Cancer Letters 380 (2016) 114–121

Contents lists available at ScienceDirect

Cancer Letters

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

Original Articles

Adipocytes contribute to the growth and progression of multiple myeloma: Unraveling obesity related differences in adipocyte signaling

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 9 June 2016
Accepted 13 June 2016

Keywords:
Multiple myeloma
Adipocyte
Obesity
Adhesion
Angiogenesis
Adipose-derived stem cells

ABSTRACT

The prevalence of obesity over the last several decades in the United States has tripled among children and doubled among adults. Obesity increases the incidence and progression of multiple myeloma (MM), yet the molecular mechanisms by which adipocytes contribute to cancer development and patient prognosis have yet to be fully elucidated. Here, we obtained human adipose-derived stem cells (ASCs) from twenty-nine normal (BMI = 20–25 kg/m^2), overweight (25–30 kg/m^2), obese (30–35 kg/m^2), or super obese (35–40 kg/m^2) patients undergoing elective liposuction. Upon differentiation, adipocytes were co-cultured with RPMI-8226 and NCI-H929 MM cell lines. Adipocytes from overweight, obese and super obese patients displayed increased PPAR-gamma, cytochrome C, interleukin-6, and leptin protein levels, and decreased fatty acid synthase protein. 8226 MM cells proliferated faster and displayed increased pSTAT-3/STAT-3 signaling when cultured in adipocyte conditioned media. Further, adipocyte conditioned media from obese and super obese patients significantly increased MM cell adhesion, and conditioned media from overweight, obese and super obese patients enhanced tube formation and expression of matrix metalloproteinase-2. In summary, our data suggest that adipocytes in the MM microenvironment contribute to MM growth and progression and should be further evaluated as a possible therapeutic target.
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Introduction

Multiple myeloma (MM), a plasma cell neoplasia, represents approximately 10% of all hematologic cancers [1]. Despite a dramatic improvement in the average prognosis of MM patients over the last two decades due to more effective drugs and treatment strategies, MM remains an incurable malignancy [2]. In addition to established risk factors such as age, African ancestry, male sex, monoclonal gammopathy of undetermined significance, and family history, in recent years, numerous epidemiological studies have identified obesity as a risk factor for MM [3–6]. Obesity significantly increases both the relative risk of developing MM and MM-associated mortality [3–6]. Despite this epidemiological association, the molecular underpinnings by which obesity contributes to MM growth and progression are relatively unknown.

MM is characterized by clonal expansion of abnormal plasma cells in the bone marrow [7]. The bone marrow microenvironment plays a supportive role in growth, migration, proliferation, survival, and drug resistance of MM cells [8,9]. Within human bone marrow adipocytes are the most abundant cell type [10]. Adipocytes, although traditionally thought of as having functions limited to energy storage, are now considered a major endocrine organ [11]. Adipocytes secrete various adipokines and inflammatory factors and reciprocal signaling between adipocytes and cancer cells is reported to contribute to tumor initiation, growth and metastasis in several types of cancer [11]. Adipocytes from overweight and obese individuals display an altered cytokine and lipid profile when compared to adipocytes from normal weight individuals, as adipocytes from individuals with a higher BMI have increased production of inflammatory markers and leptin and decreased production of anti-inflammatory cytokines and the tumor suppressor adiponectin [12,13]. This exaggerated inflammatory response may increase genomic instability, disturb DNA repair, promote tumor progression, cause local immunosuppression, or induce epigenetic changes [14,15].

In this study we co-cultured human MM cell lines with adipocytes from normal, overweight, obese or super obese patients who received elective liposuction. We found a positive correlation between BMI and adhesion and angiogenesis of MM cells. Moreover, we identified hormonal, lipid, and signaling factor dysregulation in obese adipocytes that can contribute to MM growth and progression.

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http://dx.doi.org/10.1016/j.canlet.2016.06.010
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Materials and methods

Cell lines

Human MM cell lines RPMI 8226 and NCI-H929 (ATCC; Manassas, VA) were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 1% PenStrep, and 1% GlutaMAX (Life Technologies; Grand Island, NY) at 37 °C and 5% CO_2.

Stem cell isolation

Lipoaspirates from patients undergoing elective liposuction at the office of DaVinci Plastic Surgery (Washington, DC) were used in this study. All patients gave informed consent prior to surgery. Lipoaspirate cells were washed extensively to remove contaminating red blood cells and debris. Washed lipoaspirate samples were then digested with 0.075% Type II Collagenase in HBSS (Life Technologies, Grand Island, NY) for 30–45 at 37 °C. Collagenase was neutralized with HBSS + 10% FBS, and cells were centrifuged at 1000 RPM for 5 minutes, filtered through 100 μm mesh filter, and centrifuged again. Adipose-derived stem cells (ASCs) were plated and extensively washed 24 hours later to remove any residual red blood cells. For some studies additional ASCs were purchased from Zen-Bio, Inc. (Research Triangle Park, NC). All ASCs were used at passage 2. All ASCs and subsequently differentiated adipocytes were grouped into one of four categories: normal (BMI = 20–25 kg/m^2), overweight (25–30 kg/m^2), obese (30–35 kg/m^2), and super obese (35–40 kg/m^2).

