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# FABP4-mediated ERK phosphorylation promotes renal cancer cell migration

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## Abstract

Clear cell Carcinoma (ccRCC) is the most common and lethal subtype among renal cancers. In the present study we investigated the potential role of fatty acid-binding protein 4 (FABP4), also known as adipocyte FABP (A-FABP) or aP2 on ccRCC progression. Firstly, we found that FABP4 median serum levels were significantly higher in ccRCC patients compared to HD. Based on this result and to evaluate whether FABP4 plays a role on renal cancer malignant phenotype, we analyzed proliferation and migration in 786-O and ACHN cell lines using recombinant FABP4. We found that FABP4 significantly increased cell migration, whereas it had no significant effect on proliferation. As FABP4 is mainly expressed by adipocytes, we measured FABP4 adipocyte conditioned media (Ad-CM) levels showing that Ad-CM from ccRCC (Ad-CM ccRCC) had significantly higher mean values compared to Ad-CM obtained from Healthy Donors (HD). To assess the effects of adipocyte-released FABP-4, on cancer malignant phenotype we evaluated 786-O and ACHN proliferation and migration, using Ad-CM from ccRCC and Ad-CM from HD alone or in combination with FABP4 inhibitor BMS309403. Our results showed that Ad-CM enhanced cell proliferation in ACHN, but not in 786-O and on cell motility in both cell lines and this effect was partially reverted by BMS309403 in both cell lines. Moreover, in both cell lines, FABP4 effect was associated with an increased ERK phosphorylation. Collectively these data support the role of FABP4 in ccRCC progression and its potential use as noninvasive biomarker and therapeutic target for ccRCC.

**Keywords** Adipocytes, Renal cancer, Peri-renal adipose tissue, FABP4

## Introduction

Renal carcinoma includes a heterogeneous group of malignancies from renal tubular epithelial cells that account for about 2.2% of all tumors and 1.8% of the total cancer mortality worldwide [1]. Generally, less than 10% of patients with RCC are diagnosed with the classic triad of flank pain, hematuria and palpable mass, more often the diagnosis occurs as incidental findings during ultrasonography or abdominal CT performed for another reason [2]. Accordingly, about 25% of patients present with distant metastatic disease at diagnosis [3]. Early detection of organ-confined RCC allows curative surgical resection via partial or radical nephrectomy and about 30% of patient who undergo surgical resection develop

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recurrence associated with high mortality rate [4]. Clear cell Renal Cell Carcinoma (ccRCC) is the most common and lethal subtype among renal cancers, comprising almost the 70% of cases [5].

Several epidemiological studies reported a strong association between perirenal adipose tissue (PRAT) thickness measured by computer tomography scan and ccRCC histotype in patients with renal cortical tumor [6]. In addition, it has been reported that PRAT width is associated with poor Progression Free Survival (PFS) [7] and Overall Survival (OS) in patients with surgical treated ccRCC [8]. These epidemiological data suggest that Peri-Renal Adipose Tissue (PRAT) may affect ccRCC behavior through the exchange of proteins, cytokines and metabolites with cancer cells [9], thanks to an extensive network of blood and lymph vessels connecting PRAT and ccRCC [10]. PRAT, in fact, is a peculiar visceral fat depot, defined along with pericardial Adipose Tissue (AT) and renal sinus fat an "ectopic fat" [11]. It is able to regulate kidney homeostasis in physiological conditions and involved in different metabolic diseases such as hypertension, atherosclerosis, and insulin resistance [12]. Thus, it is possible to speculate that peri-renal mature adipocytes may exert their effect on tumor microenvironment through secretion of adipokines and/or growth factors that support tumor progression.

Although it is well known that high perirenal fat accumulation is associated with increased mortality and poor PFS of patients with localized ccRCC [7, 8], the molecular mechanism underlying the relationship between perirenal adipocytes and ccRCC spread is still unclear.

