INTRODUCTION

Malignant melanoma is a heterogeneous disease with complex molecular mechanisms involving genetic alterations that control the proliferation, differentiation, and survival of tumor cells (Palmieri et al., 2015). A central mechanism of melanoma cell survival is activation of the MAPK pathway, which is responsible for mediating cellular responses to extracellular mitotic stimuli. This pathway is controlled by phosphorylation of the intracellular proteins RAS (H-RAS, K-RAS, and N-RAS), RAF (A-RAF, B-RAF, and C-RAF), MEK, and ERK (Palmieri et al., 2015; Davies et al., 2002). MAPK pathway alterations have been reported in about 20% of all cancers (Castellano, Downward, 2011). More than 60% of cutaneous melanoma patients carry the B-RAF V600E mutation (Davies et al., 2002) and about 20% carry the N-RASQ61R mutation (Ribas, Flaherty, 2011; Uguen et al., 2015; Sheen et al., 2016).

Inflammation plays an important role in melanoma cell progression, invasion, and metastasis (Fan, Mao, Yang, 2013; Quail, Joyce, 2013). The link between inflammation and melanoma development is established by intrinsic and extrinsic pathways. The former involves activation of oncogenes, including BRAF and NRAS, both of which encode members of the MAPK signaling pathway, and inactivation of tumor suppressors such as p53 (Hocker, Singh, Tsao, 2008; Madan, Lear, Szeimies, 2010; Hanahan, Weinberg, 2011). These alterations lead to inflammation by stimulating the production of inflammatory mediators,
such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), in the tumor microenvironment (Mantovani et al., 2002; Mantovani et al., 2008; Sica et al., 2006). The association between tumor progression and inflammation was first observed in 1863, when researchers discovered macrophage infiltration in neoplastic tissues, suggesting a strong correlation between tumor survival and chronic inflammation (Balkwill, Mantovani, 2001).

The tumor microenvironment is composed of a variety of cells, including fibroblasts and immune cells, mostly macrophages. Tumor-associated macrophages may represent up to 50% of solid tumors, and their activities are usually pro-tumorigenic, enhancing cancer cell survival, proliferation, and dissemination (Mantovani, Sica, Locati, 2005; Sica et al., 2006; Qian, Pollard, 2010).

A similar inflammatory condition observed in obese individuals is low-grade inflammation (Maachi et al., 2004; Klein-Platat et al., 2005; Apovian et al., 2008). White adipose tissue secretes hormones, cytokines or adipokines, and growth factors (e.g., leptin, transforming growth factor-beta, IL-1β, IL-6, adiponectin, resistin, and the pleiotropic cytokine TNF-α) (Ahima, Flier, 2000; Labrie et al., 2000). These inflammatory mediators induce macrophage infiltration, causing local inflammation and enhancing the production of angiogenic factors, an example of which is vascular endothelial growth factor (Vona-Davis, Rose, 2009). Obesity is a risk factor for tumor development and the cause of about 20% of cancer cases (Wolin, Colditz, 2008; De Pergola, Silvestris, 2013). A previous study suggested that UV radiation may promote inflammatory responses in obese subjects, increasing the levels of hormones and cytokines, mainly leptin, and thereby stimulating cutaneous carcinogenesis (Sharma, Katiyar, 2010). A previous meta-analysis showed that overweight or obese males are at a higher risk for melanoma and that adipose tissue inflammation might be responsible for the proliferation of melanoma cells in these subjects (Sergentanis et al., 2013). It has been shown that tumor cells become more aggressive and proliferate faster in the presence of white adipose tissue (Zhang et al., 2009). Adipose tissue-derived inflammatory cytokines involved in obesity (such as TNF-α) have a significant role in tumorigenesis, angiogenesis, and invasion (Mantovani et al., 2008; Moore et al., 1999; Balkwill, 2006; Mueller, 2006).

Other obesity-related factors may also influence the tumor microenvironment. Interleukins and protein-induced cytokines, for instance, stimulate the proliferation of tumor cells (Rundhaug, Fischer, 2010). Serum amyloid A (SAA), an acute-phase apolipoprotein associated with high-density lipoprotein (HDL), is produced mainly by the liver during regulation of chronic and acute inflammation (Yamada, 2006; Yamada, 1999). However, SAA is also released by hypertrophic adipocytes associated with enhanced lipolysis and insulin resistance (Filippin-Monteiro et al., 2011). Research has demonstrated that SAA induces the secretion of TNF-α and other cytokines by mononuclear cells (Cai et al., 2007; Furlaneto, Campa, 2000) and is a potential serum biomarker in human cancer patients (Chan et al., 2007).

