-R, Italy) (Siciliano et al., 2015a,b,c; Carducci et al., 2016) and allowed to adhere in the incubator for 3 days. ASC cultures were maintained at a cell density 4000 cells/cm^2 and passaged when reached 80–90% of confluence. Cell growth/viability was assessed by Trypan Blue (Siciliano et al., 2015a; De Falco et al., 2013) and measured by the automatic cell counter Countess® (Life Technology, Monza, Italy). The number of doubling cells was calculated as the following ratio: 3.322 $\log_{10}$ N/N_0 (N is the final number of cells obtained and N_0 is the number of cells plated) (De Falco et al., 2013). CM were collected from cultures maintaining consistent ratios of cell densities and media volumes between passage 3 and 6 and stored at −80 °C until use.

Immunophenotype was assessed by FACS Analysis, as we already described (Siciliano et al., 2015a,b,c). Briefly, cells were stained with the following panel of primary antibodies: CD105, CD44, CD90, CD45, CD117, CD133 (all Abcam Cat. N. ab44967, ab6124, ab23894, ab10559, ab5506, ab19898), CD31 (Sigma, St. Louis, MO, USA, Cat. N. P8590), CD34 (Miltenyi Biotech, Calderara di Reno, Bologna, Italy, Cat. N. 130-081-001), CD13 and CD73 (Biolegend, San Diego, CA, USA Cat. N. 301,703 and 344005, respectively). Anti-Rabbit Alexafluor-488 (Molecular Probe), mouse FITC-conjugated anti-mouse IgG (H + L) (Jackson ImmunoResearch, Suffolk, UK) and mouse IgG2a (Miltenyi Biotech, Calderara di Reno, Bologna, Italy) were used as secondary antibodies. Samples were acquired by cytofluorimeter (FACSAria II, BD, San Jose, CA, USA) and analysed by DiVa Software (v6.1.1, BD, San Jose, CA, USA).

Secondary colony forming efficiency assay was performed as we previously described (Siciliano et al., 2015a,b,c; Menna et al., 2014). Briefly, ASCs were seeded at passage 3 at low density (10 cells/cm^2) in complete medium and incubated for 14 days at 37 °C. Colonies produced were fixed with 4% paraformaldehyde and then stained with Giemsa (Sigma, Milan, Italy) for 1 h and counted by optical microscope. A cluster with >50 cells was considered as a colony. For adipogenic trans-differentiation test, ASCs were cultured for 2–3 weeks using StemPro® Adipogenesis Differentiation kit (GIBCO, Grand Island, NY, USA, cell density 1×10^4 cells/cm^2, Cat. N. A10070-01) (Siciliano et al., 2015a,c). Accumulation of lipid droplets was evaluated by Oil Red Oil staining (Sigma-Aldrich, St. Louis, MO, USA, Cat. N. 01391-500ml). For the quantification of the adipogenesis, at least 10 randomly selected fields were observed under an optical microscope and the number of positive adipocytes were counted and expressed as following: differentiation ratio (%) = (Number of transdifferentiated ASCs / total cell number) × 100.

For detailed analysis of migration assay, cytokine profile, tubule formation assay, immunomodulatory properties and transmission electron microscopy, see Supplementary methods.

2.3. Statistical analysis

Data were analysed by GraphPad Prism 5 software (San Diego, USA) using the unpaired student *t*-test for comparison between any two datasets. *P* values < 0.05 were considered to be significant. Data are expressed as means ± SD, unless specified. Experiments were repeated at least three times.

3. Results

3.1. Proliferation and clonogenic activity reflect biological differences between mediastinal and subcutaneous ASCs

In order to compare ASC populations derived from two distinct and anatomically distant sources of adipose tissue, we isolated both mediastinal and subcutaneous ASCs (fat inside and outside the rib cage, respectively). Interpatient variability was reduced by collecting surgical biopsies of both adipose depots from the same patient. Intrinsic

biological differences and behaviour were assessed by expanding ASC cultures under two conditions, platelet lysate (PL), a hemoderivate enriched in growth factors and cytokines (Siciliano et al., 2015a,b,c; Iudicone et al., 2014), or foetal bovine serum (FBS). Both are routinely used for *in vitro* ASC expansion and maintenance, and also differently influence stromal biological properties (Siciliano et al., 2015a). A summary of the experimental design is described in Fig. 1A.

Given that high mitotic activity is recognized as a distinct feature of ASCs (Friedenstein et al., 1976; Friedenstein et al., 1966), we evaluated the potency of PL or FBS between passages 2 and 4 to cause proliferation, which generally corresponds to the log phase of ASC growth (Marquez-Curtis et al., 2015). We observed a significant difference in the number of doubling cells between subcutaneous and mediastinal ASCs at passage 4 in the presence of PL (Fig. 1B–C, $P = 0.038$). Conversely, no significant difference in cell proliferation was found in presence of FBS (Fig. 1B, $P > 0.05$ all passages). The phenotypic analysis of cells from the fourth passage by flow cytometry, revealed that both subcutaneous and mediastinal ASCs displayed a homogenous and similar phenotype for the main surface antigens. This defined the stromal and the endothelial/hematopoietic fraction (Siciliano et al., 2015a,c; Dominici et al., 2006) with either stimulus (PL or FBS). Both ASC pools expressed comparable positive levels of CD44, CD90, CD105, CD13 and CD73, and similar negative profile for CD31, CD117, CD45 and CD34 (Supplementary Fig. 1A–B).

Interestingly, the number of secondary colonies generated at passage 3 by subcutaneous ASCs in presence of FBS was significantly higher compared to that formed by mediastinal ASCs (Fig. 2A, D–E, $P = 0.04$). Whereas, no differences between the two ASC pools was observed after preconditioning with PL (Fig. 2A, B–C $P > 0.05$).

Assessment of the ability of two ASC pools to migrate, showed comparable migratory activity either in presence of PL or FBS (Supplementary Fig. 2A–E, $P > 0.05$). As expected, the treatment with PL significantly increased the number of migrated ASCs compared to FBS regardless of the adipose depot (Supplementary Fig. 2A–E both $P < 0.0001$).

3.2. Conditioned media derived from subcutaneous and mediastinal ASCs show comparable immunostimulatory capacity, but different angiogenic and paracrine properties

Considering the ability of ASCs to promote angiogenesis and regeneration both *in vitro* and *in vivo* by paracrine effects in several pathologies including heart failure, diabetes and cancer (Ohnishi et al., 2007; Gu et al., 2014; Zimmerlin et al., 2013), we analysed the secretome profile of their CM by quantifying the levels of multiple angiogenic soluble factors, including the VEGF and TGF-β families, several interleukins and growth factors. We found that only upon treatment with PL, higher levels of IL-6 or VEGF-C were secreted by subcutaneous or mediastinal ASCs, respectively (Fig. 3A–B, $P = 0.016$ and $P = 0.048$, respectively). No significant difference was observed regarding the remaining cytokines/growth factors either in presence of PL and FBS (Supplementary Fig. 3A–D, all $P > 0.05$).