Adipocyte, chondrocyte and osteoblast differentiation and detection

ASCs were cultured in subconfluent conditions in supplemented DMEM until they reached passage 2. ASCs were functionally evaluated by inducing differentiation into the following cell types: adipocytes, chondrocytes, and osteoblasts. Adipocyte differentiation media contained 10% FBS, 1% PenStrep, 1.0 μM Dexamethasone, 0.5 mM Isobutylmethylxanthine (IBMX), 0.2 mM Indomethacin, and 10.0 μM insulin, (Sigma-Aldrich; St. Louis, MO). Adipogenesis was visualized on day 17 by Oil Red O staining. Adipocytes were paraformaldehyde-fixed, washed with 60% isopropyl alcohol, incubated in Oil Red Solution (Sigma-Aldrich; St. Louis, MO), and imaged. ASCs differentiated into chondrocytes were plated in 1×10^7 micro-mass 10 μL droplets. Cultures were grown in chondrocyte differentiation media (DMEM supplemented with 1% FBS, 1% PenStrep, 50 μg/mL L-Ascorbic Acid 2-Phosphate, 6.25 μg/mL insulin, 6.25 μg/mL transferrin, and 10 ng/mL TGFβ-1) for 17 days. Mature chondrocytes were washed, fixed, stained with Alcian Blue stain and imaged. ASCs were differentiated into osteoblasts by culturing in DMEM supplemented with 5% FBS, 1% PenStrep, 0.1 μM Dexamethasone, 50 μM L-ascorbic acid 2-phosphate, and 10 mM β-1 glycerophosphate. Mature osteoblasts were stained using Von Kossa Stain. Briefly, cells were fixed, incubated in 2% silver nitrate, exposed to UV light, washed, and imaged.

Adipocyte – MM co-culture

Fully differentiated adipocytes from normal, overweight, obese or super obese patients were cultured alone or co-cultured with RPMI 8226 or NCI-H929 MM cells (ATCC; Manassas, VA) using 0.4 μm polyethylene terephthalate hanging cell culture insert (EMD Millipore; Chicago, IL). Additional blank wells containing no adipocytes were plated with normal supplemented media to be used as controls. After 24 hours of co-culture, conditioned media, protein and RNA were collected from adipocytes (adipocyte conditioned media) or blank wells ("media alone" control). Conditioned media was filtered prior to use to remove cellular debris.

Tube formation assay

1×10^5 3B-11 endothelial cells were serum-starved in DMEM with 0.2% FBS overnight, calcein stained (Life Technologies; Grand Island, NY), plated onto basement membrane extract (BME) as described elsewhere [16], and exposed to pooled conditioned media from normal, overweight, obese or super obese adipocytes. The tube network was allowed to grow for 6–9 hours before paraformaldehyde fixing and then imaged using a fluorescent microscope (Olympus; Center Valley, PA). The tube network (number of nodes/meshes/segments) was quantified using ImageJ Angiogenesis Analyzer Plugin, HUVEC Fluo Analysis.

Western blotting

Total protein lysates were prepared from MM cell lines, MM cells that had been co-cultured with adipocytes and adipocytes alone using M-PER reagent (Thermo Fisher Scientific, Rockford, IL, USA) containing Halt protease/phosphatase inhibitor (Thermo Fisher Scientific) in accordance with the manufacturer's protocol. Proteins were quantified using BCA protein assay kit (Thermo Scientific; Waltham, MA) and samples pooled within each BMI category. Proteins were separated using 4–12% Bis-Tris protein gels (Life Technologies; Grand Island, NY). Gels were electrophoresed and transferred to PVDF membrane using the iBlot 2 Dry Blotting System (Life Technologies; Grand Island, NY). Membranes were blocked in 5% BSA. Primary antibodies (Cell Signaling; Danvers, MA) were applied in a 1:1000 dilution in 5% BSA. Anti-rabbit HRP secondary antibody was applied at a dilution of 1:2000 (Cell Signaling Technology). West Dura Chemiluminescent substrate (Thermo, Rockland, Il) was used for signal detection. Membranes were visualized using a ChemiDoc-It imaging system (UVP, Upland, CA). All bands on the Western blot were quantified using NIH ImageJ and normalized to the densitometry for the respective β-actin band. Analysis of variance (ANOVA) results for normalized samples were obtained using StatPlus One-Way ANOVA analysis with group variables. To determine whether individual comparisons (e.g. BMI 25–30 vs. 30–35) were significant, Tukey's LSD post-hoc test was conducted. All Western blots were replicated a minimum of three times.

IL-6 ELISA

IL-6 was measured through an IL-6 ELISA assay (Thermo Fisher Scientific; Grand Island, NY) according to manufacturer's protocol. Briefly, biotinylated antibody was added to a 96 well plate, followed by addition of standards or conditioned media samples from adipocytes. After incubation and washings, streptavidin-HRP was added, plate was incubated, washed, substrate added and absorbance measured at 450 nm. Concentration was determined using a standard curve.

