SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 15: 1607-1615, 2018

High-fat diet-induced adipokine and cytokine alterations promote the progression of prostate cancer *in vivo* and *in vitro*

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Received August 16, 2016; Accepted September 13, 2017

DOI: 10.3892/ol.2017.7454

Abstract. High-fat diet (HFD) -induced obesity is associated with more aggressive and lethal prostate cancer (PCa) in males, although the exact underlying mechanisms remain unclear. In the present study, transgenic adenocarcinoma of mouse prostate (TRAMP) models fed on an HFD (40% fat) or a control diet (CD; 16% fat) were generated, and cancer differentiation, local invasion and metastasis were compared at 20, 24 and 28 weeks. Mouse sera from each group were collected, and adipokines and cytokines were measured using multiplex immunoassays. HFD-sera and CD-sera were additionally processed into conditioned media (2.5% mixed sera), and *in vitro* studies were conducted to determine the proliferation, migration and invasion of cancer cells when conditioned media were used for culture. In TRAMP mice, HFD feeding increased body weight and adipose tissue deposition, and promoted the progression of PCa, specifically with regard to poorer differentiation, increased local invasion and metastasis rate. Sera from HFD-fed TRAMP mice contained increased levels of leptin, and a time-dependent increasing trend in the levels of CC chemokine ligand (CCL)3, CCL4, CCL5 and CXC chemokine ligand (CXCL)10 was observed. However, no alterations were detected in the levels of adiponectin, interleukin (IL)-4, IL-5, IL-6, IL-12p70, interferon-γ, tumor necrosis factor-α, CCL2, CCL7, CCL11, CXCL1 and CXCL2. *In vitro* studies determined that HFD-sera-conditioned medium promoted proliferation, migration and invasion of DU145 cells, as compared with CD-sera-conditioned medium and serum-free medium. In conclusion, the results of the present study suggested that the circulating adipokine and cytokine alterations in response to excess adipose tissue deposition induced by HFD feeding contributed to PCa progression.

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Key words: high-fat diet, prostate cancer, progression, adipokines, transgenic adenocarcinoma of mouse prostate

Introduction

Prostate cancer (PCa) was the second most frequently diagnosed malignant tumor and the fifth leading cause of cancer mortality in men worldwide in 2012 (1), and its development and progression have been associated with obesity through extensive previous studies [reviewed in (2)]. A wide range of epidemiological studies have demonstrated that obesity was positively associated with more aggressive PCa, with higher pathological grade (3), increased recurrence rate (4) and increased cancer-specific mortality (5), whereas the exact molecular mechanisms that contribute to PCa progression remain unclear.

Excessive adipose tissue deposition in obese patients, along with its paracrine and endocrine impact on tumor microenvironment, was suggested to exert reciprocal interactions with cancer cells and contribute to cancer progression (6,7). In particular, adipokines, a group of cytokines secreted by adipocytes, together with their associated pro-inflammatory cytokines, were demonstrated to exert multifaceted effects on cancer (8). The prostate is surrounded by marked amounts of adipose tissues, and, accordingly, the progression of PCa is more likely to be affected by the aberrant secretion and signaling of adipokines and cytokines in obese patients in comparison with non-obese patients (9,10). Leptin and adiponectin are two primary adipokines that have been studied previously: It was demonstrated that increased serum leptin levels were positively associated with tumor development and progression, whereas adiponectin inhibited the cancer growth (11). The downstream pathways and regulatory mechanisms were complicated, involving apoptosis, cell cycle, angiogenesis and migration (11).

Notably, a previous study identified that adipocytes cultivated with cancer cells exhibited an altered phenotype, with delipidation in cells, decreased adipocyte markers and overexpression of pro-inflammatory cytokines (12). This study considered that the cancer-associated adipocytes may be an important factor that contributed to cancer progression. However, the function of these cancer-associated adipocytes in cancer development and progression, involving the crosstalk of adipocytes and cancer cells, remains largely unknown.

To elucidate the effect of obesity on PCa growth and progression, a high-fat diet (HFD) was used to induce obesity in a transgenic adenocarcinoma of mouse prostate (TRAMP)

animal model, which closely resembled the natural progression of PCa in obese human patients (13). Through a series of *in vivo* and *in vitro* experiments, the hypothesis that HFD-induced obesity may promote the PCa progression via altered serum adipokines and cytokines was tested. The trends in serum adipokine and cytokine alterations, which were jointly affected by HFD-induced obesity and PCa progression were also investigated.

Materials and methods

Animals and diets. All of the animal studies were approved by the Institutional Animal Care and Use Committee from Department of Laboratory Animal Science, Fudan University (Shanghai, China). A total of 108 TRAMP mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA), bred and maintained under pathogen-free conditions at the Department of Laboratory Animal Science, Fudan University. Each mouse was kept and raised separately in a cage, with cork dust bedding and a 12-h light/12-h dark cycle, with an environmental temperature of 22-26˚C and humidity of 50-60%. All male TRAMP mice were selected by genotyping as previously described (14), and then randomly assigned to two groups, where the mice were fed on a control diet (CD) or an HFD *ad libitum* at 5 weeks of age. CD (16% calories from fat) and HFD (40% calories from fat) was supplied by Puluteng Biotechnology, Co., Ltd. (http://www.slaccas.com; Shanghai, China; Table I).