Given the dual ability of ASCs to promote angiogenesis (Kwon et al., 2014) and to act as immunomodulators (Aggarwal and Pittenger, 2005; Meisel et al., 2004; Krampera et al., 2006), we evaluated both the *in vitro* angiogenic potential and the possible effect on PBMC activation of the same CM derived from PL and FBS-cultured ASCs. Accordingly, we tested the influence of the ASC-derived CM on the ability of HUVECs to form networks, and their specific capacity to modulate PBMCs proliferation

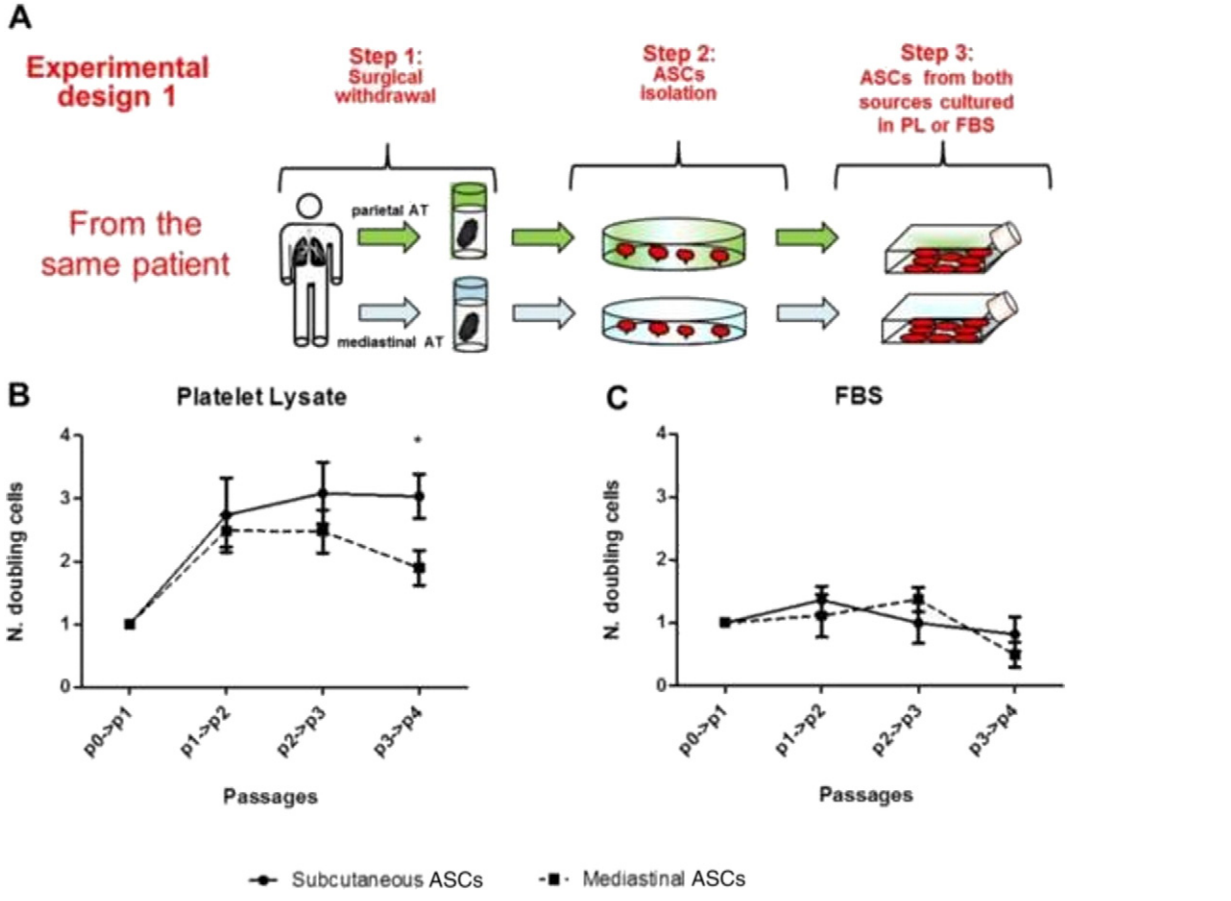

Fig. 1. A–C. Experimental design 1 and ASC growth curve. (A) The cartoon displays the experimental plan 1 designed for the study. (B) ASC growth trend until passage 4. Platelet lysate-cultured subcutaneous ASCs show a significantly higher number of doubling cells compared to mediastinal ASCs. (C) FBS does not reproduce similar effect between the two ASCs pools. The number of doubling cells was calculated according to the following formula: 3.322 $\log_{10}$ N/N0, where N is the number of cells obtained and N0 is the number of cell plated. AT, adipose tissue. *$P < 0–05$.

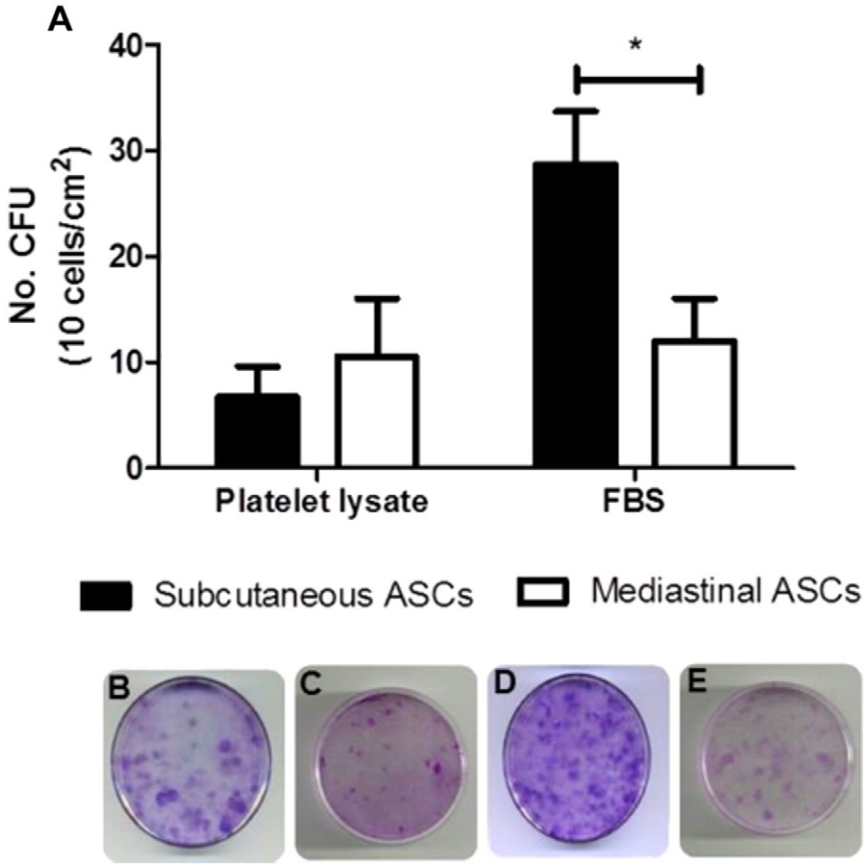

Fig. 2. A–E. Colony Forming Unit-fibroblast assay (CFU-F). (A) The graph shows that the number of secondary colonies generated at passage 3 by subcutaneous ASCs is significantly higher compared to that produced by mediastinal ASCs when treating with FBS. Conversely, no significant difference was found if ASCs were maintained in Platelet lysate. Representative images of the clones generated upon PL (B–C) or FBS (D–E) treatment after Giemsa staining. Magnification 5×. *$P < 0.05$.

by carboxyfluorescein diacetate succinimidyl ester (CFSE) assay. Results showed that only CM derived from mediastinal ASCs, significantly increased the number of *fenestrae* and total network length (either in presence of PL or FBS), compared to those derived from subcutaneous ASCs (Fig. 4A–F, *fenestrae* $P = 0.0072$ and $P = 0.0005$, respectively; network length $P = 0.045$ and $P = 0.022$, respectively). No difference was observed regarding the number of branching points. Additionally, in order to verify whether or not the improved angiogenic capacity of CM derived from the mediastinal ASC pool was related to VEGF-C, we added a VEGF-C neutralizing antibody to the supernatants before performing matrigel assay on HUVECs. Results showed a significant reduction of both *fenestrae* and tubule length respect to untreated CM, and independently from treatment (Fig. 4G–H all $P < 0.001$).

Moreover, CM derived from ASCs, positively stimulated or decrease the number of cell divisions of PBMCs over the time similarly to the trend exerted by PL or FBS alone and regardless the tissue of origin (Supplementary Fig. 4A–B, $P > 0.05$). The percentage of the $CD4^+$ and $CD4^-$ subpopulations was not affected (data not shown).