Zymography

To measure MMP-2 enzymatic activity, zymography was conducted on adipocyte conditioned media samples using 10% Tris-Glycine gels containing 0.1% gelatin (Life Technologies; Grand Island, NY). Fifteen micrograms of protein in β-mercaptoethanol-free 2X loading buffer were electrophoresed at 125 V for 90 min in Tris-Glycine/sodium dodecyl sulfate running buffer. After renaturation, developing solution was added and gels were washed, stained using Invitrogen SimplyBlue™ SafeStain (Life Technologies; Grand Island, NY) and destained in deionized water. Densitometry was performed on all bands using NIH ImageJ.

Viability

To assess MM cell viability, WST-1 Reagent was used (Cayman Chemical; Ann Arbor, MI) per manufacturer's instructions. Briefly, 2.5×10^4 MM cells were grown in normal supplemented media or adipocyte-conditioned normal supplemented media in 96-well plates. After 48 hours, 10 μL of Wst-1 Reagent was added to each well, incubated at 37 °C and measurements taken at 450 nm.

Adhesion assay

MM cells were grown in adipocyte conditioned media in 96-well plates. 2×10^4 H929 or 1×10^4 8226 cells were plated in 100 μL of adipocyte conditioned media and grown for 2 days. Non-adherent MM cells were removed and cells were washed and fixed with paraformaldehyde for 15 minutes at room temperature. Images were taken and number of adherent cells was obtained using the NIH ImageJ with Cell Counter Plugin.

Real-time polymerase chain reaction (qPCR)

SuperScript® III Platinum® SYBR® Green Two-Step qPCR Kit w/ROX was used per manufacturer's instructions (Life Technologies; Grand Island, NY) using 1 μg of purified total RNA as template for amplifications. Reverse transcribed cDNA was diluted 1:4 with ultrapure water. qPCR was performed on an Mx3005P QPCR System (Agilent Technologies). Threshold cycle values were calculated with the MxPro QPCR software (v. 4.10) using the Pfaffl method, and dissociate curves for each reaction were checked to confirm that only a single PCR product was obtained [17]. Threshold cycle values were normalized with GAPDH, and relative expression was calculated comparing differences between adipocyte samples. An unpaired t-test between triplicates was used to determine level of significance. All experiments were repeated a minimum of three times. The specific oligonucleotide primers obtained from Life Technologies (Carlsbad, CA) are displayed in Table 1.

Statistical analysis

Viability, qPCR, zymogram, adhesion, and tube formation data were analyzed through analysis of variance (StatPlus One-Way ANOVA). *P* values of 0.05 and below were determined to be significant. To determine whether individual comparisons (e.g. BMI 25–30 vs. 30–35) were significant, Tukey's LSD post-hoc test was conducted.

Results

BMI negatively correlates with ASC viability and positively correlates with markers of differentiation and lipid signaling in adipocytes

Cells from twenty-nine patients undergoing elective liposuction were used in this study (Table 2). Samples from patients were divided into four categories based on BMI of the patient: normal (BMI = 20–25 kg/m^2), overweight (BMI = 25–30 kg/m^2), obese (BMI = 30–35 kg/m^2), and super obese (BMI = 35–40 kg/m^2). Patients

Table 1
qPCR primer sequences.

Primer	Forward (5′-3′)	Reverse (3′-5′)	Amplicon (bp)
GAPDH	AAGGTGAAGGTCGGAGTCAA	AATGAAGGGGTCATTGATGG	108
Leptin	GTAGGAATCGCAGCGCC	AAAGATAGGGCCAAAGCCAC	106
PPARγ	CCAGAAAGCGATTCCTTCAC	CGGAGCTGATCCCAAAGTT	109
Cytochrome C	CCACAGGCATTCCTCTGTCCA	TGTGTATTTGATTGGGAATGGTGC	380
Adiponectin	TGGTGAGAAGGGTGAGAA	AGATCTTGGTAAAGCGAATG	221

with chronic diseases or illnesses were excluded from this study. The mean age of patients did not statistically differ between categories (normal = 39.1 +/− 11.0; overweight = 45.9 +/− 10.9; obese = 39.7 +/− 8.1; super obese = 41.8 +/− 13). In order to confirm the pluripotent nature of isolated ASCs, ASCs were differentiated into the following cell types: adipocytes, chondrocytes, and osteoblasts (Fig. 1A). ASC viability in individual patients was performed and revealed an inverse relationship between viability and BMI, with cells from overweight and obese individuals growing 10% slower than cells from normal weight individuals and super obese individuals growing 26% slower (Fig. 1B). To see if ASC viability and markers of adipocyte differentiation and lipid signaling are inversely correlated we measured RNA and protein expression in adipocytes (Fig. 1C, 1D). qPCR revealed significant ($p \leq 0.05$) increases in expression of PPARγ (2.1, 9.6 and 10.4), leptin (1.6, 2.5 and 3.6) and adiponectin (3.1, 3.2 and 4) in overweight, obese and super obese adipocytes compared to normal adipocytes. Cytochrome C was elevated in obese subjects 2.1 fold, but not in the other treatment groups. Through Western analysis we found significantly higher leptin (1.3, 2.1 and 3.8), cytochrome C (1.3, 1.5 and 1.9) and PPARγ (2.2, 5, 2.1 fold respectively) in cells from overweight, obese and super obese patients compared to cells from normal weight individuals. Moreover, we found fatty acid synthase to be negatively correlated with BMI, as overweight, obese and super obese adipocytes expressed significantly lower (3.7, 5, 3.8 fold respectively) fatty acid synthase protein compared to adipocytes from normal weight patients.