Although many studies and epidemiological data have confirmed the association between cancer and obesity (Chan et al., 2007; Bhaskaran et al., 2014), the relationship between high body mass index (BMI) and malignant melanoma has been little investigated, with reports showing modest or inconsistent associations (Calle et al., 2003; Renehan et al., 2008; Roberts, Dive, Renehan, 2010). In the current study, we demonstrate that serum from subjects with a BMI higher than 40 kg/m² promotes an increase in the migration capacity of SK-Mel-28 and SK-Mel-147 bearing the oncogenes BRAF<sup>V600E</sup> (Davies et al., 2002; Daveri et al., 2015; Gorden et al., 2003) and NRAS<sup>Q61R</sup> (Nissan et al., 2014), respectively.

**MATERIAL AND METHODS**

**Reagents**

Recombinant human TNF-α was purchased from BD Biosciences (San Jose, USA). Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Gibco (Grand Island, USA). Penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Cultilab (Campinas, Brazil). All other reagents were from Merck (Darmstadt, Germany) unless stated otherwise.
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Subjects and sample collection

This study used serum from morbidly obese patients and lean individuals. All 20- to 65-year-old patients scheduled to undergo Roux-en-Y gastric bypass surgery at the Professor Polydoro Ernani de São Thiago University Hospital (Florianópolis, Brazil) were eligible for inclusion if their BMI was greater than 40 kg/m² before surgery. Exclusion criteria were cancers, acquired immunodeficiencies (such as HIV infection), and acute disorders. The lean control group was composed of healthy volunteers with a BMI between 18.5 and 24.9 kg/m². Exclusion criteria were chronic or acute diseases and continuous medication. Anthropometric parameters were determined for all participants. This study was approved by the ethics committee of the Federal University of Santa Catarina, Florianópolis, Brazil (protocol no. 24279013.7.0000.0121). Written informed consent was obtained from all subjects before participation.

Blood was collected in tubes without anticoagulant and centrifuged at 1500 × g for 10 min. After centrifugation, the serum was withdrawn and inactivated for 30 min at 56 °C. Samples from obese (n = 10) and lean (n = 6) patients were pooled separately and diluted to a final concentration of 10% (v/v) in DMEM containing 100 U/mL penicillin and 100 µg/mL streptomycin.

Serum assays

Total cholesterol, triglycerides, HDL cholesterol, and glucose were measured by enzymatic analysis (Dimension RXL Max, Siemens, Berlin, Germany). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation. Serum concentrations of TNF-α and MCP-1 were determined using a commercial quantitative sandwich immunoassay kit (DuoSet ELISA, R&D Systems, Minneapolis, USA) with a sensitivity of 2.0 pg/mL for TNF-α and 1.0 pg/mL for MCP-1. Serum concentrations of SAA were quantified using a commercially available kit (hSAA ELISA kit, Sigma–Aldrich, St. Louis, USA) with a sensitivity of 4.0 ng/mL.

Cell culture

SK-Mel-28, a BRAF-mutated human melanoma cell line, was obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil) and SK-Mel-147, an NRAS-mutated human melanoma cell line, was kindly provided by Professor Ana Campa (São Paulo University, São Paulo, Brazil). Cells were maintained in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10 mM HEPES at 37 °C and 5% CO₂.

Migration (scratch) assay

Cells were seeded at a density of 3 × 10⁵ cells/well in 12-well plates in triplicate. On the following day, a path was scratched in each well with a 200 µL pipette tip, old culture medium was replaced by 10% pooled serum or 50 pg/mL TNF-α in FBS-free DMEM, and plates were incubated for 24 h. Microphotographs were taken at 0 and 24 h after treatment. Cell migration was analyzed using ImageJ® software (Rueden et al., 2017) according to Grada et al. (2017), with some modifications. The difference in cell layer width was calculated as the mean distance between the borders of the scratch path at 0 and 24 h after incubation. Results were compared with those of untreated cells (control, 100% gap closure). Calculation parameters are provided as Supplementary material (Figure 1S).