3.3. Influence of the tissue origin on adipogenic differentiation and ultrastructural characteristics

We then investigated whether the source of adipose tissue could influence the intrinsic ASC differentiation potential towards the adipogenic lineage. Results showed that both ASC pools exhibited equal adipogenic differentiation ratio independently from treatment

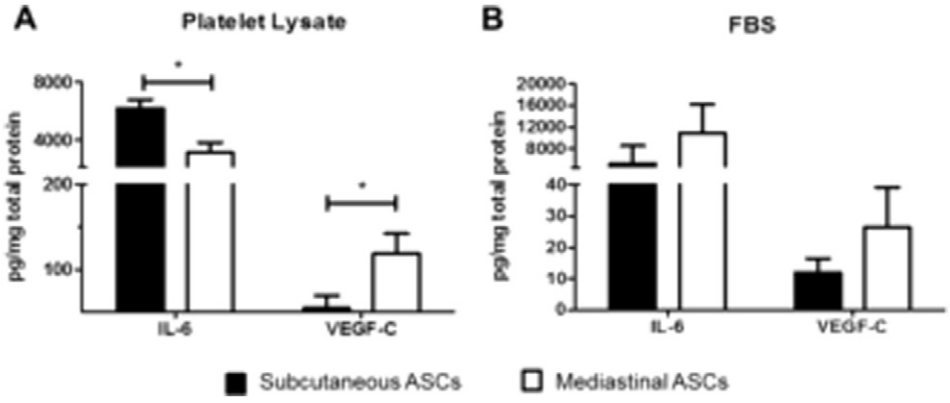

Fig. 3. A–B. Secretome profile of conditioned media derived from subcutaneous and mediastinal ASC cultures. (A) The graph displays that only upon platelet lysate treatment subcutaneous and mediastinal ASCs secrete significantly higher levels of IL-6 and VEGF-C, respectively and not when cultured with FBS (B).

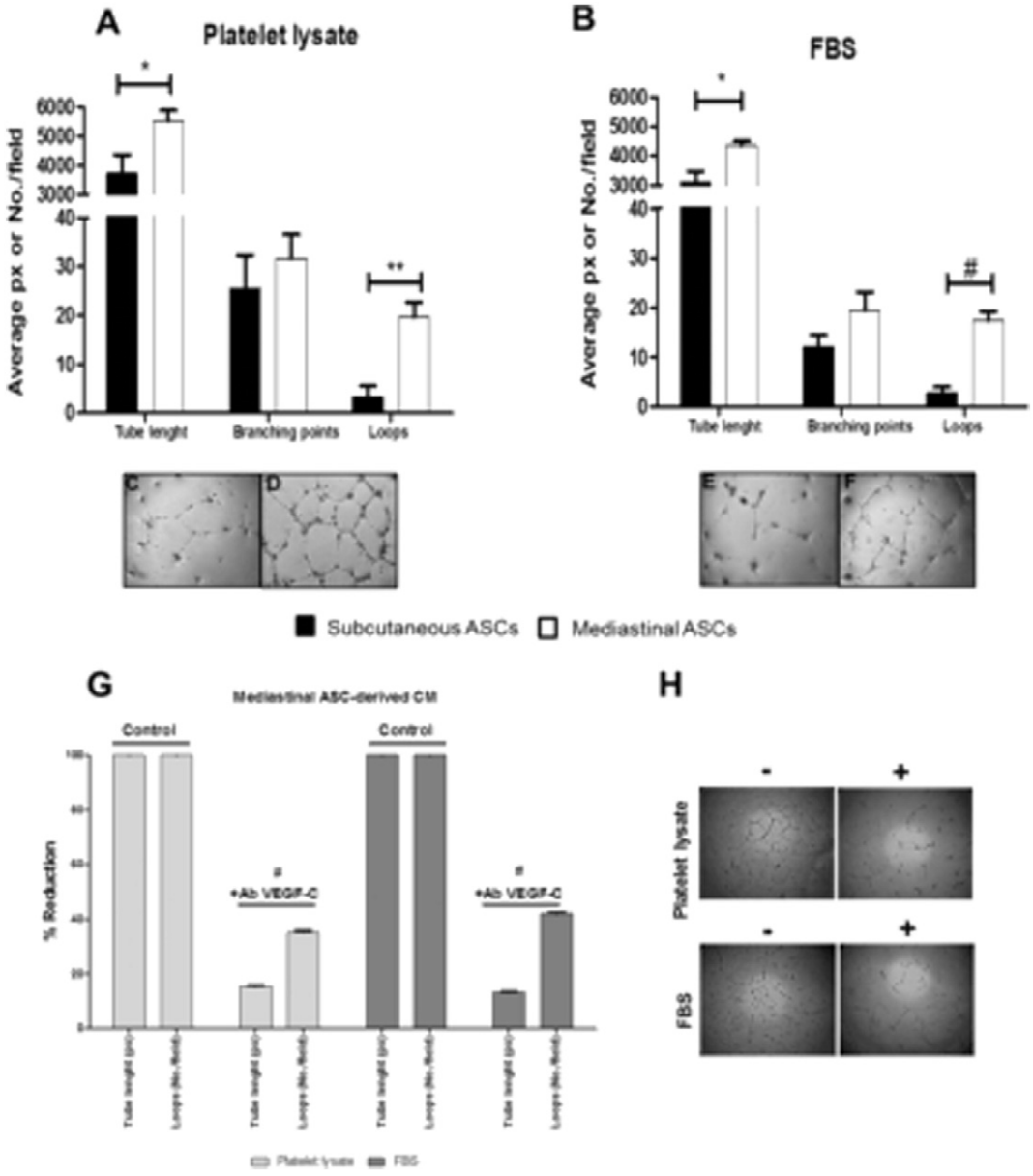

Fig. 4. A–H. Angiogenic ability of CM derived from subcutaneous and mediastinal ASC cultures on HUVECs. (A–B) The graphs show that only CM derived from mediastinal ASCs are able to significantly increase the number of *fenestrae* and tube length formed by HUVECs in a matrigel assay regardless the treatment. (C–F) Representative optical images of HUVECs in a matrigel assay cultured with CM derived from the two ASC pools. (G) The graph shows that the pre-treatment with the VEGF-C neutralizing antibody of the CM derived from the mediastinal ASC pool is able to significantly reduce the number of loops and tube length. (H) Representative optical images of the matrigel assay in absence and in presence of VEGF-C neutralizing antibody. Magnification 5×. $^{**}P < 0.01$, $^{\#}P < 0.0001$.

(Fig. 5A–B, all $P > 0.05$). Remarkably, PL-cultured mediastinal ASCs displayed a significantly increased proportion of adipogenic cells compared to those cultured in FBS ($P = 0.04$).

Additionally, we evaluated the intrinsic ultrastructure characteristics performing a qualitative analysis by performing Transmission electron microscopy (TEM). Given the influence of the genetic background on the ultrastructural features of the ASC population (Gesta et al., 2007), we compared the two ASC pools derived from a single patient (patient 55) according to the experimental design 2 (Supplementary Fig. 5A). The analysis confirmed that both ASC pools appeared heterogeneous. Morphological phenotype was not terminally committed (for instance towards a specific lineage), but interestingly mediastinal ASCs showed a significant higher average diameter compared to the subcutaneous pool regardless of the treatment (Supplementary Fig. 5B, $P = 0.0011$ PL; $P = 0.0005$ FBS). In addition, PL-cultured subcutaneous ASCs displayed a large number of autophagosomes engulfed with cellular components and autolysosomal vacuoles (Fig. 6A, C), whereas in mediastinal ASCs a greater number of primary lysosomes (identified as electrondense black vacuoles) and dilated *cisternae* of the endoplasmic reticulum, that were centrally localized and in the proximity of the primary lysosomes, predominated (Fig. 6B, D). Interestingly, in the subcutaneous ASC pool the euchromatin coexisted with a discrete amount of condensed heterochromatin (Fig. 6E), whereas finely dispersed and unclamped euchromatin was clearly present in the mediastinal counterpart (Fig. 6F). Nuclear indentations were equally visible (Fig. 6E–F). Cellular surface in both PL-cultured ASC populations was characterized by the presence of numerous non-intestinal-like microvilli associated to blebs, as well as *filopodia* and microvesicles (Fig. 6G–H). In addition, mitochondria present in PL-cultured mediastinal ASCs displayed active physiological characteristics, such as abundant non-dilated crests and regular shape, in contrast to the subcutaneous ASCs where crests were less visible (Fig. 6I–L).