The discovery of extracellular FABP4 (e-FABP4) as adipocyte-released factor changed the game in research of a mechanistic link between cancer and AT. This small protein of 15 kDa is a fatty acid carrier, secreted by adipocytes, macrophages and endothelial cells, it is involved in metabolic disorders associated to visceral adiposity such as insulin resistance and atherosclerosis. Interestingly, e-FABP4 has been identified as driver of cell proliferation and migration in breast [13], colon [14], and ovarian cancer [15].

In the present study, we investigated the effect of FABP4 and adipocyte-released FABP4 on ccRCC cell proliferation and migration.

## Materials and methods

### Materials

Media, sera and antibiotics were purchased from GIBCO (Thermo Fischer Scientific, Waltham, MA, USA) and from BioWhittaker (Lonza, Basel, Switzerland). Antibody against phospho-ERK1/ERK2 was purchased from Cell Signaling Technology (Cell Signaling Technology, Danvers, MA, USA) and antibody against ERK was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) reagents were acquired from Bio-Rad (Hercules, CA, Usa). All other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA). Human FABP4 Quantikine ELISA kit was obtained from R&D Biotechne (Bio-Techne, Minneapolis MN, USA). Recombinant FABP4 was purchased from Elabscience. FABP4 inhibitor was obtained from Sigma-Aldrich (Merck, Darmstadt, Germany).

### Conditioned media

We prospectively collected PRAT from 19 patients who underwent radical nephrectomy and 10 healthy donors who underwent minimally invasive surgery to remove a living donor's kidney at the Department of Urology at University of Naples "Federico II" and Department of Andrology and Kidney Transplantation Unit of University of Bari "Aldo Moro" between 2022 and 2024. All patients consented to participate in the study and this procedure was approved by the Ethical Committee of University of Naples "Federico II" (protocol number 380/20) and the University of Bari "Aldo Moro" (protocol number 5648/2018). Clinical and pathological characteristics including age, sex, BMI, Fuhrman grade and pathological stage were prospectively collected. Surgical specimens were processed and evaluated by genitourinary pathologists to assess tumor histotype and tumor staging according to 2018 American Joint Committee on Cancer (AJCC) [16]. Only patients with a ccRCC histotype were included in this study. To obtain mature adipocytes from PRAT, human specimens were processed under a sterile laminar flow hood, where AT was washed twice with sterile Phosphate Buffer Salin (PBS) to remove red blood cells and debris and dissected into 1 mm segments. The resulting homogeneous mixture was digested with Collagenase Type IV (1 mg/mL) and kept in a shaking incubator at 37 °C for 1 h. After digestion, the adipocytes released from the tissue floated on cell suspension forming a yellow layer on top. The adipocyte suspension was washed twice with PBS and the adipocytes obtained were incubated with RPMI or EMEM supplemented with 100 IU/mL penicillin and 100 IU/mL streptomycin and 0.25% Bovine Serum Albumin (BSA) for 16 h to obtain Adipocytes Conditioned Medium (Ad-CM). The Ad-CM was collected and stored at -80 °C until the analysis.

### Cell culture

786-O and ACHN human ccRCC cells were kindly provided by Professor Camillo Porta (University of Bari Aldo Moro, Italy) and cultured in RPMI (GIBCO) and EMEM (GIBCO) respectively supplemented with 10% Fetal Bovin Serum (FBS), 100 IU/mL penicillin and 100 IU/mL streptomycin in a humidified incubator a 37 °C with 5% $CO_2$.

### Cell proliferation assay

Cell proliferation was assessed using Cell Proliferation ELISA BrdU colorimetric kit (Roche Basle, Swiss). 786-O and ACHN were seeded in 96 well plates at a density of $1 \times 10^3$ cells per well and incubated with medium containing 10% FBS, 0.25% BSA, recombinant FABP4 and Ad-CM obtained from ccRCC patients (Ad-CM ccRCC) or HD (Ad-CM HD) alone or in combination with BMS309403. In the last 2 h of incubation cells were labeled with 10 µM bromodeoxyuridine (BrdU). Thereafter, cells were fixed and the incorporated BrdU was detected by colorimetric ELISA according to manufacturer's instructions.