To evaluate the effect of HFD on the development and progression of prostate cancer, TRAMP mice were sacrificed at 20, 24 and 28 weeks of the age according to previous studies (CD-fed TRAMP: 20-week, 24-week, 28-week, three groups, n=12 mice/group; HFD-fed TRAMP: 20-week, 24-week, 28-week, three groups, n=24 mice/group) (15,16).

Systemic evaluation, serum and tissue preparation. All TRAMP mice were not fed overnight, and received weight and blood glucose examinations prior to sacrifice. At the time of sacrifice, each TRAMP mouse was anaesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg), scanned by GE eXplore Locus micro-computed tomography (CT) scanner (GE Healthcare BioSciences, Pittsburg, PA, USA) for systemic evaluation, and euthanized by asphyxiation with CO_2. During euthanasia, the mice were kept in a 3 l-cage, then 100% CO_2 was introduced at a flow rate of 0.75 l per min. The final concentration of CO_2 reached was ~75%. The mortality of the mice was confirmed by checking breathing and the dilation of the pupil. Subsequently, blood was harvested from the portal vein using a 1-ml syringe, and the serum was collected and stored at -80˚C for future analysis. The tumor, genitourinary tract, epididymal fat, liver and lung were removed from the TRAMP mice, weighed and fixed for histopathological analysis. The epididymal fat pad was dissected between the distal major blood vessel and the epididymis from each side.

Histopathological analysis. Prostate and other prepared tissues were fixed in 10% buffered formalin, processed in an alcohol (80, 90 and 100%)-xylene series and embedded in paraffin (at 56˚C for 4 h). A series of 2-μm sections were prepared, and stained with hematoxylin (0.5%) for 10 min and eosin (0.5%) for 2 min (H&E) at 26˚C. A light microscope was used at a magnification of x40 and x200. For evaluation of tumor differentiation, local invasion and detection of distant metastasis, these sections were additionally analyzed and cross-checked by two pathologists from Huashan Hospital, Fudan University (Shanghai, China). The differentiation of prostate cancer was identified based on Gleason score and categorized into three groups, including well-differentiated (Gleason score, <7), moderately differentiated (Gleason score, 7) and poorly differentiated (Gleason score, >7) (17).

Table I. Nutrient and energy compositions of the control and high-fat diets.

Component	Control diet	High-fat diet
Nutrients (g)		
Fat	7	20
Carbohydrates[a]	64	45
Protein	20	22
Energy (%)		
Fat	16	40
Carbohydrates	64	40
Protein	20	20

[a]Excluding dietary fiber.

Serological analysis. Concentrations (in pg/ml) of different adipokines and associated cytokines in mouse sera were measured by commercial ProcartaPlex Multiplex Immunoassays (eBioscience; Thermo Fisher Scientific, Inc., Waltham, MA, USA), which use the Luminex technology with Multi-Analyte Profiling beads and enable the simultaneous detection and quantification of multiple cytokines in one sample. The equipment was used according to the manufacturer's protocol. A total of 30 μl serum from each mouse was examined following the manufacturer's protocol. The adipokines and cytokines investigated included leptin, adiponectin, interleukin (IL)-4, IL-5, IL-6, IL12-p70, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, CC chemokine ligand (CCL)2, CCL3, CCL4, CCL5, CCL7, CCL11, CXC chemokine ligand (CXCL)1, CXCL2 and CXCL10.

Cell culture and culture medium preparation. DU145 cells, an androgen-independent human prostate cancer cell line, were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The DU145 cells were cultured in Ham's F-12K (Kaighn's) medium (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin and streptomycin combination. Cells were incubated at 37˚C in a humidified atmosphere of 5% CO_2.

In the series of *in vitro* experiments, cells were treated with three different culture media: i) Serum-free medium (SFM); ii) HFD-sera-conditioned medium (HFD-CM) which contained SFM plus 2.5% mixed sera from all experimental 28-week HFD-fed TRAMP mice; and iii) CD-sera-conditioned

medium (CD-CM) which contained SFM plus 2.5% mixed sera from all experimental 28-week CD-fed TRAMP mice.

Cell Counting Kit-8 (CCK-8) proliferation assay. Cell proliferation was determined using a CCK-8 assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Exponential phase DU145 cells were seeded in 96-well plates (10,000 cells/well) in culture medium at 37˚C overnight, and then the medium was replaced by SFM for 18 h. Subsequently, cells were treated with SFM, CD-CM or HFD-CM, and incubated for 0, 24, 48, 72 and 96 h. Each group was tested in triplicate in three replicate wells. CCK-8 solution (10 μl) was added to each well at each time point as mentioned above. Following incubation at 37˚C for another 2.5 h, the optical density (OD) values were determined at a wavelength of 450 nm using a microplate reader BioTek, ELx800 (BioTek Instruments, Winooski, VT, USA) at each time point.

Wound-healing assay. Cell migration was studied by conducting a wound-healing assay. DU145 cells were seeded on 6-well plates (50,000 cells/well) in culture medium. After 24 h, culture medium was replaced by SFM for 18 h. A wound was generated by scratching down the cells in the center of the well with a sterile pipette tip, and the detached cells were washed away with PBS. Then, the cells were incubated in the following experimental conditions for 96 h: SFM, CD-CM and HFD-CM. Images were captured using Nikon TE2000 inverted microscope (magnification, x40) connected to a Nikon Coolpix 4500 camera (Nikon Corporation, Tokyo, Japan) at 0, 24, 48, 72 and 96 h. The distance between the scratched regions were additionally measured with Spot Imaging 5.2 software (Diagnostic Instruments, Sterling Heights, MI, USA).