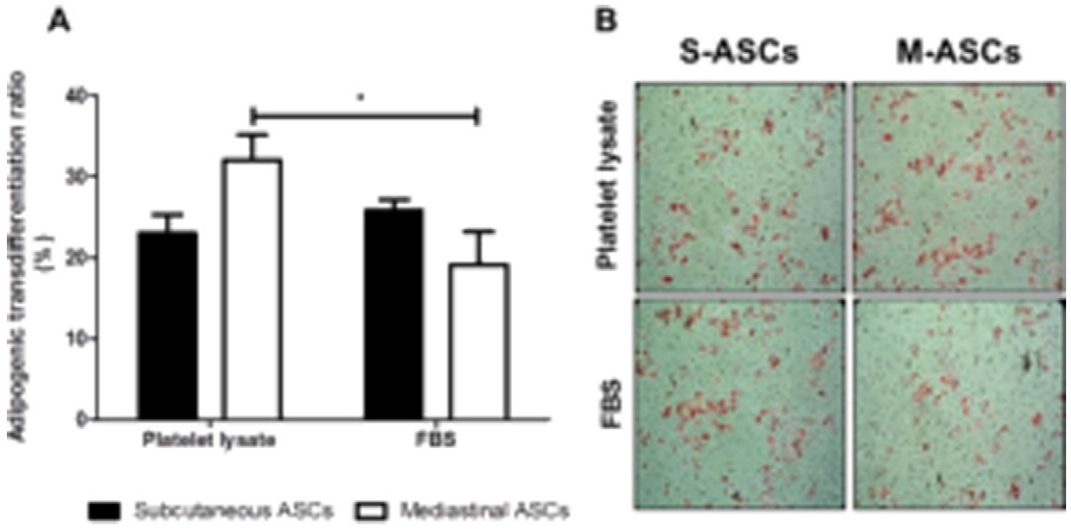

Fig. 5. A–B. Adipogenic differentiation of ASC pools. (A) The graph displays that the ratio of ASCs transdifferentiated towards the adipogenic lineage is similar in both pools regardless the treatment. Only ASCs derived from the mediastinal fat tissue exhibit higher ratio of transdifferentiated cells in platelet lysate compared to FBS. (B) Representative optical images of ASCs transdifferentiated in adipogenic cells and stained with Oil Red Oil. Magnification 10×. $^{*}P < 0.05$.

Whereas, in FBS-cultured subcutaneous counterpart abundant microvesicles and blebs appeared as short and small protrusions uniformly distributed, accompanied by less numerous *filopodia* and vacuoles (Fig. 7A). The mediastinal counterpart showed a greater number of small autolysosomal vacuoles, *filopodia*, as well as few primary lysosomes and blebs (Fig. 7B). Of note, both ASC pools exhibited indented nuclei (repeated invaginations of the nuclear membrane towards the cytoplasmic membrane) mainly containing a finely dispersed and unclamped euchromatin (Fig. 7A–B). Mediastinal ASCs also displayed mitochondrial crests which were barely visible and several lamellar bodies in the form of onion-like structures, in contrast to well-defined mitochondrial lamellar crests and absent lamellar bodies observed in the subcutaneous pool (Fig. 7C–D).

4. Discussion

In this study, we evaluated if different and distant anatomic regions of fat depots, albeit limited to the thoracic cavity, might reflect a heterogeneous array of biological, functional and ultrastructural features of ASCs. Our results support previous findings on putative differences between ASC pools isolated from multiple adipose sources (Ritter et al., 2015; Baglioni et al., 2012), but additionally they highlighted for the first time novel insights on intrinsic biological variations between intra- and extra-thoracic adipose compartments, indicating a different behaviour in relation not only to tissue of origin but also to the type of treatment employed. Notably, we described the properties of the mediastinal fat, considered as cardiac visceral fat with a different ontogenetic origin with respect to the subcutaneous depot (Chau et al., 2014; Atalar et al., 2013). To date, the function and biology of the mediastinal tissue, and particularly of the corresponding ASC pool, is poorly defined. This is likely due to technical limitations, as it represents a less accessible source of adipose tissue respect to other body regions. Yet, the mediastinal fat exerts a metabolic role *in situ* antagonistic to the protective subcutaneous counterpart (Chau et al., 2014; Atalar et al., 2013; Sironi et al., 2012; Sicari et al., 2011; Cheung et al., 2013). Remarkably, its enhanced deposition has been significantly associated to cardiovascular risk. Our study has implemented these differences in the thoracic cavity, as we showed that the angiogenic capability of mediastinal ASCs stands out from other properties, evoking the potential physiological/metabolical relevance of the mediastinal fat according to its proximity to the heart (Siciliano et al., 2015b,c; Baglioni et al., 2012; Patel et al., 2013). In contrast to other reports (Baglioni et al., 2012; Li et al., 2014), we described that the conditioning with specific stimuli (PL or FBS) unveils changes in stem cell plastic ability or functionality. This is dependent upon the intrinsic biological background of ASCs, and distinguishable from their common biological properties. Conditioning with supplements alone was insufficient to determine all of the biological differences observed. Accordingly, PL is known to be a superior inducer of ASC growth compared to FBS (Siciliano et al., 2015a; Bieback, 2013). Nevertheless only PL-cultured subcutaneous ASCs displayed a proliferative advantage compared to the mediastinal counterpart. This enhanced metabolic profile of subcutaneous ASCs, which was amplified by PL treatment, likely resembled the biological and physiological role played by the subcutaneous fat, such as exerting a feeder-like support for skin-resident cells during wound healing processes (Richmond and Harris, 2014). Interestingly, the same subcutaneous pool showed superior clonogenic activity (specifically a secondary colony forming efficiency capacity) compared to FBS treated mediastinal counterpart. This does not influence *per se* the capacity to stimulate ASCs to clone as PL (Siciliano et al., 2015a). Additionally, we found that the two ASC pools secreted partially different paracrine microenvironments, in addition to contrasting pro-angiogenic properties. Despite the fact that the majority of the secretome profile is largely shared by both populations, CM derived from PL-cultured subcutaneous ASCs contained higher levels of IL-6, but a decreased amount of VEGF-C compared to CM derived from mediastinal ASCs. Such feature conferred enhanced angiogenic ability in mediastinal ASCs regardless of the treatment. Interestingly, this was strictly related to VEGF-C, since the use of the neutralizing antibody significantly reduced the pro-angiogenic property. As we have already shown that IL-6 is absent in media supplement with 10% PL or FBS (Carducci et al., 2016), it is likely that the dissimilar combination of growth factors and/or cytokines between PL and FBS modulates the production of IL-6 in the two ASC pools, thus emphasising an intrinsic diversity between the two niches related to the anatomical region. Recently, the analysis of the secretome profile generated by ASCs has shifted researchers' attention to the clinical meaning (Feisst et al., 2015). More importantly to the role exerted by ASCs during inflammation. In this regard, the ASC pool is able to respond to inflamed microenvironments developed by a damaged tissue by combining anti-inflammatory humoral factors and angiogenic function within the adipose tissue (Tanaka, 2015; Han et al., 2015). Although the nature of this cross-talk requires further investigation, it undoubtedly involves the activation of specific signalling pathways such as PPAR-γ, ERK or p38 (Ye and Gimble, 2011), resulting in a reshuffling of positive and negative consequences, spanning from an enhanced coordination of *in*