### Scratch assay

786-O and ACHN ccRCC cell lines were seeded in 12 well plates at density of $1 \times 10^5$ cells per well and allowed to form a monolayer, in vitro "wound" was created by a straight-line scratch across the cell monolayer using a sterile 20 µl filter tip. Cells were treated with Mytomicin C 10 µg/mL to stop proliferation for 2 h before the experiment and throughout its duration. Then cells were washed three times with PBS to clear cell debris and fresh medium was added. Cells were incubated with medium containing 10% FBS, 0.25% BSA, recombinant FABP4 and Ad-CM obtained from ccRCC patients (Ad-CM ccRCC) or HD (Ad-CM HD) alone or in combination with BMS309403 and allowed to close the wound for 24 h. The images were acquired at 0 (control) and 24 h (post wounding) using a camera connected to the microscope. The distance toward the wound edges was measured at three fixed points for each image with three images taken for each well then it was analyzed using ImageJ (Bio-Rad, Hercules, CA, USA).

### FABP4 ELISA

Ad-CM and serum FABP4 content was measured using ELISA assay (Bio-Techne Minneapolis MN, USA) according to the manufacturer's instructions.

### Western blot

786-O and ACHN were seeded in 12 well plates ($1 \times 10^5$ cells/ well) and allowed to form a monolayer for 24 h and serum starved for 16 h. Cells were treated with indicated stimuli for 24 h. After treatment cells were washed with ice-cold PBS and harvested in a Laemmli buffer containing β-mercaptoethanol, phosphatase inhibitors (0.5 mM sodium vanadate, 2 mM sodium pyrophosphate, 5 mM β-glycerolphosphate, and 50 mM sodium fluoride) and the protease inhibitor and inhibitor phenylmethylsulfonyl fluoride (Sigma–Aldrich). As already described [17], 50 µg of protein samples were separated using 10% SDS page and blotted on a nitrocellulose membrane (Fisher scientific). Membranes were blocked for 1 h in TBS tween (10 mM Tris-HCl, pH 7.4, and 140 mM NaCl) containing 5% of non-fat dry milk and then incubated with indicated antibodies (GAPDH Santa Cruz, ERK1/ERK2 Santa Cruz, pERK1/pERK2 cell signaling). Detection of blotted proteins was detected by ECL according to manufacturer's instruction and densitometric analysis was performed using Image Lab software 3.0 (Bio-Rad, Hercules, CA, USA).

The bands were analysed by densitometric analysis using the imaging software Image Lab software 3.0 (Bio-Rad, Hercules, CA, USA). Based on pixel counts, quantitative data for each sample were obtained.

### Sera collection

After obtaining informed consent, a brief medical history was recorded. Sera were collected at the time of admission of each patient using BD vacutainer blood collection tubes with no additives (Becton Dickinson, Oxfordshire, UK). All patients were asked to respect 12 h before blood collection. Serum samples were centrifuged and immediately stored below 80 °C until they were analyzed.

### Statistical analysis

Statistical analyses were performed using the GraphPad Prism software (version 9.0, San Diego, CA, USA). Difference among means were compared using a Student's *t* test, Mann-Whitney, one-way ANOVA test or Kruskal-Wallis test. Values are reported as mean ± standard deviation (SD) from at least 3 independent experiments. A two-sided *p*-value of less than 0.05 was considered statistically significant.

## Results

### Clinical characteristics of CcRCC patients and healthy donors

We obtained serum samples from 35 patients who underwent radical nephrectomy between 2022 and 2024 in the Department of Urology at the University of Naples "Federico II" and from 23 Healthy Donors (HD). The clinical characteristics of enrolled subjects are reported in Table 1.