Table 2
Patient information from lipoaspirate samples.

ID Number	BMI	Location	Gender	Age
1016	17.59	Abdomen	F	33
20206	18.8	Thigh	F	25
116	20.83	Abdomen	F	56
605	21	Abdomen	F	37
82010	21.1	Abdomen	F	42
321	22	Abdomen	F	41
1108	23.29	Abdomen	F	57
417	24.51	Abdomen	F	26
322	24.7	Abdomen	F	32
413	24.71	Abdomen	F	42
401	26	Abdomen	F	66
404	26.58	Abdomen	F	42
402	27.2	Abdomen	F	38
1107	27.44	Abdomen	F	40
913	27.5	Abdomen	F	57
206	28.12	Abdomen	F	25
502	28.15	Abdomen	F	42
1015	28.97	Abdomen	F	57
41102	31.52	Abdomen	M	31
912	31.6	Breast	M	27
21606	32.1	Abdomen	F	46
70804	33	Abdomen	M	42
32003	33.4	Thigh	F	40
60303	34.2	Thigh	F	50
50709	34.8	Abdomen	F	42
117	35.15	Neck	M	33
302	36.58	Abdomen	F	61
72709	38	Abdomen	F	39
40403	39.5	Abdomen	M	34

MM cell viability is increased in adipocyte conditioned media and positively correlates with BMI

The effect of adipocyte secreted factors on MM viability was assessed (Fig. 2A, 2B). When RPMI-8226 MM cells were cultured for 48 hours in conditioned media from adipocytes, viability increased 52% (normal; $p = 0.031$), 56% (overweight; $p = 0.009$), 66% (obese; $p = 0.002$), and 78% (super obese; $p = 5 \times 10^{-5}$) compared to when cultured alone. In a similar fashion, RPMI-929 MM viability was increased when MM cells were exposed to conditioned media from adipocytes, achieving increases of 40.8% (normal; $p = 0.002$), 52.8% (overweight; $p = 0.0003$), 65.1% (obese; $p = 0.002$), and 42.2% (super obese; $p = 0.048$). Moreover, this increase in viability was associated with increases in p-STAT-3 and STAT-3 protein levels (Fig. 2D). p-STAT-3 was undetectable in MM cells alone. The levels of p-STAT-3 were elevated 5, 3.8 and 1.8 fold in overweight, obese and super obese co-cultured cells compared to MM cells co-cultured with adipocytes from normal-weight individuals. Further, STAT-3 total protein levels were similar in 8226 MM cells and 8226 MM cells co-cultured with adipocytes from normal weight individuals but increased in MM cells co-cultured with adipocytes from overweight (3 fold), obese (3.8 fold) and super obese (4.9 fold) patients. In a similar fashion, IL-6, an activator of STAT-3, was measured in conditioned media from adipocytes (Fig. 2C). Higher BMIs were associated with increased secretion of IL-6, as IL-6 levels increased 3.8 fold (overweight; $p \leq 0.001$) 4.6 fold (obese; $p \leq 0.01$), and 4.1 fold (super obese; $p \leq 0.002$) when compared to adipocytes from normal weight individuals.

MM cells grown in adipocyte conditioned media from obese and super obese patients display increased adhesion

Induction of adhesion molecules aid in the homing of MM cells to bone marrow [18]. In normal supplemented media a small percentage of MM cells weakly adhere to tissue culture flasks, amounting to 1.5% of total NCI-H929 cells and 10% of RPMI-8226 cells. However, when MM cells are grown in adipocyte-conditioned media, overall adhesion increases with increasing BMI (Fig. 3A–3E). Media conditioned by obese adipocytes increased adhesion of NCI-H929 and RPMI-8226 cells 1.5 to 2 fold ($p \leq 0.05$) and media from super obese adipocytes increased adhesion of NCI-H929 and RPMI-8226 2 to 2.5 fold ($p \leq 0.05$) compared to supplemented media. Further, this increased adhesion correlated with increases in the amount of Integrin α4 protein in RPMI-8226 MM cells co-cultured with adipocytes compared to MM cells alone.

3B11 endothelial cells display increased angiogenic potential in response to adipocyte conditioned media from overweight, obese and super obese individuals

White fat has been shown to stimulate angiogenesis [19,20]. Thus, we performed an endothelial tube formation assay to identify whether adipocytes from overweight, obese and super obese individuals drive increased tube formation compared to adipocytes from normal individuals (Fig. 4A,B). 3B11 endothelial cells subjected to