Matrigel-Transwell invasion assay. Cell invasion was studied by conducting a Matrigel-Transwell assay with BD Biocoat Matrigel Chambers (Discovery Labware; BD Biosciences, Franklin Lakes, NJ, USA). A total of ~120,000 DU145 cells were suspended and spread to the upper chamber under serum-free conditions, and the lower chamber of the well was filled with SFM, CD-CM or HFD-CM as a chemoattractant. After 24 h, inserted cells were fixed in 95% ethanol (at 26˚C for 30 min) and stained with 1.0% crystal violet (at 26˚C for 10 min). The images of inserts were captured using a Nikon TE2000 inverted microscope connected to a Nikon Coolpix 4500 camera (Nikon Corporation). Subsequently, cells were counterstained with 33% acetic acid, and the OD of the counterstained solution was additionally examined using a microplate reader at a wavelength of 570 nm.

Statistical analysis. Results are presented as the mean ± standard error of the mean. Cell experiments were conducted in triplicate. The TRAMP mouse sample size was determined by a previous study (14) that characterized the mouse model, and all mice were randomized into different groups in a blinded manner. Analyses were conducted with an unpaired two-tailed Student's t-test, χ^2 test, Fisher's exact test or two-way analysis of variance followed by Sidak's/Tukey's multiple comparisons test, using GraphPad Prism (version 6.0c; GraphPad Software, La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

HFD feeding increases body weight and fat deposition in TRAMP mice. HFD feeding increased the body weight of TRAMP mice compared with CD feeding. The differences in mean body weight between HFD-fed and CD-fed TRAMP mice were all of statistical significance in the 20-week (23.6±3.4 vs. 20.8±1.8 g; $P<0.05$), 24-week (28.3±2.4 vs. 25.2±2.3 g; $P<0.001$) and 28-week groups (33.8±4.7 vs. 28.2±2.1 g; $P<0.001$) (Fig. 1A). This difference appeared to be time-dependent (Fig. 1B). Blood glucose levels of the mice following fasting overnight did not differ markedly between CD-fed and HFD-fed groups (Fig. 1C). As expected, HFD-fed TRAMP mice presented with increased genitourinary tract weight compared CD-fed mice in the 24- and 28-week groups (Fig. 1D). Additionally, epididymal fat weight was also increased in HFD-fed mice in all comparisons (Fig. 1E).

HFD feeding promotes tumor progression in TRAMP mice. Micro-CT scanning, optic observation and histopathological studies of dissected tissues were applied to evaluate the tumor development and progression. Prostate cancer was detected in all TRAMP mice from the CD-fed or HFD-fed groups. In the older TRAMP mice, PCa tended to be more advanced and aggressive, irrespective of the CD or HFD. In addition, HFD-fed mice possessed PCa with poorer differentiation as compared with CD-fed mice at the same age (Fig. 2A). An increased number of events of local invasion and metastasis were detected in HFD-fed mice compared with their CD-fed counterparts (Fig. 2B and C). The H&E staining sections also revealed that the PCa of HFD-fed mice was poorly differentiated compared with CD-fed mice at the same age (Fig. 2D). Extracapsular invasion, seminal vesical invasion and metastasis to retroperitoneal lymph nodes or lung were common in the 28-week HFD-fed TRAMP mice in the present study (Fig. 2E and F).

HFD induces adipokine and cytokine alterations in mouse sera. As a great variety of adipokines and cytokines that are associated with tumor development and progression may be altered by HFD feeding, multiple immunoassays were applied for the detection of cytokines in TRAMP mouse sera. The comparisons of cytokine levels depended on three different ages of mice: The 20-week group, where the majority of the CD-fed and HFD-fed mice experienced organ-confined PCa, the 28-week group, where half of the mice experienced local invasion and metastasis of PCa, and the 24-week group, with intermediate cancer status. A time-dependent trend in changes in adipokines and cytokines was detected. HFD feeding increased leptin levels >2-fold in all age groups, and the increase appeared to be even more marked in older mice (Fig. 3A). CCL3 levels were increased in the CD-fed mice in the 24-week group compared with the HFD-fed mice at the same time, whereas in the 28-week group, CCL3 levels were increased in the HFD-fed mice compared with the CD-fed mice (Fig. 3J). Similarly, levels of CCL4 and CCL5 were increased in the CD-fed mice in the 24-week group; however, this difference was diminished and the levels were slightly increased in the HFD-fed mice in the 28-week group (Fig. 3K and L). CXCL10 levels were significantly increased