### Serum FABP4 concentration in CcRCC patients compared to HD

Growing evidence suggests that circulating levels of FABP4 are increased in cancer patients. To investigate the circulating FABP4 levels in ccRCC patients, we collected and analyzed serum samples from 35 ccRCC patients and 23 HD. As shown in Fig. 1A, ccRCC patients had a significantly higher median serum FABP4 levels compared to HD [18680 pg/mL (IQ: 24930 – 13388); 14090 (IQ: 18733 – 11288); *p* value 0.0292]. Moreover, to verify whether conditions other than cancer could lead to an increased serum FABP4 concentration in our study

**Table 1** Clinical characteristics of the study population

| | ccRCC patients (*n* 35) | | | | Healthy Donors (*n* 23) |
|---|---|---|---|---|---|
| Sex | | | | | |
| Male, *n* (%) | 26 (74) | | | | 12 (52) |
| Female, *n* (%) | 9 (26) | | | | 11 (48) |
| Age (years) | 63 ± 11 | | | | 55 ± 10 |
| BMI (Kg/m^2) | 27 ± 4 | | | | 25 ± 3 |
| Comorbidity *n* (%) | 27 (77) | | | | 0 |
| Diabetes mellitus *n* (%) | 6 (17) | | | | 0 |
| Hypertension *n* (%) | 26 (74) | | | | 0 |
| Others | 0 | | | | |
| Pathology stage *n* (%) | pT1 | pT2 | pT3 | ND | |
| | 16 (46) | 3 (9) | 4 (11) | 12 (34) | |
| Fuhrman grade, *n* (%) | G1 | G2 | G3 | G4 | |
| | 7 (20) | 13 (37) | 10 (29) | 5 (14) | |

cohort, we evaluated FABP4 levels stratifying patients according to BMI (B) (10 BMI ≤ 25 vs. 20 BMI > 25), Type 2 Diabetes (T2D) (C) (29 TD- vs. 6 TD+), and hypertension (D) (9 Hypertension- vs. 26 Hypertension+). No significant differences were found between patients with BMI ≤ 25 and those with BMI > 25 (p= 0.54), subjects with hypertension vs. those without hypertension (p= 0.62) and subjects with T2D and those without T2D (p= 0.99).

#### Ad-CM effect on ACHN and 786-O cell lines proliferation

To evaluate the biological importance of circulating extracellular FABP4 (e-FABP4) in ccRCC progression, we determined whether recombinant FABP4 affected ccRCC cell proliferation by performing BrdU assay on 786-O and ACHN cell lines. Serum-starved cells were incubated with a medium containing 10% FBS, 0.25% BSA and recombinant FABP4 10000 pg/mL alone or in combination with BMS309403, a synthetic antagonist of FABP4[19]. As shown in Fig. 2, FABP4 treatment had no significant effect on cell proliferation in 786-O and ACHN cells (p= 0.061 and 0.995).

#### Human Recombinant FABP4 effect on migration of ccRCC cell lines

Then, we evaluated the effect of FABP4 on cancer cell migration, by scratch assay incubating cells with human recombinant FABP4 (10000 pg/mL) alone or in combination with BMS309403. As shown in Fig. 3, e-FABP4 enhanced cell migration both in 786-O and ACHN cell lines.

#### E-FABP4 concentration in Ad-CM isolated from CcRCC patients compared to Ad-CM from HD

To assess the FABP4 secretion by peri-renal adipocytes, we measured FABP4 concentrations in Ad-CM obtained from PRAT of 10 HD and 19 ccRCC patients (A). As shown in Fig. 4, Ad-CM from ccRCC had a higher e-FABP4 mean concentration compared to Ad-CM from HD [10150 pg/mL ± 3835; 5578 ± 2969; *p* value 0,0048], suggesting that tumor proximity affects the e-FABP4 content of perirenal adipocytes.