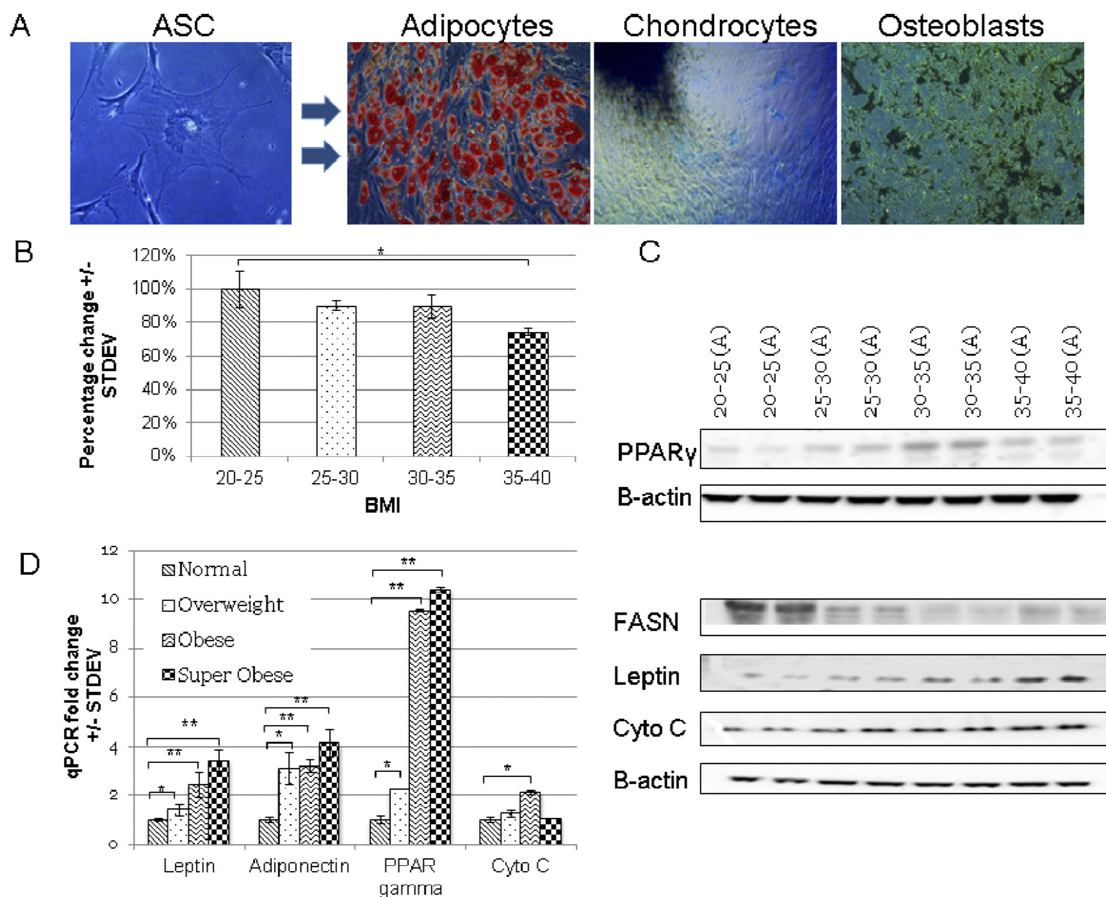

Fig. 1. Functional evaluation of pluripotency (A) and WST-1 viability (B) in ASCs after 48 hours. PPARγ, fatty acid synthase (FASN), leptin, and cytochrome C protein expression in lysates pooled by BMI ($n \geq 4$ patients/BMI) and run in duplicate (C). qPCR analysis of leptin, adiponectin, PPARγ, and cytochrome C in patients pooled by BMI category ($n \geq 4$ patients/BMI category). In addition to statistical differences between normal adipocytes and adipocytes from individuals with higher BMIs, all BMI-categories for Leptin, PPAR-γ, and Adiponectin (except between overweight and obese) were found to be statistically different from one another ($p \leq 0.05$) (D). *$p \leq 0.05$, **$p \leq 0.01$.

adipocyte conditioned media from individuals with higher BMIs formed more extensive networks. After eight hours of exposure to conditioned media from overweight, obese and super obese adipocytes, 3B11 cultures formed 2–3 times more nodes than cells that were exposed to media from normal adipocytes ($p \leq 0.01$ for obese and super obese, $p \leq 0.05$ for overweight). Additionally, conditioned media from obese and super obese samples had statistically higher numbers of segments ($p \leq 0.01$ for obese, $p \leq 0.05$ for overweight and super obese) and conditioned media from obese samples had significantly higher numbers of mesh ($p \leq 0.05$). Further, since MM patients with elevated MMP-2 expression display increased bone marrow angiogenesis [21] we measured active MMP-2 through zymography (Fig. 4C/D). We found MMP-2 expression to mirror the angiogenesis data, with overweight, obese and super obese patients having 2–3 times higher MMP-2 activity ($p \leq 0.05$) than normal adipocytes.

Discussion

The obesity epidemic is increasingly a global problem, as obesity has more than doubled worldwide in the last 35 years and obesity-related cancer deaths have dramatically increased in modern times [22,23]. BMI is routinely used as a measure of adiposity. Epidemiological studies have correlated BMI with cancer mortality, as each 5 kg/m^2 increase in BMI is associated with an increase of 10% in cancer-related death [24]. With obesity rates on the rise and the epidemiological connection between obesity and MM, it is important to identify the molecular mechanisms driving MM disease progression.