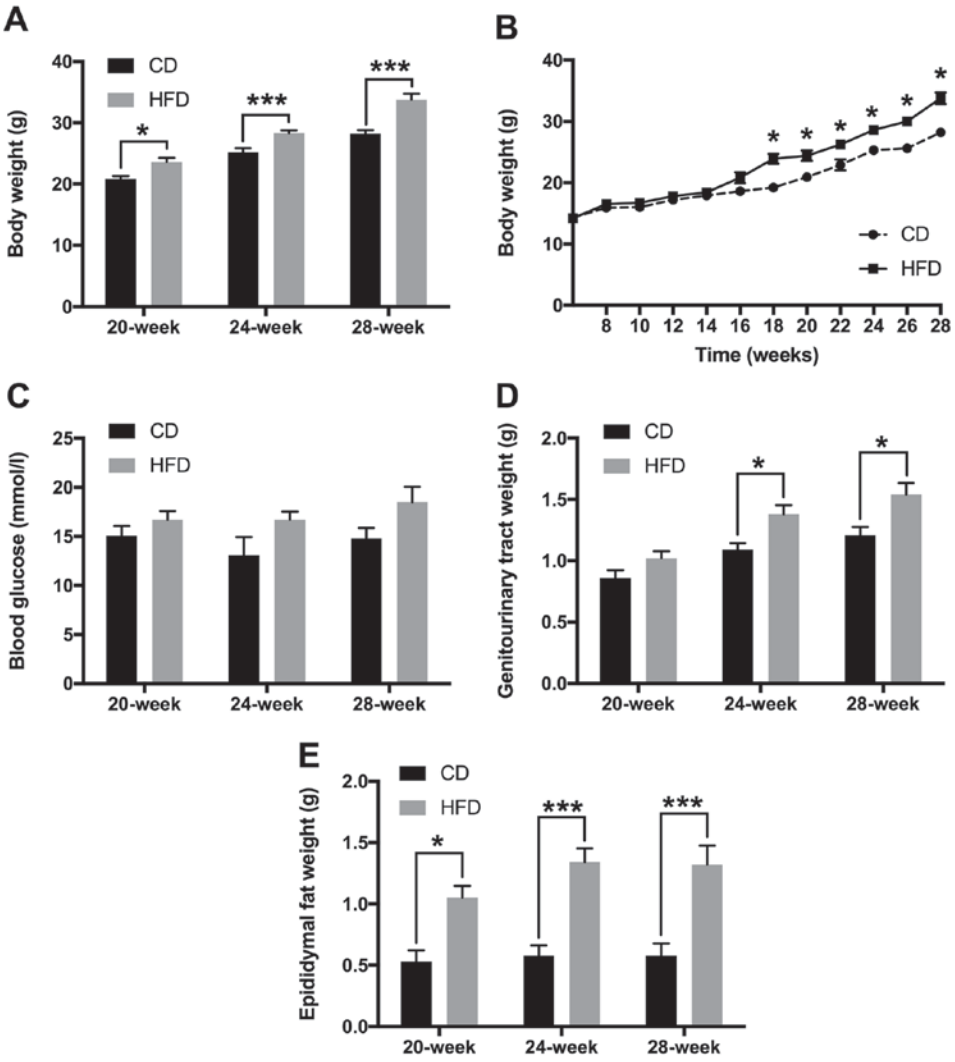

Figure 1. TRAMP mice fed on an HFD demonstrated increased body, genitourinary tract and epididymal fat weight. (A) Body weight of TRAMP mice fed on a control diet (CD) or HFD across different groups. (B) Change in body weight of 28-week CD-fed and HFD-fed TRAMP mice. (C) The blood glucose level of each mouse was examined at the time of sacrifice following fasting overnight. (D) The genitourinary tract of each mouse was dissected after sacrifice and weighed. (E) Epididymal fat was dissected following sacrifice and weighed for each mouse. Results are expressed as the mean ± standard error of the mean. *P<0.05 and ***P<0.001. HFD, high-fat diet; CD, control diet; TRAMP, transgenic adenocarcinoma of mouse prostate.

in HFD-fed mice compared with CD-fed counterparts in the 28-week group (Fig. 3Q). The present study failed to detect any difference between CD-fed and HFD-fed mice in levels of adiponectin, IL-4, IL-5, IL-6, IL-12p70, IFN-γ, TNF-α, CCL2, CCL7, CCL11, CXCL1 and CXCL2 (Fig. 3).

HFD-CM promotes proliferation, migration and invasion of DU145 cells. A series of *in vitro* studies were conducted to determine the proliferative, migratory and invasive abilities of DU145 cells cultured in SFM, CD-CM and HFD-CM. CD-CM and HFD-CM stimulated the proliferation of DU145 cells compared with SFM after 72 h of culture, and the increase in the HFD-CM group was more marked compared with that of the CD-CM counterparts (Fig. 4A). As for cell migration, cells cultured in HFD-CM exhibited a more marked wound closure speed compared with cells cultured in CD-CM and SFM following 48 h of culture (Fig. 4B and C). In addition, HFD-CM also promoted significantly different cell invasion abilities in the Transwell-Matrigel assay compared with CD-CM and SFM (Fig. 4D and E).

Discussion

A positive association between obesity and PCa incidence and aggressiveness has been demonstrated previously (18). However, owing to the complexity and variability of molecular biological networks, elucidating the exact role of obesity in PCa progression is challenging.