Clonogenic assay

Colony formation was assessed by plating 300 cells/well in triplicate in a six-well plate one day before cell treatment with TNF-α (50 pg/mL) in DMEM medium. After 7 days of incubation, colonies were fixed with 10% formalin, stained with Giemsa, and counted.

Cell cycle analysis

Cells (2 × 10⁶ cells/well) in DMEM supplemented with 10% FBS were plated in triplicate in a 24-well plate and incubated for 24 h. After this period, the medium was replaced by DMEM without FBS for cell cycle
synchronization for 24 h. Then, cells were treated with FBS-free DMEM (control) or TNF-α (50 pg/mL) in FBS-free DMEM and incubated for a further 24 h. Cells were washed in PBS, fixed at 70% ethanol for 30 min at −20 °C, and washed and re-suspended in PBS containing 10 µg/mL RNase. Propidium iodide (10 µg/mL) was added for flow cytometric analysis (FACScanto™ II system, BD Biosciences, San Jose, USA), performed at the Multiuser Laboratory for Biological Studies of the Federal University of Santa Catarina (LAMEB/UFSC). A total of 10 000 events were acquired for each measurement, and cell cycle data were analyzed using Flowing software version 2 (University of Turku, Turku, Finland).

**BRAF and NRAS expression in SK-Mel-28 and -147 cells**

Cells were treated with 10% obese or lean patient serum in FBS-free DMEM. After 48 h of incubation, total RNA was extracted with TRIzol® (Gibco, Grand Island, USA). cDNA was then synthesized using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, New Jersey, USA). For quantitative real-time PCR, the following primers were used: \textit{NRAS}, 5′-ACAAACTGGTGGTGTTGGGA-3′ and 5′-ATTGTCACTGCGCTTTTC-3′; \textit{BRAF}, 5′-CTCCTTGAGATCGGCTGTTTGTT-3′ and 5′-GCCTGGATGGGTGTTTG-3′; and \textit{GAPDH} (constitutive housekeeping gene), 5′-CAATTGACCCCTTCATTGACC-3′ and 5′-GACAAGCTTCCCGTTCG-3′. Each amplification reaction was performed in duplicate together with a negative control (absence of cDNA) using SYBR Green Master Mix (Applied Biosystems, New Jersey, USA). Reaction conditions were as follows: 95 °C for 10 min, 45 cycles of 95 °C for 10 s (melting), and 57 °C for 1 min (annealing and elongation). Melting curve analyses from 76 to 84 °C were performed at the end of each run as quality control. The cycle threshold (Ct) within the log phase was set at 0.1. Results are expressed as arbitrary units relative to the housekeeping gene.

**Statistical analysis**

Demographic and serum data from obese and lean patients are presented as median values, and groups were compared by the Mann–Whitney U-test. Cell assay data are presented as the mean and standard deviation of three to five independent experiments. Comparisons were performed by Student’s t-test and correlations were investigated by Spearman’s rank correlation coefficient (r) using GraphPad Prism version 4 (Graph Pad Software, Inc., San Diego, USA). The level of significance was set at p < 0.05.