In addition, to clarify whether conditions other than cancer could lead to an increased e-FABP4 secretion by peri-renal adipocytes, we analyzed the difference in e-FABP4 concentration by stratifying patients according to BMI (B) (10 BMI < 25 vs. 19 BMI ≥ 25), T2D (C) (22 T2D + VS 4 T2D-) and hypertension (D) (16 T2D- VS 13 T2D+). As shown in Fig. 4, in our study cohort no significant differences were found in patients with BMI ≤ 25 and those with BMI > 25 (p 0.21), and with hypertension vs. those without hypertension (p 0.37), subjects with T2D and those without T2D (p 0.62).

#### Ad-CM from CcRCC patients effect on proliferation of CcRCC cell lines

To investigate the effect of extracellular FABP4 secreted by perirenal adipocytes on ccRCC proliferation, we performed a BrdU assay by incubating cells with Ad-CM from HD and ccRCC with or without BMS309403. As shown in Fig. 5, Ad-CM treatment had no significant effect on cell proliferation in 786-O cells (p= 0.061 and 0.995, respectively). Conversely, Ad-CM from both HD and ccRCC enhanced cell proliferation in ACHN (p= 0.0039 and 0.0089, respectively). Notably, the Ad-CM effect on ACHN proliferation was not reverted by BMS309403, suggesting that adipocyte-released factors other than FABP4 may affect ACHN cell proliferation.

#### Ad-CM from CcRCC patients' effect on migration of CcRCC cell lines

We evaluated the effect of adipocyte-released e-FABP4 on cancer cell migration by scratch assay incubating cells with Ad-CM from HD and ccRCC alone or in combination with BMS309403.