The bone marrow microenvironment plays an important role in the proliferation, survival, progression, and drug resistance of MM cells [8,9]. With increasing age, adipocytes become the principal component in bone marrow [25]. Adipocytes are known to adapt their cell signaling profile as an individual's BMI increases [26]. This change in the secretion of inflammatory, adipokine and hormonal factors can directly impact disease progression within MM. Numerous reports have shown that obesity is positively correlated with leptin expression, and adipocytes, partially through their expression of soluble leptin, contribute to MM pathogenesis [27,28]. However, whether BMI affects the degree to which adipocytes influence MM cancer growth and progression is unknown.

In this study we used differentiated human adipocytes obtained from adipose-derived stem cells of normal, overweight, obese or super obese patients undergoing elective liposuction. Adipose-derived stem cells are biologically similar to bone marrow mesenchymal stem cells in that both types of stem cells have capacity for multi-lineage differentiation and display similar fibroblast-like morphology and surface antigen expression [29,30]. Although there are some differences in proliferative ability, differentiation potential, immunomodulatory activity, or secreted proteins between these alternate sources of stem cells, adipose-derived mesenchymal stem cells are easier to obtain, have higher yields and are

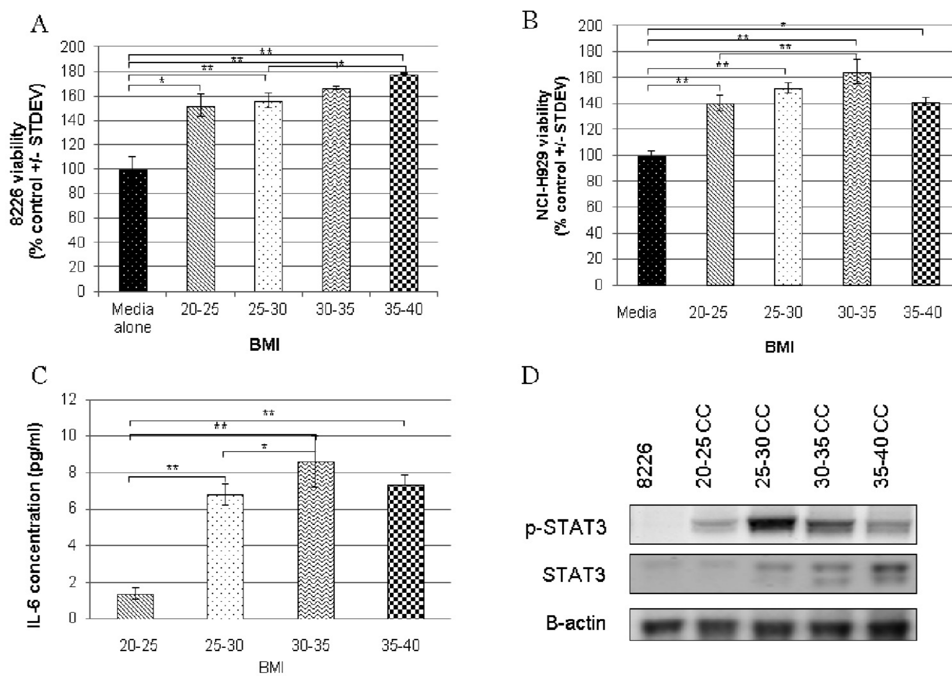

Fig. 2. Wst-1 assay measuring RPMI-8226 (A) and NCI-H929 (B) viability in MM cells or MM cells cultured in normal, overweight, obese or super obese adipocyte conditioned media for 48 hours. Data were collected from individual patients and pooled by BMI category. IL-6 secreted protein levels in conditioned media from adipocytes (C). Western analysis of p-STAT-3 and STAT-3 protein in cell lysates from RPMI-8226 MM cells alone and MM cells co-cultured with adipocytes of various BMI categories (D). Western data was normalized to B-actin. *p ≤ 0.05, **p ≤ 0.01.