High dietary fat, comprising a large part of western diets, induces obesity by increasing adipose tissue deposition via adipocyte hypertrophy and hyperplasia (6). In turn, the excess fat deposition tends to additionally alter the function of the adipocytes, and accordingly affects cancer progression (6). Several studies were conducted in murine xenograft models, implicating that diet-induced obesity was associated with PCa progression (19,20). Additionally, genetically engineered animal models were of even greater utility, owing to their normal immune system function, and orthotopic and spontaneous development of tumors. The first study indicating a promoting role of HFD feeding in PCa development in TRAMP mice was conducted in 2010 (21). Subsequently,

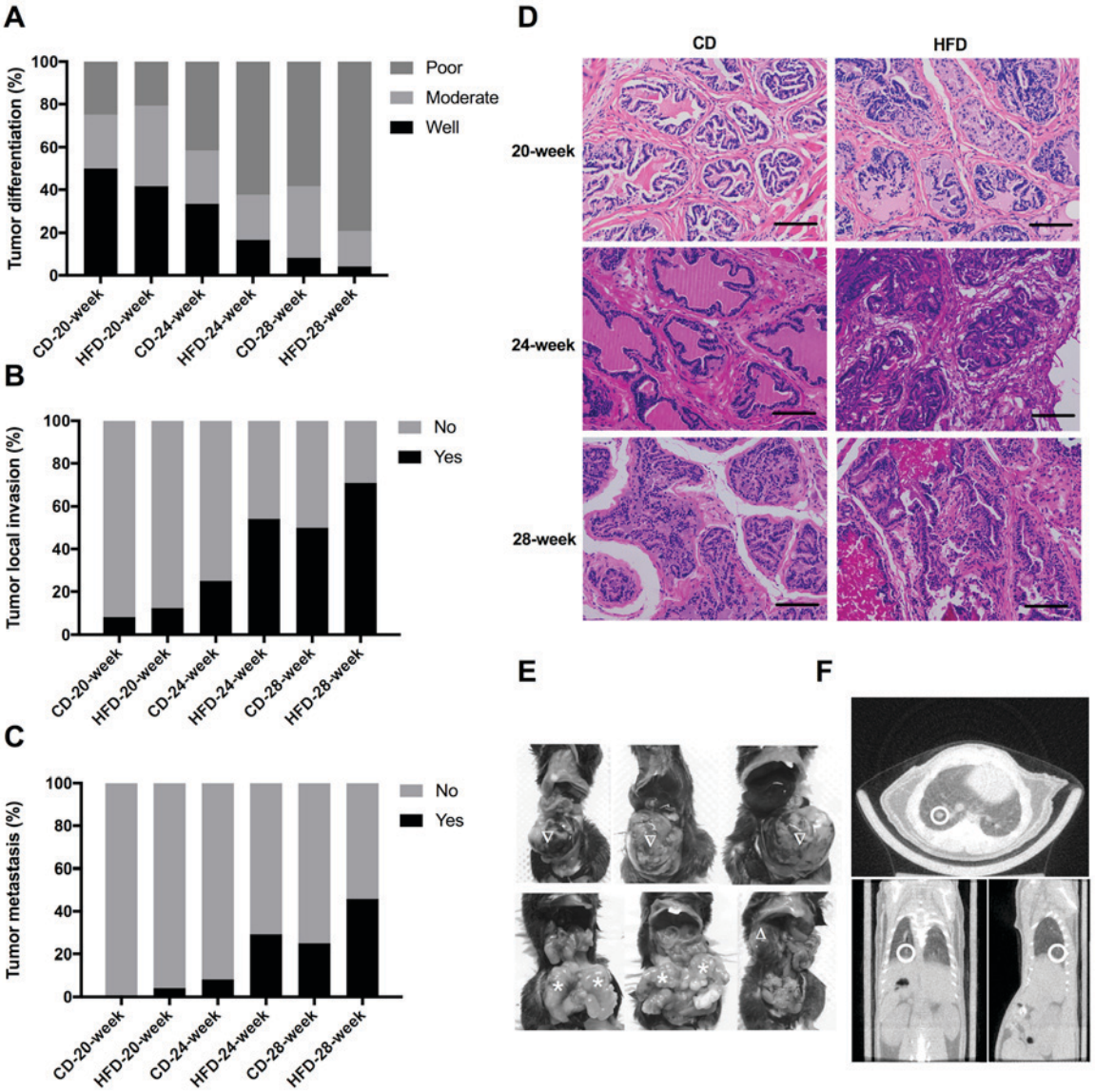

Figure 2. TRAMP mice fed on an HFD exhibited more advanced pathological carcinogenic lesions, increased rate of local invasion and metastasis of prostate cancer. (A) TRAMP mice fed on an HFD or a CD until 20, 24 or 28 weeks were sacrificed, and each mouse prostate was dissected, fixed, sectioned and stained with hematoxylin and eosin. The tumor differentiation was additionally classified into poor, moderate and well according to histopathological studies of stained sections. (B) The local invasion of prostate cancer was determined by histopathological studies of dissected prostate and surrounding tissues. (C) The metastasis of prostate cancer was determined by micro-CT scanning outcomes and histopathological studies of dissected liver, lung and other organs. (D) Representative pathological images of mice prostate in different groups (magnification, x200; scale bar, 100 μm). (E) Representative images of prostate cancer mass (∇), bilateral seminal vesical invasion (*), and retroperitoneal lymph node enlargement (Δ) in the 28-week HFD-fed TRAMP mice group. (F) Representative micro-CT images of lung metastasis from a 28-week HFD-fed TRAMP mouse (demarcated by a white circle). Micro-CT, micro-computed tomography; HFD, high-fat diet; CD, control diet; TRAMP, transgenic adenocarcinoma of mouse prostate.