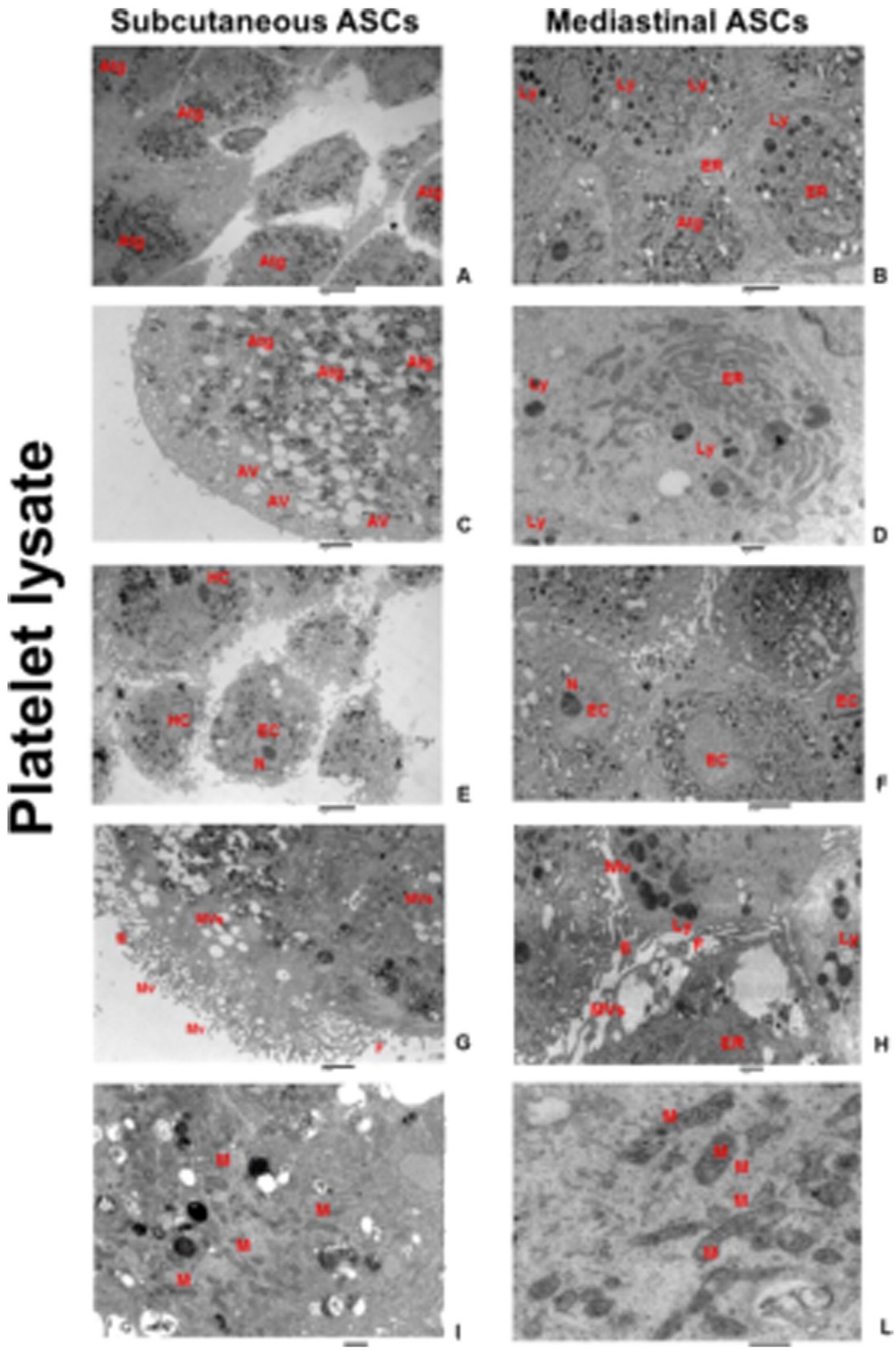

Fig. 6. A–L. Ultrastructure analysis of PL-cultured subcutaneous and mediastinal ASCs by electron microscopy (Patient 55). Mediastinal ASCs mainly display a greater number of primary lysosomes (A, C), as well as regular nuclei where euchromatin is abundant (E) compared to the subcutaneous pool, where autophagosome and autolysosomal vacuoles are more frequent (B, D) in combination with irregular nuclei and a discrete amount of heterochromatin (F). (G–H) The subcutaneous counterpart display more advanced autophagy-associated structures, as well as non-intestinal type microvilli, blebs and filopodia than mediastinal ASCs. Structural detail of mitochondria present in mediastinal (I) and subcutaneous ASCs (L), displaying abundant non-dilated and barely visible crests, respectively. Atg = autophagosomes, AV = autolysosome vacuoles, M = mitochondria, ER = cisternae of endoplasmic reticulum, F = filopodia, MVs = microvesicles, Mv = microvilli, Ly = primary lysosomes, B = blebs, N = nucleus, HC = heterochromatin, EC = euchromatin. Acronyms in TEM panels indicate a representative cellular structure.

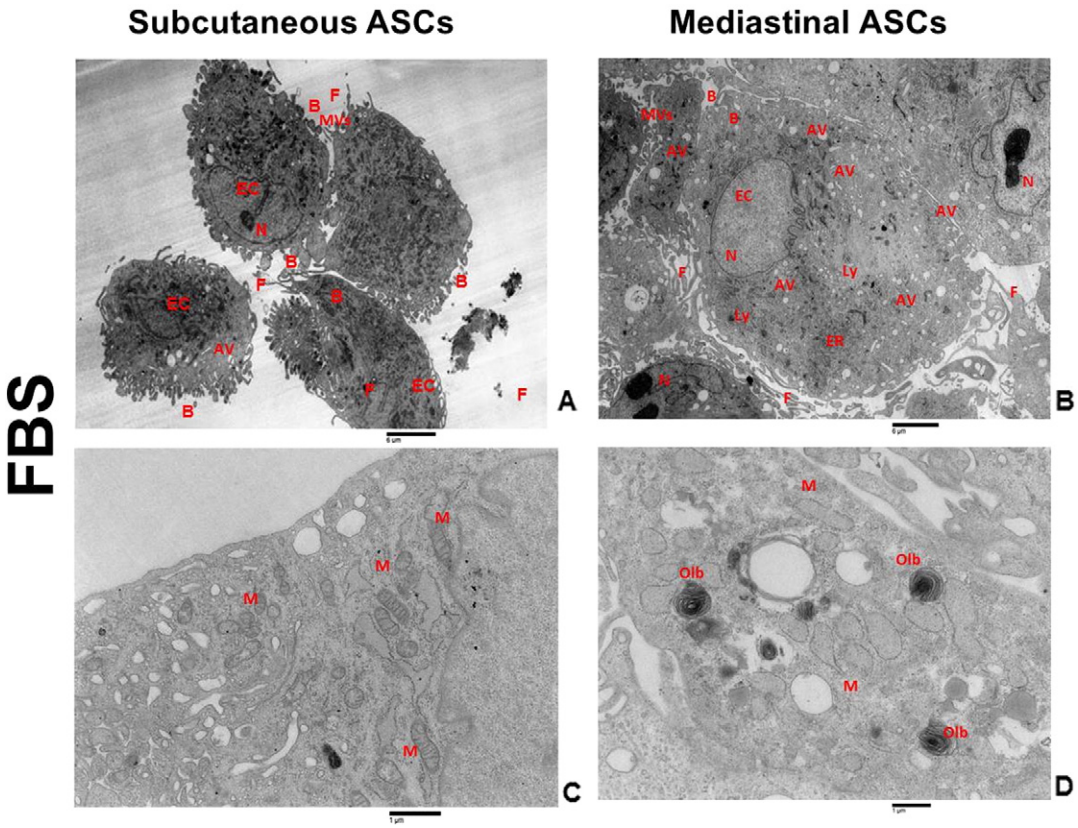

Fig. 7. A–D. Ultrastructure features of FBS-cultured subcutaneous and mediastinal ASCs by electron microscopy (Patient 55). (A) Subcutaneous ASCs display numerous short and small cytoplasmic protrusions, blebs and microvesicles, differently from the mediastinal counterpart (B) where small autolysosomal vacuoles and filopodia predominate. Of note, in the subcutaneous pool lamellar structures, such as onion-like bodies, are absent but accompanied by a larger number of mitochondria with well-defined lamellar crests (C) respect to the mediastinal correspondent (D). AV = autolysosomal vacuoles, M = mitochondria, ER = cisternae of endoplasmic reticulum, Olb = onion-like bodies, F = filopodia, MVs = microvesicles, Ly = primary lysosomes, B = blebs, N = nucleus, EC = euchromatin. Acronym in TEM panels indicates a representative cellular structure.