**RESULTS**

**Demographic characteristics and serum parameters**

Ten obese and six lean subjects were recruited. Most participants were female (80%) and all were White. Differences in BMI, gender, age, and biochemical parameters related to obesity are shown in Table I. The median BMI of obese and lean subjects was 47.3 and 21.7 kg/m², respectively (p = 0.0002). Serum levels of obesity-related inflammatory proteins (TNF-α, SAA, and MCP-1) were determined. No differences in MCP-1 levels were found between groups (p = 0.6354). The levels of TNF-α (p = 0.0226) and SAA (p = 0.0002), however, differed between obese and lean subjects. Obese individuals had higher serum levels of triglycerides (p = 0.0016), LDL cholesterol (p = 0.0120), and glucose (p = 0.0002) than lean subjects. Moderate correlations were found between BMI and inflammatory cytokines SAA (p = 0.0029, r_s = 0.6941) and TNF-α (p = 0.0303, r_s = 0.5416) and between BMI and triglycerides (p = 0.0058, r_s = 0.6750), LDL cholesterol (p = 0.0445, r_s = 0.5250), and glucose (p = 0.0051, r_s = 0.6637) (Supplementary material, Table IS and IIS, Figure 2S).
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**TABLE I - Demographic characteristics and serum parameters of obese and lean subjects included in the study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese patients</th>
<th>Lean subjects</th>
<th>p-value</th>
<th>p-value for BMI correlation</th>
<th>rs value for BMI correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>10</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.5 (31–61)</td>
<td>29.5 (23–40)</td>
<td>0.0506</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender (N)</td>
<td>F (8) M (2)</td>
<td>F (4) M (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>131.3 (105–175)</td>
<td>63.1 (58–72)</td>
<td>0.0010*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>47.3 (40–5.9)</td>
<td>21.7 (19.3–24.3)</td>
<td>0.0002*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCP-1 (pg/mL)</td>
<td>21.9 (9.5–59.8)</td>
<td>18.38 (8.74–41.61)</td>
<td>0.6354</td>
<td>0.4246</td>
<td>0.2147</td>
</tr>
<tr>
<td>SAA (ng/mL)</td>
<td>368.15 (15.6–1882.3)</td>
<td>10.2 (6.6–13.22)</td>
<td>0.0002*</td>
<td>0.0029*</td>
<td>0.6941</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>11.15 (3–39.8)</td>
<td>3.0 (2.0–10)</td>
<td>0.0226*</td>
<td>0.0303*</td>
<td>0.5416</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.13 (0.98–4.77)</td>
<td>0.96 (0.57–2.01)</td>
<td>0.0016*</td>
<td>0.0058*</td>
<td>0.6750</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.69 (1.45–4.79)</td>
<td>0.96 (0.57–1.08)</td>
<td>0.0120*</td>
<td>0.0445*</td>
<td>0.5250</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.9324 (0.82–1.08)</td>
<td>0.84 (0.45–1.35)</td>
<td>0.6889</td>
<td>0.6384</td>
<td>0.1323</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.35 (4.7–7.0)</td>
<td>4.1 (3.8–4.4)</td>
<td>0.0002*</td>
<td>0.0051*</td>
<td>0.6637</td>
</tr>
</tbody>
</table>

BMI, body mass index; F: Female; M: Male; MCP-1, monocyte chemoattractant protein-1; SAA, serum amyloid A; TNF-α: *tumor necrosis factor-alpha; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Values are the median, followed by the range in parentheses. Differences were analyzed by the Mann–Whitney U-test. Significant differences are denoted by asterisk (* p < 0.05). Serum reference values are as follows: TG ≥ 8.3 mmol/L, LDL-C ≤ 7.2 mmol/L, HDL-C ≥ 3.3 mmol/L, glucose = 3.9–5.8 mmol/L

**Effects of obese patient serum on melanoma cell migration**

The scratch assay was performed using *BRAF<sup>V600E</sup>* (SK-Mel-28) and *NRAS<sup>Q61R</sup>* (SK-Mel-147) melanoma cells in DMEM medium supplemented with pooled serum from obese or lean subjects (Supplementary Material, Figures 3S and 4S). As shown in Figure 1, SK-Mel-28 and -147 showed different migration patterns: SK-Mel-28 exhibited a more aggressive behavior than SK-Mel-147. When cells were exposed to pooled serum from obese or lean subjects, SK-Mel-147 (*p* = 0.0408) and SK-Mel-28 (*p* = 0.0111) showed increased cell migration after 24 h of treatment.
FIGURE 1 - A: Migration of BRAF- (SK-Mel-28) and NRAS-mutated (SK-Mel-147) melanoma cells treated with pooled serum from morbidly obese or lean individuals for 24 h. Results are expressed as wound width in mm. Data are the mean ± standard error of five independent experiments. *p=0.0408 for SK-mel-147 cells and *p=0.0111 for SK-mel-28 (unpaired one-tailed t-test). B and C: Representative micrographs of the migration assay showing (B) SK-Mel-28 and (C) SK-Mel-147 cell monolayers (100× magnification) before and after treatment with pooled serum from morbidly obese or lean individuals.