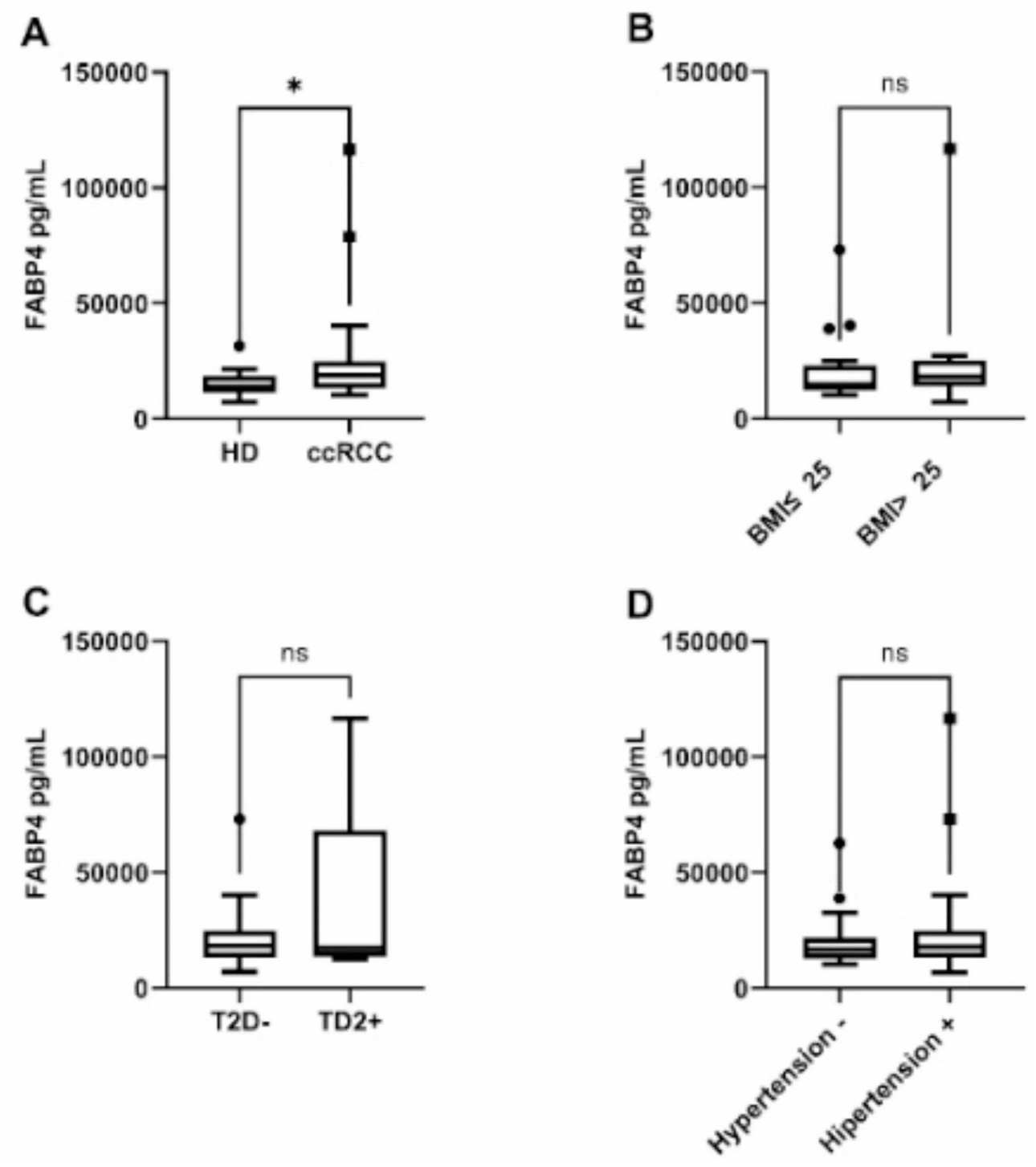


**Fig. 1** Serum FABP4 levels of ccRCC patients were higher compared to HD (**A**), whereas no significant differences were observed stratifying patient according to BMI (**B**), diabetes (**C**) and hypertension (**D**). Box plots denote median and 25th to 75th percentiles (boxes) and Tukey whiskers. The *p* value was evaluated using Mann–Whitney test. Ns indicates a *p*-value > 0.05 and *Indicates a *p* value < 0.05