situ tissue regeneration and metabolic homeostasis, to the inhibition of adipogenic differentiation of the fat depot (Han et al., 2015; Cao, 2007; Greenberg and Obin, 2006; Gimble et al., 2007). As a result, the role played by the ASC-derived secretome (still to be properly defined) would include functional properties beyond just inflammation (Trayhurn and Wood, 2004; Lehrke and Lazar, 2004; Neels and Olefsky, 2006). Remarkably, whether or not alternative sources of fat depots exhibit similar inflammatory features, requires to be addressed. In the light of our results, we believe that the higher amount of IL-6 secreted by subcutaneous ASCs could reflect their physiological ability to trigger acute repairing processes (Richmond and Harris, 2014; Kim et al., 2009; Fain, 2010), whereas higher levels of VEGF-C (a main regulator of lymphangiogenesis (Iudicone et al., 2014; Huang et al., 2001)) secreted by mediastinal ASCs would mirror the influence of the multiple lymph nodes in the mediastinal region, although angiogenesis and lymphangiogenesis represent distinct processes yet mainly controlled by the VEGF family (Veikkola et al., 2000; Conrad et al., 2009). The poorly explored interaction between fat and lymphoid tissue has been reported to impact adipose tissue vascularization, as well as function and immunological responses during inflammatory processes (Schaffler et al., 2006).

Finally, although observed only in a single donor, we verified if differences in the two ASC pools mirrored dissimilar intracellular composition and organization. Ultrastructural differences have been reported to reflect differentiative potential and mechanical properties (Pasquinelli et al., 2007; Gonzalez-Cruz et al., 2012). Interestingly, we found that mediastinal ASCs showed a higher cell diameter than subcutaneous ASCs regardless of the treatment, likely coherent with a different anatomic profile. However, the combination of the supplement and the different origin of the fat depot seemed to influence certain ultrastructural characteristics of ASCs. Accordingly, we observed changes in mitochondrial *cristae* morphology in both ASC pools (suggesting different oxidative states, as well as a preferential anaerobic/aerobic metabolism on the basis of the treatment), but also increased microvilli and microvesicles in presence of PL. These are hallmarks of a more active autophagic flux in ASCs, and confirming the known ability of PL to boost metabolic growth and differentiation compared to FBS (2, 38). A major prevalence of primary lysosomes (suggesting early autophagy) in mediastinal ASCs, *versus* abundantly and accumulated autophagosomes in the subcutaneous counterpart (markers of advanced autophagy) was found, indicating enhanced, and perhaps faster turnover of this latter in recycling proteins or organelles. As FBS represents a cellular distress compared to PL, it is also plausible that changes in the autophagic process observed upon FBS may reflect an attempt of the cultures to survive in harsher microenvironments, as recently shown upon other treatments (Molaei et al., 2015). To date, autophagy in ASCs represents a relevant topic. It significantly contributes to the dysregulation of the adipose tissue by controlling lipid accumulation, and balancing white and brown fat deposition (Singh et al., 2009; Maixner et al., 2016). Intriguingly, autophagy is enhanced in obesity and associated to visceral fat distribution (Kovsan et al., 2011), thus encouraging further investigations on the ability of the stromal subset during adipogenic commitment.

5. Conclusions

The heterogeneity and the specificity among different anatomical depots, including new insights on the biological potency of the ASC pool, represent a relevant issue to explore with potential clinical implications.

The understanding of the basic intrinsic properties of ASCs by discriminating between multiple sources of fat depots and the modality by which extrinsic factors modulate their biological features, could represent a tool to elucidate either their physiological and pathological role in many disorders.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2016.07.010.

Disclosure of potential conflicts of interest

Prof. G. Frati holds a patent concerning platelet lysate in regenerative medicine (Pub. number WO/2013/042095, International Application number PCT/IB2012/055062).

Acknowledgments

We thank Fondazione Roma for continuous support. We also acknowledge Prof. Mara Riminucci, Prof. Luca Pierelli, Dr. C. Murdoch, Dr. Paola Iudicone and Eve Technologies for the analysis of growth factors and cytokines.

This study was financially supported by Sapienza University of Rome prot. N. C26A1429M2 granted to Elena De Falco.

References

Aggarwal, S., Pittenger, M.F., 2005. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105, 1815–1822.

Atalar, F., Gormez, S., Caynak, B., Akan, G., Tanriverdi, G., Bilgic-Gazioglu, S., Gunay, D., Duran, C., Akpinar, B., Ozbek, U., Buyukdevrim, A.S., Yazici, Z., 2013. Mediastinal adipose tissue expresses a pathogenic profile of 11 beta-hydroxysteroid dehydrogenase Type 1, glucocorticoid receptor, and CD68 in patients with coronary artery disease. Cardiovasc. Pathol. Off. J. Soc. Cardiovasc. Pathol. 22, 183–188.

Baglioni, S., Cantini, G., Poli, G., Francalanci, M., Squecco, R., Di Franco, A., Borgogni, E., Frontera, S., Nesi, G., Liotta, F., Lucchese, M., Perigli, G., Francini, F., Forti, G., Serio, M., Luconi, M., 2012. Functional differences in visceral and subcutaneous fat pads originate from differences in the adipose stem cell. PLoS One 7, e36569.

Bieback, K., 2013. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transfus. Med. Hemother. 40, 326–335.

Cao, Y., 2007. Angiogenesis modulates adipogenesis and obesity. J. Clin. Invest. 117, 2362–2368.

Carducci, A., Scafetta, G., Siciliano, C., Carnevale, R., Rosa, P., Coccia, A., Mangino, G., Bordin, A., Vingolo, E.M., Pierelli, L., Lendaro, E., Ragona, G., Frati, G., De Falco, E., Jan 1, 2016. GMP-grade platelet lysate enhances proliferation and migration of tenon fibroblasts. Front. Biosci. (Elite Ed.) 8, 84–99.

Chau, Y.Y., Bandiera, R., Serrels, A., Martinez-Estrada, O.M., Qing, W., Lee, M., Slight, J., Thornburn, A., Berry, R., McHaffie, S., Stimson, R.H., Walker, B.R., Chapuli, R.M., Schedl, A., Hastie, N., 2014. Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat. Cell Biol. 16, 367–375.

Cheung, L., Gertow, J., Werngren, O., Folkersen, L., Petrovic, N., Nedergaard, J., Franco-Cereceda, A., Eriksson, P., Fisher, R.M., 2013. Human mediastinal adipose tissue displays certain characteristics of brown fat. Nutr. Diabetes 3, e66.

Chudek, J., Wiecek, A., 2006. Adipose tissue, inflammation and endothelial dysfunction. Pharmacol. Rep. 58, 81–88 Suppl.

Collas, P., 2010. Programming differentiation potential in mesenchymal stem cells. Epigenetics 5, 476–482.

Conrad, C., Niess, H., Huss, R., Huber, S., von Luettichau, I., Nelson, P.J., Ott, H.C., Jauch, K.W., Bruns, C.J., 2009. Multipotent mesenchymal stem cells acquire a lymphendothelial phenotype and enhance lymphatic regeneration in vivo. Circulation 119, 281–289.

De Falco, E., Scafetta, G., Napoletano, C., Puca, R., Vingolo, E.M., Ragona, G., Iorio, O., Frati, G., 2013. A standardized laboratory and surgical method for in vitro culture isolation and expansion of primary human Tenon's fibroblasts. Cell Tissue Bank. 14, 277–287.