Effect of obese patient serum on BRAF and NRAS expression

SK-Mel-28 and SK-Mel-147 melanoma cells were exposed to pooled serum from obese or lean subjects, and the expression of BRAF and NRAS was assessed after 24 h of treatment. Exposure to obese or lean subject serum (p = 0.2802) did not influence BRAF expression in SK-Mel-28 cells, as shown in Figure 2A. However, NRAS expression was 2-fold higher in SK-Mel-28 cells exposed to obese patient serum than cells exposed to lean subject serum (p = 0.0125, Figure 2B). Figure 2C and D shows the results for SK-Mel-147. Exposure to obese patient serum upregulated wild-type BRAF (p = 0.0024) and NRAS (p = 0.0142) expression compared with exposure to lean subject serum.
Mitogenic effects enhanced by TNF-α in melanoma cells

Because TNF-α has been associated with obesity-related low-grade inflammation and metastatic melanoma, we assessed the impact of TNF-α on the clonogenic growth, migration, and cell cycle of BRAF- and NRAS-mutated melanoma cell lines (Figure 3). Exposure to TNF-α did not affect the migration capacity of SK-Mel-28 (p = 0.3419) or SK-Mel-147 (p = 0.0665) (Figure 3IB and 3IIB, respectively) or the proportion of cells in each cell cycle phase (Figure 3IC and 3IIC). However, the cytokine increased the clonogenicity (Figure 3IA) of BRAF-mutated cells (p = 0.0005). NRAS-mutated cells were not affected by TNF-α.
DISCUSSION

The presence of pro-inflammatory mediators derived or not from adipose tissue appears to contribute to the in vitro progression and aggressiveness of melanoma cells. Our results demonstrated the effects of obese patient serum on the migration of mutated melanoma cell lines. The migration capacity and expression of two major oncogenes related to tumor aggressiveness and poor prognosis (Heppt et al., 2017) were enhanced in BRAF- and NRAS-mutated melanoma cells. We also tested the effect of recombinant TNF-α, as the cytokine is associated with tumor progression and aggressiveness for its pro-inflammatory properties in the tumor microenvironment (Rossi et al., 2018). However, the results showed that TNF-α, the major obesity-related cytokine (Hotamisligil, Shargill, Spiegelman, 1993), had only a mild influence on the aggressiveness of tumor cells.

The experiments were performed aiming to establish a link between circulating obesity-related cytokines (which are often pro-inflammatory) and melanoma progression (Ahima, Flier, 2000). Ten obese subjects were recruited, and we hypothesized that their serum contained pro-inflammatory factors that might cause alterations in melanoma cells.

SK-Mel-28 and SK-Mel-147 are extremely aggressive melanoma cell lines with a high capacity to metastasize and a poor response to anti-tumor treatments. In this study, SK-Mel-147 cells bearing NRASQ61R and SK-Mel-28 bearing BRAFV600E were exposed to serum from obese or lean individuals. Obese patient serum increased the migration capacity of both cell lines. This effect might be related to the presence of pro-inflammatory factors in the serum. Previous studies reported similar in vitro
results. Price and colleagues (2012) showed that serum from obese mice containing high concentrations of IL-6, vascular endothelial growth factor, and leptin was able to increase prostate cancer cell proliferation, invasion, migration, and matrix metalloproteinase activity. Similar results were also reported by Lamarre et al. (2007), who found that serum from obese rats significantly increased the mitogenic response of prostate cancer cells in vitro.

Obese patient serum is characterized by high glucose and insulin levels, factors associated with obesity and diabetes that might have increased the migration of SK-Mel-28 and SK-Mel-147. Previous studies revealed that high levels of blood glucose and insulin promoted growth and proliferation in tumor cells (Han et al., 2011; Okumura et al., 2002) via stimulation of epidermal growth factor (EGF) receptors by increasing EGF mRNA expression. Glucose, insulin, and insulin-like growth factor 1 (IGF-1) were shown to enhance colon cancer cell viability and resistance to chemotherapy (Volkova et al., 2014). IGF-1 increased melanoma survival by inhibiting apoptosis of BRAF-mutated A375 melanoma cells (Hilmi et al., 2008).

Acute reactive protein serum levels peak 36 to 48 h after an acute event and return to baseline after 4 days, a finding that is suggestive of the role of these proteins in the restoration of homeostasis post-inflammation (Wu et al., 2015). In contrast, in low-grade inflammation, which predominates in obese individuals, the levels of pro-inflammatory SAA, IL-6, and TNF-α remain consistently high, potentially contributing to tumor development and aggressiveness (Kern et al., 2019).