Cho *et al* (20) revealed that an HFD containing lard increased PCa development and progression, and decreased survival rates in TRAMP mice. Consistent with previous studies, the results of the present study indicated that HFD feeding increased body weight and visceral fat deposition (e.g., increased epididymal fat) in TRAMP mice. TRAMP mice fed on an HFD presented with poorer differentiation, increased rates of local invasion and distant metastasis of PCa at the age of 20, 24 and 28 weeks. Taken together, these results confirmed that HFD-induced obesity stimulates the progression of PCa. Conversely, Bonorden *et al* (22) failed to detect any association between HFD and tumor development in TRAMP mice. In that study, HFD-fed mice were divided into obesity-resistant, overweight and obesity-prone groups according to their body weight. These subgroups afforded novel perspectives on the effects of the response of the body to HFD feeding, and additional bias, which requires future validation and exploration.

In addition to triacylglycerol storage, it was established that white adipose tissue also functions in appetite control, immune and metabolism regulation by secreting and affecting a large number of adipokines and cytokines (6). Multiple previous studies indicated that these adipokines and cytokines were critical in regulating tumor growth and spread, the results of which are as follows. Xu *et al* (14) identified that pro-inflammatory cytokines, including IL-1α, IL-1β, IL-6 and TNF-α, were increased in HFD-fed mice in comparison with CD-fed mice, indicating that these cytokines might contribute to PCa progression. Price *et al* (9) demonstrated that PCa