Despres, J.P., 2006. Is visceral obesity the cause of the metabolic syndrome? Ann. Med. 38, 52–63.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D., Horwitz, E., 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8, 315–317.

Elahi, K.C., Klein, G., Avci-Adali, M., Sievert, K.D., MacNeil, S., Aicher, W.K., 2016. Human mesenchymal stromal cells from different sources diverge in their expression of cell surface proteins and display distinct differentiation patterns. Stem Cells Int. 2016, 5646384.

Fain, J.N., 2010. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. Mediat. Inflamm. 2010, 513948.

Feisst, V., Meidinger, S., Locke, M.B., 2015. From bench to bedside: use of human adipose-derived stem cells. Stem Cells and Cloning : Advances and Applications. Vol. 8, pp. 149–162.

Friedenstein, A.J., Piatetzky II, S., Petrakova, K.V., 1966. Osteogenesis in transplants of bone marrow cells. J. Embryol. Exp. Morphol. 16, 381–390.

Friedenstein, A.J., Gorskaja, J.F., Kulagina, N.N., 1976. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp. Hematol. 4, 267–274.

Gesta, S., Bluher, M., Yamamoto, Y., Norris, A.W., Berndt, J., Kralisch, S., Boucher, J., Lewis, C., Kahn, C.R., 2006. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc. Natl. Acad. Sci. U. S. A. 103, 6676–6681.

Gesta, S., Tseng, Y.H., Kahn, C.R., 2007. Developmental origin of fat: tracking obesity to its source. Cell 131, 242–256.

Gibellini, L., De Biasi, S., Nasi, M., Carnevale, G., Pisciotta, A., Bianchini, E., Bartolomeo, R., Polo, M., De Pol, A., Pinti, M., Cossarizza, A., 2015. Different origin of adipogenic stem cells influences the response to antiretroviral drugs. Exp. Cell Res. 337, 160–169.

Gimble, J.M., Katz, A.J., Bunnell, B.A., 2007. Adipose-derived stem cells for regenerative medicine. Circ. Res. 100, 1249–1260.

Gonzalez-Cruz, R.D., Fonseca, V.C., Darling, E.M., 2012. Cellular mechanical properties reflect the differentiation potential of adipose-derived mesenchymal stem cells. Proc. Natl. Acad. Sci. U. S. A. 109, E1523–E1529.

Greenberg, A.S., Obin, M.S., 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am. J. Clin. Nutr. 83, 461S–465S.

Gu, C., Huang, S., Gao, D., Wu, Y., Li, J., Ma, K., Wu, X., Fu, X., 2014. Angiogenic effect of mesenchymal stem cells as a therapeutic target for enhancing diabetic wound healing. Int. J. Low. Extrem. Wounds 13, 88–93.

Han, T.T., Toutounji, S., Amsden, B.G., Flynn, L.E., 2015. Adipose-derived stromal cells mediate in vivo adipogenesis, angiogenesis and inflammation in decellularized adipose tissue bioscaffolds. Biomaterials 72, 125–137.

Harada, K., Baba, Y., Ishimoto, T., Kosumi, K., Tokunaga, R., Izumi, D., Ida, S., Imamura, Y., Iwagami, S., Miyamoto, Y., Sakamoto, Y., Yoshida, N., Watanabe, M., Baba, H., 2015. Low visceral fat content is associated with poor prognosis in a database of 507 upper gastrointestinal cancers. Ann. Surg. Oncol. 22, 3946–3953.

Huang, H.Y., Ho, C.C., Huang, P.H., Hsu, S.M., 2001. Co-expression of VEGF-C and its receptors, VEGFR-2 and VEGFR-3, in endothelial cells of lymphangioma. Implication in autocrine or paracrine regulation of lymphangioma. Lab. Investig. 81, 1729–1734.

Iudicone, P., Fioravanti, D., Bonanno, G., Miceli, M., Lavorino, C., Totta, P., Frati, L., Nuti, M., Pierelli, L., 2014. Pathogen-free, plasma-poor platelet lysate and expansion of human mesenchymal stem cells. J. Transl. Med. 12, 28.

Karantalis, V., Hare, J.M., 2015. Use of mesenchymal stem cells for therapy of cardiac disease. Circ. Res. 116, 1413–1430.

Kim, W.S., Park, B.S., Sung, J.H., 2009. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch. Dermatol. Res. 301, 329–336.

Kovsan, J., Bluher, M., Tarnovscki, T., Kloting, N., Kirshtein, B., Madar, L., Shai, I., Golan, R., Harman-Boehm, I., Schon, M.R., Greenberg, A.S., Elazar, Z., Bashan, N., Rudich, A., 2011. Altered autophagy in human adipose tissues in obesity. J. Clin. Endocrinol. Metab. 96, E268–E277.

Krampera, M., Cosmi, L., Angeli, R., Pasini, A., Liotta, F., Andreini, A., Santarlasci, V., Mazzinghi, B., Pizzolo, G., Vinante, F., Romagnani, P., Maggi, E., Romagnani, S., Annunziato, F., 2006. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 24, 386–398.

Kwok, K.H., Lam, K.S., Xu, A., 2016. Heterogeneity of white adipose tissue: molecular basis and clinical implications. Exp. Mol. Med. 48, e215.

Kwon, H.M., Hur, S.M., Park, K.Y., Kim, C.K., Kim, Y.M., Kim, H.S., Shin, H.C., Won, M.H., Ha, K.S., Kwon, Y.G., Lee, D.H., Kim, Y.M., 2014. Multiple paracrine factors secreted by mesenchymal stem cells contribute to angiogenesis. Vasc. Pharmacol. 63, 19–28.

Lapidus, L., Bengtsson, C., Larsson, B., Pennert, K., Rybo, E., Sjostrom, L., 1984. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br. Med. J. (Clin. Res. Ed.) 289, 1257–1261.

Lee, M.J., Wu, Y., Fried, S.K., 2013. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol. Asp. Med. 34, 1–11.

Lehrke, M., Lazar, M.A., 2004. Inflamed about obesity. Nat. Med. 10, 126–127.

Li, X., Bai, J., Ji, X., Li, R., Xuan, Y., Wang, Y., 2014. Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. Int. J. Mol. Med. 34, 695–704.

Lim, S., Meigs, J.B., 2013. Ectopic fat and cardiometabolic and vascular risk. Int. J. Cardiol. 169, 166–176.

Luna, A.C., Madeira, M.E., Conceicao, T.O., Moreira, J.A., Laiso, R.A., Maria, D.A., 2014. Characterization of adipose-derived stem cells of anatomical region from mice. BMC Res. Notes 7, 552.

Maixner, N., Bechor, S., Vershinin, Z., Pecht, T., Goldstein, N., Haim, Y., Rudich, A., 2016. Transcriptional dysregulation of adipose tissue autophagy in obesity. Physiology 31, 270–282.

Marquez-Curtis, L.A., Janowska-Wieczorek, A., McGann, L.E., Elliott, J.A., 2015. Mesenchymal stromal cells derived from various tissues: biological, clinical and cryopreservation aspects. Cryobiology 71, 181–197.

Meisel, R., Zibert, A., Laryea, M., Gobel, U., Daubener, W., Dilloo, D., 2004. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103, 4619–4621.

Meissburger, B., Perdikari, A., Moest, H., Muller, S., Geiger, M., Wolfrum, C., 2016. Regulation of adipogenesis by paracrine factors from adipose stromal-vascular fraction - a link to fat depot-specific differences. Biochim. Biophys. Acta 1861, 1121–1131.

Menna, C., De Falco, E., Pacini, L., Scafetta, G., Ruggieri, P., Puca, R., Petrozza, V., Ciccone, A.M., Rendina, E.A., Calogero, A., Ibrahim, M., 2014. Axitinib affects cell viability and migration of a primary foetal lung adenocarcinoma culture. Cancer Investig. 32, 13–21.