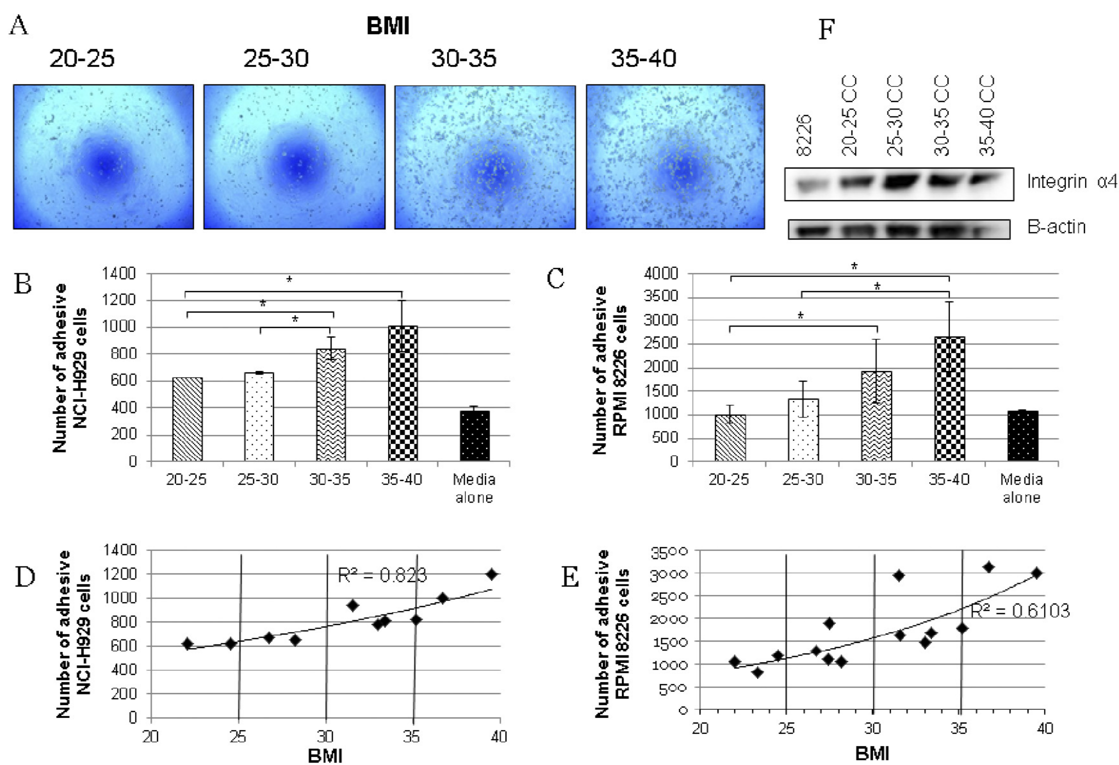

Fig. 3. Representative images from RPMI-8226 adhesion after 48 hours in adipocyte conditioned media (A). NCI-H929 (B) and RPMI-8226 (C) adhesion in MM cells cultured in adipocyte conditioned media from individual patients. NCI-H929 (D) and RPMI-8226 (E) correlation coefficient in individual patients. Western blot of Integrin α4 in RPMI-8226 MM cellular lysates alone or protein from MM cells cultured in adipocyte conditioned media. * p ≤ 0.05.

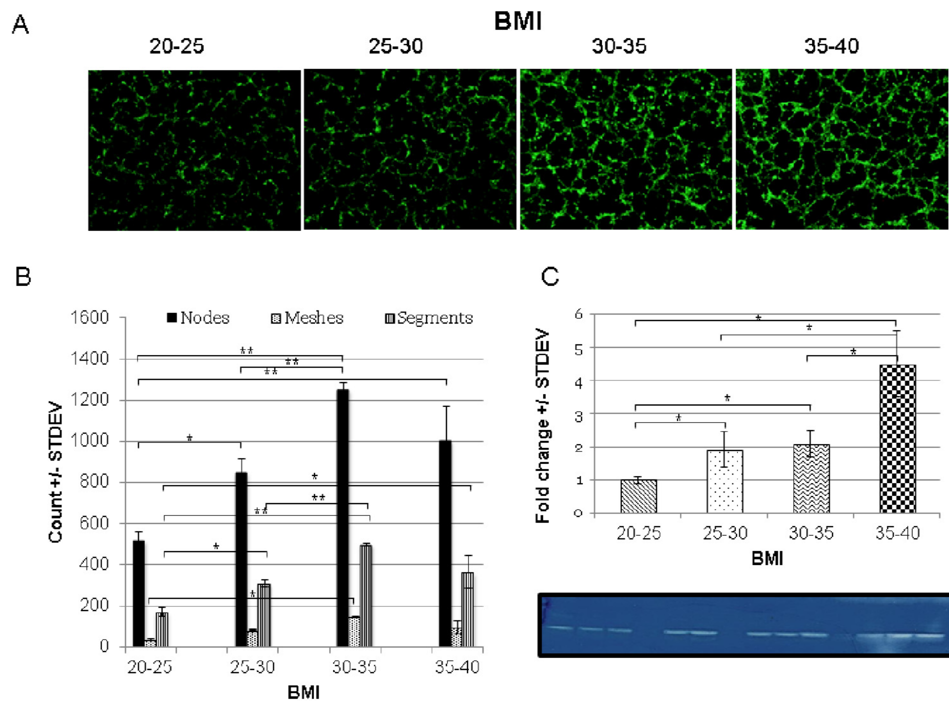

Fig. 4. Representative images of endothelial tube formation assay (A). 3B-11 endothelial cells were cultured in adipocyte conditioned media for 8 hours, Calcein stained and imaged with a fluorescent microscope at 5× magnification. Quantification of 3B-11 endothelial tube formation assay to display number of nodes, meshes and segments $^{*}p \le 0.05$, $^{**}p \le 0.01$ (B). Representative zymogram for MMP-2 activity in conditioned media from adipocytes of increasing BMIs (C).

reportedly as effective as bone marrow for clinical applications [29–35].