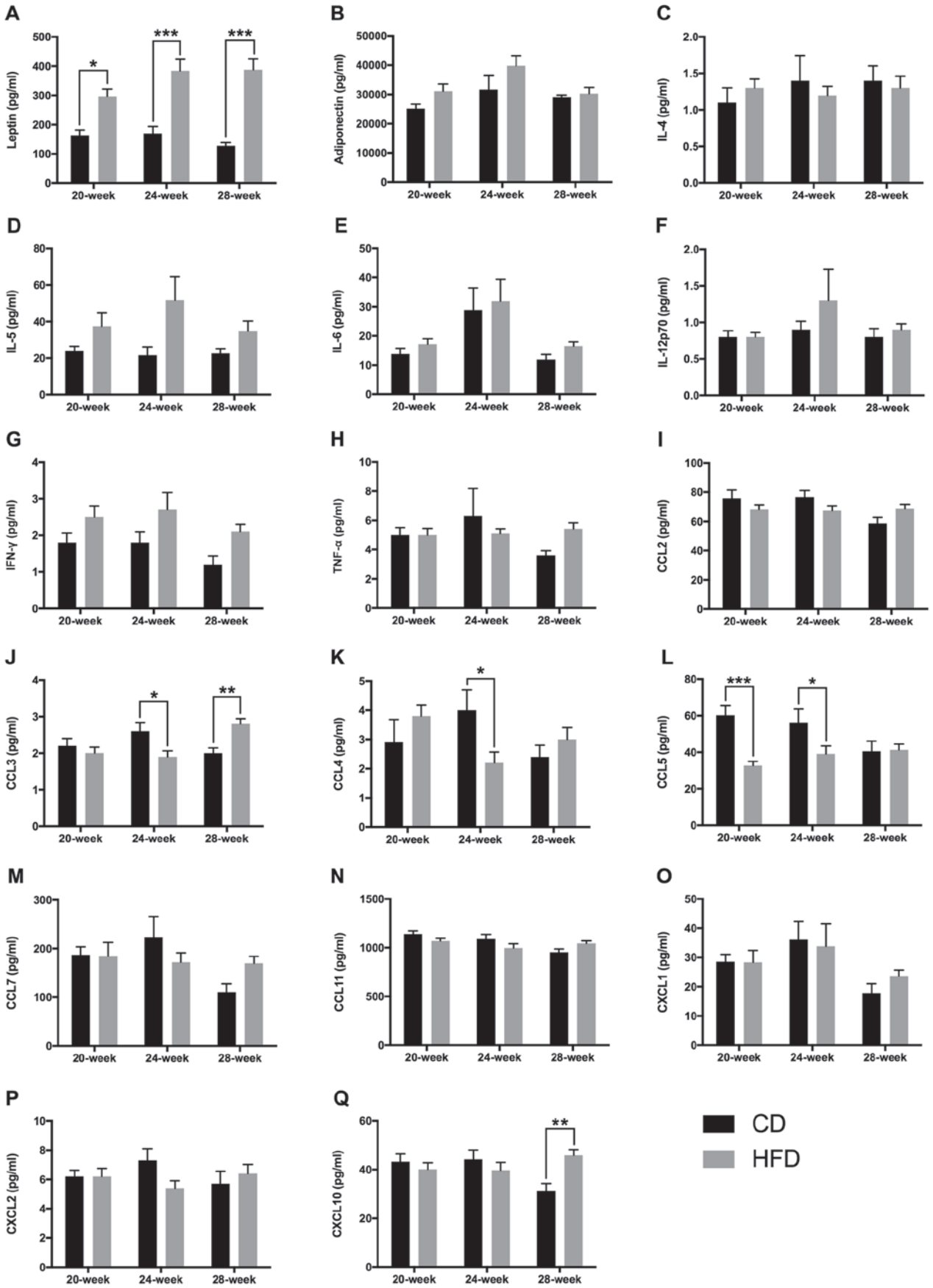

Figure 3. High-fat diet-induced adipokine and cytokine alterations in TRAMP mouse sera. TRAMP mice fed on an HFD or CD were sacrificed at 20, 24 and 28 weeks. Mouse sera were collected, and serum adipokines and cytokines were examined using ProcartaPlex Multiplex Immunoassays. The adipokines and cytokines under examination included (A) leptin, (B) adiponectin, (C) IL-4, (D) IL-5, (E) IL-6, (F) IL-12p70, (G) IFN-γ, (H) TNF-α, (I) CCL2, (J) CCL3, (K) CCL4, (L) CCL5, (M), CCL7, (N) CCL11, (O) CXCL1, (P) CXCL2 and (Q) CXCL10. Results are the mean ± standard error of the mean. *P<0.05, **P<0.01 and ***P<0.001. IL, interleukin; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; HFD, high-fat diet; CD, control diet; TRAMP, transgenic adenocarcinoma of mouse prostate.

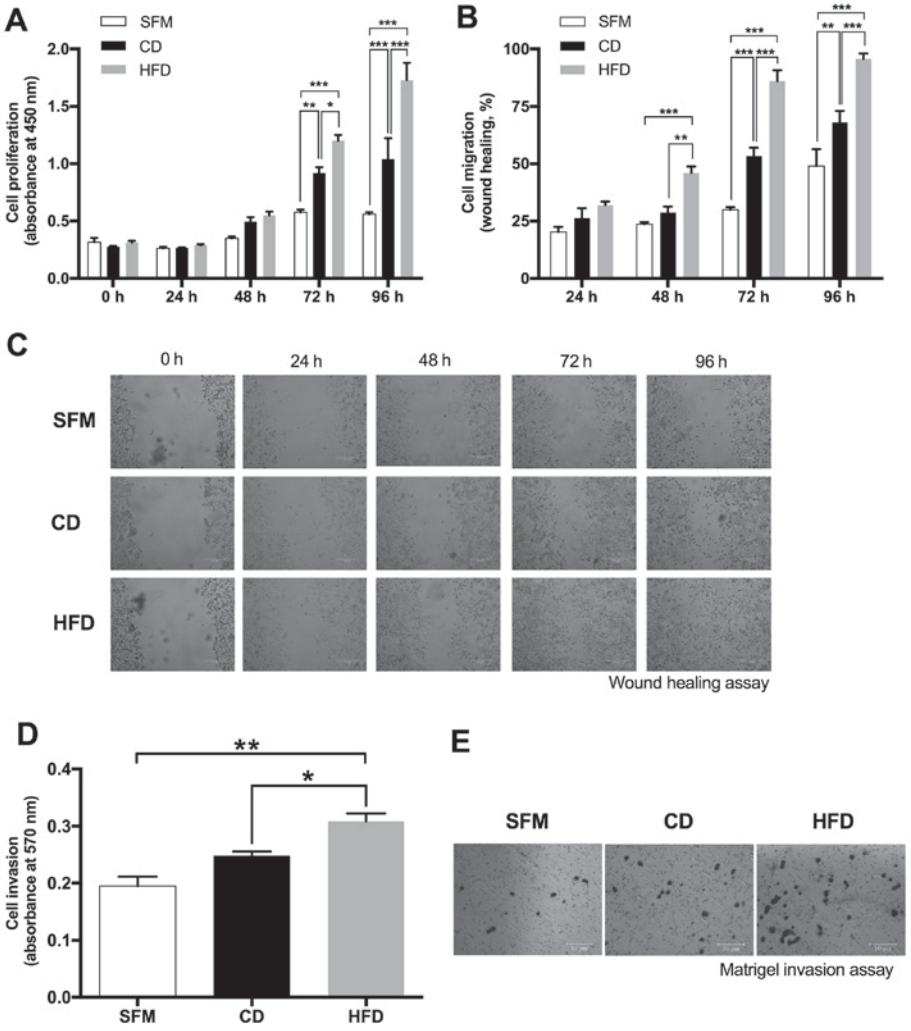

Figure 4. Sera from high-fat diet-fed TRAMP mice promoted proliferation, migration and invasion of prostate cancer cells. (A) DU145 cells were treated with SFM, CM containing 2.5% mixed sera from CD-fed TRAMP mice or CM containing 2.5% mixed sera from HFD-fed TRAMP mice. Cell viability was determined using a Cell Counting Kit-8 assay. (B) Effects of SFM, CD-CM and HFD-CM culture on the migration of DU145 cells as determined using a wound-healing assay. (C) Representative images of the wound-healing experiment demonstrating the migration of DU145 cells cultured with SFM, CD-CM and HFD-CM, respectively. (D) Invasion of DU145 cells was determined using a Matrigel-Transwell invasion assay. SFM, CD-sera-CM or HFD-sera-CM was added to the lower chamber of the Transwell as the chemoattractant. (E) Representative images of the Matrigel-Transwell experiment, demonstrating the invasive abilities of DU145 cells cultured with SFM, CD-CM and HFD-CM, respectively. Results are presented as the mean ± standard error of the mean (n=3). *P<0.05, **P<0.01 and ***P<0.001. SFM, serum-free medium; CD, control diet; HFD, high-fat diet; CM, conditioned media; TRAMP, transgenic adenocarcinoma of mouse prostate.