Misra, A., Garg, A., Abate, N., Peshock, R.M., Stray-Gundersen, J., Grundy, S.M., 1997. Relationship of anterior and posterior subcutaneous abdominal fat to insulin sensitivity in nondiabetic men. Obes. Res. 5, 93–99.

Molaei, S., Roudkenar, M.H., Amiri, F., Harati, M.D., Bahadori, M., Jaleh, F., Jalili, M.A., Mohammadi Roushandeh, A., 2015. Down-regulation of the autophagy gene, ATG7, protects bone marrow-derived mesenchymal stem cells from stressful conditions. Blood Res. 50, 80–86.

Neels, J.G., Olefsky, J.M., 2006. Inflamed fat: what starts the fire? J. Clin. Invest. 116, 33–35.

Ohnishi, S., Ohgushi, H., Kitamura, S., Nagaya, N., 2007. Mesenchymal stem cells for the treatment of heart failure. Int. J. Hematol. 86, 17–21.

Pasquinelli, G., Tazzari, P., Ricci, F., Vaselli, C., Buzzi, M., Conte, R., Orrico, C., Foroni, L., Stella, A., Alviano, F., Bagnara, G.P., Lucarelli, E., 2007. Ultrastructural characteristics of human mesenchymal stromal (stem) cells derived from bone marrow and term placenta. Ultrastruct. Pathol. 31, 23–31.

Patel, A.N., Yockman, J., Vargas, V., Bull, D.A., 2013. Putative population of adipose-derived stem cells isolated from mediastinal tissue during cardiac surgery. Cell Transplant. 22, 507–511.

Perrini, S., Laviola, L., Cignarelli, A., Melchiorre, M., De Stefano, F., Caccioppoli, C., Natalicchio, A., Orlando, M.R., Garruti, G., De Fazio, M., Catalano, G., Memeo, V., Giorgino, R., Giorgino, F., 2008. Fat depot-related differences in gene expression, adiponectin secretion, and insulin action and signalling in human adipocytes differentiated in vitro from precursor stromal cells. Diabetologia 51, 155–164.

Pinnick, K.E., Nicholson, G., Manolopoulos, K.N., McQuaid, S.E., Valet, P., Frayn, K.N., Denton, N., Min, J.L., Zondervan, K.T., Fleckner, J., Mol, P.C., McCarthy, M.I., Holmes, C.C., Karpe, F., 2014. Distinct developmental profile of lower-body adipose tissue defines resistance against obesity-associated metabolic complications. Diabetes 63, 3785–3797.

Pou, K.M., Massaro, J.M., Hoffmann, U., Vasan, R.S., Maurovich-Horvat, P., Larson, M.G., Keaney Jr., J.F., Meigs, J.B., Lipinska, I., Kathiresan, S., Murabito, J.M., O'Donnell, C.J., Benjamin, E.J., Fox, C.S., 2007. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 116, 1234–1241.

Richmond, J.M., Harris, J.E., 2014. Immunology and skin in health and disease. Cold Spring Harb Perspect. Med. 4, a015339.

Ritter, A., Friemel, A., Fornoff, F., Adjan, M., Solbach, C., Yuan, J., Louwen, F., 2015. Characterization of adipose-derived stem cells from subcutaneous and visceral adipose tissues and their function in breast cancer cells. Oncotarget 6, 34475–34493.

Russo, V., Yu, C., Belliveau, P., Hamilton, A., Flynn, L.E., 2014. Comparison of human adipose-derived stem cells isolated from subcutaneous, omental, and intrathoracic adipose tissue depots for regenerative applications. Stem Cells Transl. Med. 3, 206–217.

Schaffler, A., Muller-Ladner, U., Scholmerich, J., Buchler, C., 2006. Role of adipose tissue as an inflammatory organ in human diseases. Endocr. Rev. 27, 449–467.

Schweizer, R., Tsuji, W., Gorantla, V.S., Marra, K.G., Rubin, J.P., Plock, J.A., 2015. The role of adipose-derived stem cells in breast cancer progression and metastasis. Stem Cells Int. 2015, 120949.

Sicari, R., Sironi, A.M., Petz, R., Frassi, F., Chubuchny, V., De Marchi, D., Positano, V., Lombardi, M., Picano, E., Gastaldelli, A., 2011. Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI vs echo study. J. Am. Soc. Echocardiogr. Off. Publ. Am. Soc. Echocardiogr. 24, 1156–1162.

Siciliano, C., Ibrahim, M., Scafetta, G., Napoletano, C., Mangino, G., Pierelli, L., Frati, G., De Falco, E., 2015a. Optimization of the isolation and expansion method of human mediastinal-adipose tissue derived mesenchymal stem cells with virally inactivated GMP-grade platelet lysate. Cytotechnology 67, 165–174.

Siciliano, C., Chimenti, I., Bordin, A., Ponti, D., Iudicone, P., Peruzzi, M., Rendina, E.A., Calogero, A., Pierelli, L., Ibrahim, M., De Falco, E., 2015b. The potential of GMP-Compliant platelet lysate to induce a permissive state for cardiovascular transdifferentiation in human mediastinal adipose tissue-derived mesenchymal stem cells. Biomed. Res. Int. 162439. http://dx.doi.org/10.1155/2015/162439 (Epub 2015 Oct 1. PMID: 26495284).

Siciliano, C., Chimenti, I., Ibrahim, M., Napoletano, C., Mangino, G., Scafetta, G., Zoccai, G.B., Rendina, E.A., Calogero, A., Frati, G., De Falco, E., 2015c. Cardiosphere conditioned media influence the plasticity of human mediastinal adipose tissue-derived mesenchymal stem cells. Cell Transplant. 24, 2307–2322.

Singh, R., Xiang, Y., Wang, Y., Baikati, K., Cuervo, A.M., Luu, Y.K., Tang, Y., Pessin, J.E., Schwartz, G.J., Czaja, M.J., 2009. Autophagy regulates adipose mass and differentiation in mice. J. Clin. Invest. 119, 3329–3339.

Sironi, A.M., Petz, R., De Marchi, D., Buzzigoli, E., Ciociaro, D., Positano, V., Lombardi, M., Ferrannini, E., Gastaldelli, A., 2012. Impact of increased visceral and cardiac fat on cardiometabolic risk and disease. Diabet. Med. J. Br. Diabet. Assoc. 29, 622–627.

Tanaka, Y., 2015. Human mesenchymal stem cells as a tool for joint repair in rheumatoid arthritis. Clin. Exp. Rheumatol. 33, S58–S62.

Tchkonia, T., Giorgadze, N., Pirtskhalava, T., Tchoukalova, Y., Karagiannides, I., Forse, R.A., DePonte, M., Stevenson, M., Guo, W., Han, J., Waloga, G., Lash, T.L., Jensen, M.D., Kirkland, J.L., 2002. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. Am. J. Phys. Regul. Integr. Comp. Phys. 282, R1286–R1296.

Toyoda, M., Matsubara, Y., Lin, K., Sugimachi, K., Furue, M., 2009. Characterization and comparison of adipose tissue-derived cells from human subcutaneous and omental adipose tissues. Cell Biochem. Funct. 27, 440–447.

Trayhurn, P., Wood, I.S., 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br. J. Nutr. 92, 347–355.

Veikkola, T., Karkkainen, M., Claesson-Welsh, L., Alitalo, K., 2000. Regulation of angiogenesis via vascular endothelial growth factor receptors. Cancer Res. 60, 203–212.

Ye, J., Gimble, J.M., 2011. Regulation of stem cell differentiation in adipose tissue by chronic inflammation. Clin. Exp. Pharmacol. Physiol. 38, 872–878.

Zimmerlin, L., Park, T.S., Zambidis, E.T., Donnenberg, V.S., Donnenberg, A.D., 2013. Mesenchymal stem cell secretome and regenerative therapy after cancer. Biochimie 95, 2235–2245.