The two most critical pro-inflammatory cytokines in obese patient sera are SAA (Gómez-Ambrosi et al., 2006; Lappalainen et al., 2008) and MCP-1 (Kim et al., 2006). In the present study, obese patient serum contained high levels of SAA, MCP-1, and TNF-α, corroborating the low-grade inflammation state observed in morbidly obese patients (BMI ≥ 40 kg/m²). Some of these pro-inflammatory factors were strongly correlated with BMI (e.g., SAA, r² = 0.6941 and p = 0.0029), evidence of their suitability as markers of adiposity.

In the present study, BRAF and NRAS expressions were upregulated by exposure to obese patient serum, probably associated with the action of pro-inflammatory cytokines. Future studies should investigate the expression of p53 and characterize the relationship between p53 mutation in SK-Mel-28 and enhanced migration capacity (Daveri et al., 2015). The oncopgenic protein RAS is known to cause changes in the polymerization, organization, and contraction of actin, polymerization and stability of microtubules, and transcriptional regulation of gene expression; together, these changes contribute to enhancing the motility of tumor cells (Castellano et al., 2016).

Because of the limited number of serum samples, we focused our investigations on the effects of obesity-related factors on cell morphology, proliferation, migration capacity, and clonogenicity. Literature data show that BRA-F and N-RAS proteins, which were highly expressed in this study, enhance MAPK signaling (Wellbrock, Karasarides, Marais, 2004). B-RAF^{V600E} is the most effective mutant in activating ERK in both melanocytes and melanoma cells, as stated by Whitwam et al. (2007) and revised by Ryan et al. (2016).

On the basis of the obesity-induced effects observed on melanoma cells and in an attempt to refine results related to the mitogenic profile, we carried out several experiments to assess clonogenicity, migration ability, and cell cycle alterations in melanoma cells (SK-Mel-28 and SK-Mel-147) treated with recombinant TNF-α. Cells were initially treated with 25 pg/mL TNF-α, twice the concentration found in patients’ sera, but cells were not affected (data not shown). However, when cells were treated with 50 pg/mL TNF-α, the clonogenicity of SK-Mel-28 was increased. In previous studies using in vitro models of tumor invasion and migration, TNF-α concentrations of 100 to 500 pg/mL produced significant effects (Katerinaki et al., 2003; Zhu et al., 2004). It was not our aim to assess the effects of different concentrations of TNF-α on melanoma cells, but we observed that the concentrations of TNF-α in the serum of obese individuals seem to be insufficient to alter the migration profile of tumor cells.

Katerinaki and colleagues (2003) showed that TNF-α increased the migration ability of HLB (wild type B-RAF) by 21%. Zhu et al. (2004) found that HBL melanoma cells showed increased migration capacity after TNF-α exposure. Likewise, Cantón et al. (2003) reported a 180% increase in
uveal melanoma cell invasion in the presence of TNF-α. In the present study, significant effects were only observed on \textit{BRAF}-mutated cell clonogenicity, suggesting that other cytokines are involved in the pro-tumor properties of serum from obese individuals. Obesity-related factors secreted by hypertrophic adipocytes, such as SAA, could be involved in the enhanced migratory pattern and \textit{NRAS} and \textit{BRAF} expression in melanoma cells (Castellano \textit{et al.}, 2016). SAA was associated with the migration and invasion of human and mouse breast cancer cells because of its role in stimulating the transcription of RANTES, an inflammatory chemokine, and MMP2, a critical matrix metalloproteinase involved in metastasis (Hansen \textit{et al.}, 2015).

In conclusion, this study reported an increase in the migratory ability of melanoma cells exposed to serum rich in obesity-related factors. Furthermore, we demonstrated the action of inflammatory mediators in the expression of oncogenes \textit{NRAS} and \textit{BRAF}, associated with tumor aggressiveness. The cytokines found in obese patient serum may be responsible for the increase in \textit{NRAS} expression in SK-Mel-28 cells. This result suggests that inflammatory proteins may serve as secondary targets in melanoma therapy. TNF-α made a mild contribution to melanoma progression and aggressiveness at concentrations found in the serum of obese individuals. Because of the controversial and pleiotropic roles of this cytokine, anti-tumor strategies aimed at blocking TNF-α should be examined with caution in future studies, particularly when using \textit{in vitro} models to screen for antitumor activity.

**CONFLICT OF INTERESTS**

All authors declare that there is no conflict of interest regarding the publication of this paper.

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