cells exposed to sera from obese C57BL/6 mice, containing increased levels of leptin and pro-inflammatory cytokines and decreased levels of insulin-like growth factor-binding protein 1 and tissue inhibitor of metalloproteinase 1, promoted cell proliferation, invasion, migration and epithelial-mesenchymal transition. Previously, cancer-associated adipocytes have gained attention for their dysmorphisms and altered functions when located adjacent to cancer cells; they appear to form reciprocal signaling pathways with cancer cells and promote a more aggressive cancer phenotype (23,24). Cho *et al* (20) conducted a series of *in vitro* studies and identified that adipose tissue-conditioned medium from HFD-fed mice promoted the proliferation, migration of PCa cells and angiogenesis. Similarly, the results of the present study revealed that the HFD-CM promoted proliferation, migration and invasion in DU145 cells compared with CD-CM or SFM. Owing to the limited quantity of mouse sera, the experiments were not extended to more cell lines or more serum concentrations.

Notably, the present study initiated comprehensive comparisons of different serum adipokine and cytokine levels among CD-fed and HFD-fed TRAMP mice at the age of 20, 24 and 28 weeks. As PCa developed and progressed spontaneously in TRAMP mice, the present study may reveal the complex adipokine and cytokine alterations affected jointly by obesity and cancer progression, which best imitated the actual trends of cytokine changes in obese patients with cancer. In the analysis, leptin levels were significantly increased in all three HFD feeding groups. However, the trends in CCL3, CCL4, CCL5 and CXCL10 appeared to be complicated, where a notable reversal in the changing trends was detected. Specifically, the levels of these adipokines were generally increased in CD-fed mouse sera in the 20-week or 24-week group compared with the HFD-fed mouse sera, whereas levels became increased in the HFD-fed mouse sera compared with the CD-fed mouse sera in the 28-week group. These trends could implicate the joint effects of HFD-induced excess adipose tissue deposition and tumor progression.

In the present study, five adipokines, including leptin, CCL3, CCL4, CCL5 and CXCL10, were identified to be involved in the stimulation of PCa progression by HFD. Leptin, associated with levels of adipose tissue in human body, was increased in obese patients (11). Its functions primarily involve satiety regulation and energy expenditure, and also serve a role in reproduction, hematogenesis and carcinogenesis (11). Kato *et al* (10) identified that leptin exposure increased the cell migration and invasion and maintenance of the mesenchymal phenotype of ovarian cancer cells via the activation of Janus kinase/signal transducer and activator of transcription, phosphoinositide 3-kinase/protein kinase B and RhoA/RhoA kinase pathways. Increased concentrations of CCL3, also known as macrophage inflammatory protein-1α, an inflammatory chemokine, were measured in the omentum and liver of obese patients, trafficking pro-inflammatory cells and driving the development and progression of oesophagogastric adenocarcinoma (25). Vielma *et al* (26) identified that CCL5, IL-6 and IL-10 secretions from spleen cells were stimulated by adipocytes and additionally enhanced by premalignant lesion cell supernatant, which provides evidence that obesity-associated inflammation, with the inflammatory environment of premalignant cells may promote the development and progression of cancer.

The results of the present study suggested that HFD induced excessive adipose tissue deposition and obesity in TRAMP mice, which additionally promoted development and progression of PCa. Concurrently, it was identified that DU145 cells cultured in HFD-CM, with altered adipokine and cytokine levels, increased the proliferative, migratory and invasive abilities of DU145 cells compared with the counterparts in CD-CM. On the basis of these results, it is hypothesized that leptin, CCL3, CCL4, CCL5 and CXCL10 levels, which were demonstrated to exhibit significantly different levels of a cocktail of cytokines in obesity-related and malignancy-associated sera between HFD- and CD-fed mice, and may contribute together to cancer progression.

The aim of the present pilot study was to explore the function of adipokine and cytokine alterations in obesity-stimulated PCa progression. The application of HFD-induced obesity and spontaneous development of PCa in TRAMP mice mimicked the natural course of PCa in patients with obesity, and accordingly the adipokine and cytokine alterations identified in the present study may contribute to the reciprocal effect of adipocytes and cancer cells on cancer progression. Further studies are warranted to identify the specific cytokines and their associated signaling pathways in mediating the aggressiveness of cancer cells. Consequently, these cytokines and signaling pathways may be targeted to reverse the negative effects of obesity on cancer progression.

To conclude, the results of the present study demonstrated that HFD-fed TRAMP mice presented with increased body weight, excess fat deposition, poorer differentiation, increased local invasion and metastasis rates of PCa. Concurrently, HFD feeding time-dependently altered the levels of various adipokines and cytokines in TRAMP mice, and *in vitro* studies indicated that HFD-CM promoted the proliferative, migratory and invasive abilities of prostate cancer cells. These results suggested that the circulating adipokine and cytokine alterations in response to excess adipose tissue deposition induced by HFD feeding contributed to PCa progression.

Acknowledgements

The present study was sponsored by Shanghai Sailing Program (grant no. 17YF1401700), National Natural Science Foundation of China (grant no. 81272835), Outstanding Talents Cultivation Project of Shanghai Medical System (grant no. XBR2013092), Talents Development Foundation of Shanghai (grant no. 201302) and Scientific Research Innovation Project of Shanghai Education Committee (grant no. 14ZZ010).

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