Despite the beneficial role of ASCs in cell-based therapies, ASCs have also been implicated in tumor development or progression [36–40]. Several mechanisms have been proposed to explain how ASCs interact with cancer cells and affect their microenvironment [41]. These include differentiation into cancer-associated myofibroblasts (CAFs), paracrine signaling, secretion of adipokines that promote vascularization and creation of an inflammatory microenvironment [41]. Moreover, reports have found that ASCs isolated from obese humans or animals increase tumorigenesis to a greater extent than cells from normal weight individuals, typically displaying increased expression of adipokines, chemokines and/or matrix metalloproteinases [37–40,42]. As others have shown, we found ASC viability to be inversely correlated with BMI [43]. Some reports suggest that long term storage of cryopreserved samples can affect viability of ASCs [44]. However, this is not a factor in our model as similar BMI-related differences in viability were found when studies were conducted in fresh ASCs as well as those used at passage 2 after short-term cryopreservation (less than 4 months). Since ASCs from obese and super obese patients proliferated slower than cells from normal weight individuals we sought to identify whether they were primed to have higher adipogenic differentiation potential. When grown in adipocyte differentiation media we found that ASCs from obese and super obese patients started differentiating and accumulating lipid (as assessed by Oil Red staining) on average 2–3 days earlier than ASCs from normal weight individuals (unpublished observations), yet by day 17 all ASCs completed differentiation to roughly the same degree regardless of BMI. However, molecular differences were still apparent in fully differentiated adipocytes, as overweight, obese and super obese adipocytes had higher levels of leptin, cytochrome C and PPARγ mRNA and protein expression and lower levels of FASN protein compared to adipocytes from normal weight individuals. In a similar manner, inflammatory proteins (IL-6, p-STAT-3, STAT-3) were increased in adipocytes from patients with higher BMIs. Thus, although all cells were differentiated and cultured at the same time under the same conditions, the adipocytes mirrored the patient from which they came. In particular, cells from obese and super obese patients had altered lipid profiles and increased inflammatory signaling, suggesting an epigenetic effect. Whether methylation of key genes such as PPARγ contributes to these differences has yet to be determined.

It is well established that reciprocal signaling between MM cells and cells of the BM microenvironment contributes to tumor growth and progression [45,46]. All adipocyte conditioned media increased growth of MM cell lines regardless of BMI status with super obese conditioned media having the largest effect on RPMI-8226 cell lines and conditioned media from obese adipocytes having the most pronounced effect on RPMI-H929 cells. Similar results were found in MM cells cultured with ASCs (data not shown). Interestingly, we found increases in MM adhesion when MM cells were exposed to conditioned media from obese/super obese adipocytes and increases in tube formation when endothelial cells were subjected to conditioned media from overweight, obese or super obese cells compared to conditioned media from adipocytes of normal weight individuals. Adhesion molecules expressed by MM cells can aid in homing to bone marrow and adipocytes [18]. It has previously been shown that MM cells that adhere to bone marrow have increased survival, proliferation, migration, and drug resistance [18]. Integrins on MM cells help bind MM cells to components of the extracellular matrix. We found increased levels of α4 integrin in 8226 MM cells subjected to media from adipocytes. α4 integrin, when heterodimerized with β1 integrin, constitutes VLA-4. α4 integrin has been shown to be important in cell adhesion mediated drug resistance and blockage of this integrin restores drug sensitivity to bortezomib [47]. Additionally, integrin adhesion enhances IL-6 mediated STAT3 signaling in myeloma cells [48]. Whether upregulation of integrin proteins or other adhesive factors contribute to MM-drug resistance in obese and super obese patients has yet to be identified.

Angiogenesis is a tightly regulated process important in MM progression [49,50]. Elevated bone marrow angiogenesis is associated with increased disease severity and poor clinical outcome in MM patients [49–52]. During angiogenesis endothelial cells proliferate rapidly in response to signals from the tumor and its microenvironment. Within the microenvironment, adipocytes have been shown to increase angiogenesis of cancer cells, and thus adipose tissue has emerged as a potential therapeutic target [20]. Adipocytes can induce vascularization through secretion of angiogenic factors, adipokines such as leptin and cytokines such as IL-6 [19]. Among other angiogenic factors, overexpression of matrix metalloproteinases (MMPs) has been implicated in MM [50]. MMPs are a family of serine proteases that degrade the extracellular matrix, facilitating tumor invasion, metastasis and angiogenesis [53]. Patients with active MM have heightened MMP-2, which can increase bone marrow angiogenesis [21]. Since we found increased angiogenic potential in adipocytes from obese and super obese patients, we used zymography to measure active MMP-2 expression. In accordance with the angiogenesis data, we found increased active MMP-2 to be correlated with increasing BMI.

One area of research that warrants further investigation concerns body fat location from which the lipoaspirates were taken. Although the majority of our samples came from abdominal fat, a few samples were from other locations (thigh, arms, neck, breast). In qPCR, viability and adhesion assays we found abdominal adipose was more supportive of cancer growth than samples from other fat depots (unpublished findings). This agrees with other reports that suggest abdominal obesity promotes cancer tumorigenicity more than obesity in other bodily locations [38]. The genetic differences between abdominal and non-abdominal adipocytes, as they relate to cancer susceptibility and clinical outcome, have not been fully characterized, although others have found abdominal adipocytes to have higher expression of genes involved in immune responses, insulin signaling and amino acid metabolism [54].

In conclusion, this study provides new insight into the link between obesity and MM development/progression by demonstrating that adipocytes contribute to MM cancer growth and progression, with the most pronounced effect occurring in cells from obese and super obese individuals. Improving our understanding of reciprocal signaling between adipocytes and MM cells may offer new therapeutic strategies for treating this disease.

Conflict of interest

The author declares no competing financial interests.

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