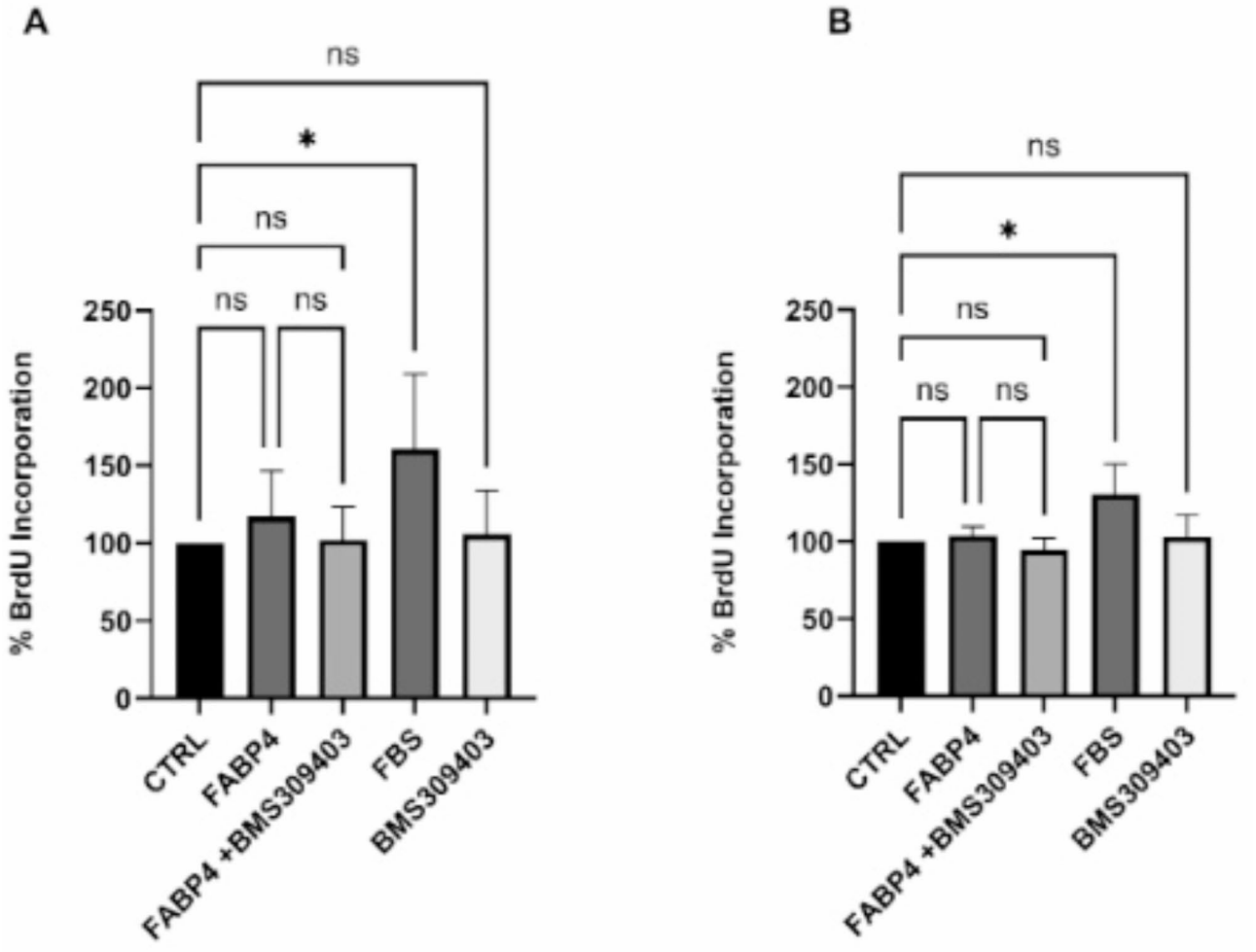


**Fig. 2** Human recombinant FABP4 does not affect cell proliferation in 786-O and ACHN. 786-O (**A**) and ACHN (**B**) cells were seeded ($1 \times 10^3$cells/ well) in 96-well. and incubated with medium containing 10% FBS, 0.25% BSA and recombinant FABP4 10000 pg/mL alone or in combination with BMS309403 40 μM for 24 h. Cell proliferation was calculated as percentage of BrdU incorporation compared to control. Data represent the mean ± SD of 5 independent experiments for 786-O and 3 independent experiments for ACHN. The *p*-value was calculated using the one-way ANOVA test. Ns indicates a *p*-value > 0.05, * Indicates a *p* value < 0.05

We showed that Ad-CM from ccRCC enhanced cell migration in both 786-O and ACHN. In addition, we found that treatment with FABP4 inhibitor partially reverts the effect of Ad-CM from ccRCC on cell motility (Fig. 6). Collectively, these results suggest that e-FABP4 could be one of the drivers of the motility-promoting effect of Ad-CM from ccRCC on 786-O and ACHN.

#### Adipocyte-released FABP4 effect on ERK phosphorylation

Since many authors have reported that ERK kinases play a pivotal role in extracellular FABP4 signal transduction, we evaluated ERK phosphorylation after treatment with FABP4 and Ad-CM with or without BMS309403. As shown in Fig. 7, both Ad-CM from ccRCC and FABP4 treatment significantly increased ERK phosphorylation in 786-O and ACHN. In addition, FABP4 inhibitor mitigated ERK phosphorylation induced by Ad-CM from ccRCC and recombinant FABP4 in both cell lines.

## Discussion

White visceral AT plays a key role in promoting tumor spread, especially in cancers developing in abdominal area such as ovarian, colorectal and RCC [18]. Several studies have shown that AT behaves as endocrine organ, releasing a plethora of active compounds that may affect the tumor cell malignant phenotype [18, 19]. This crosstalk may partially explain the correlation between visceral adiposity and OS or PFS in cancer patients [7, 8, 20, 21]. There is growing evidence that AT promotes malignant behavior of ccRCC cells. Zi and coworkers showed that PRAT CM isolated from ccRCC patients significantly increased migration in ACHN and CAKi-2 cell lines through the activation of Wnt β-catenin pathway

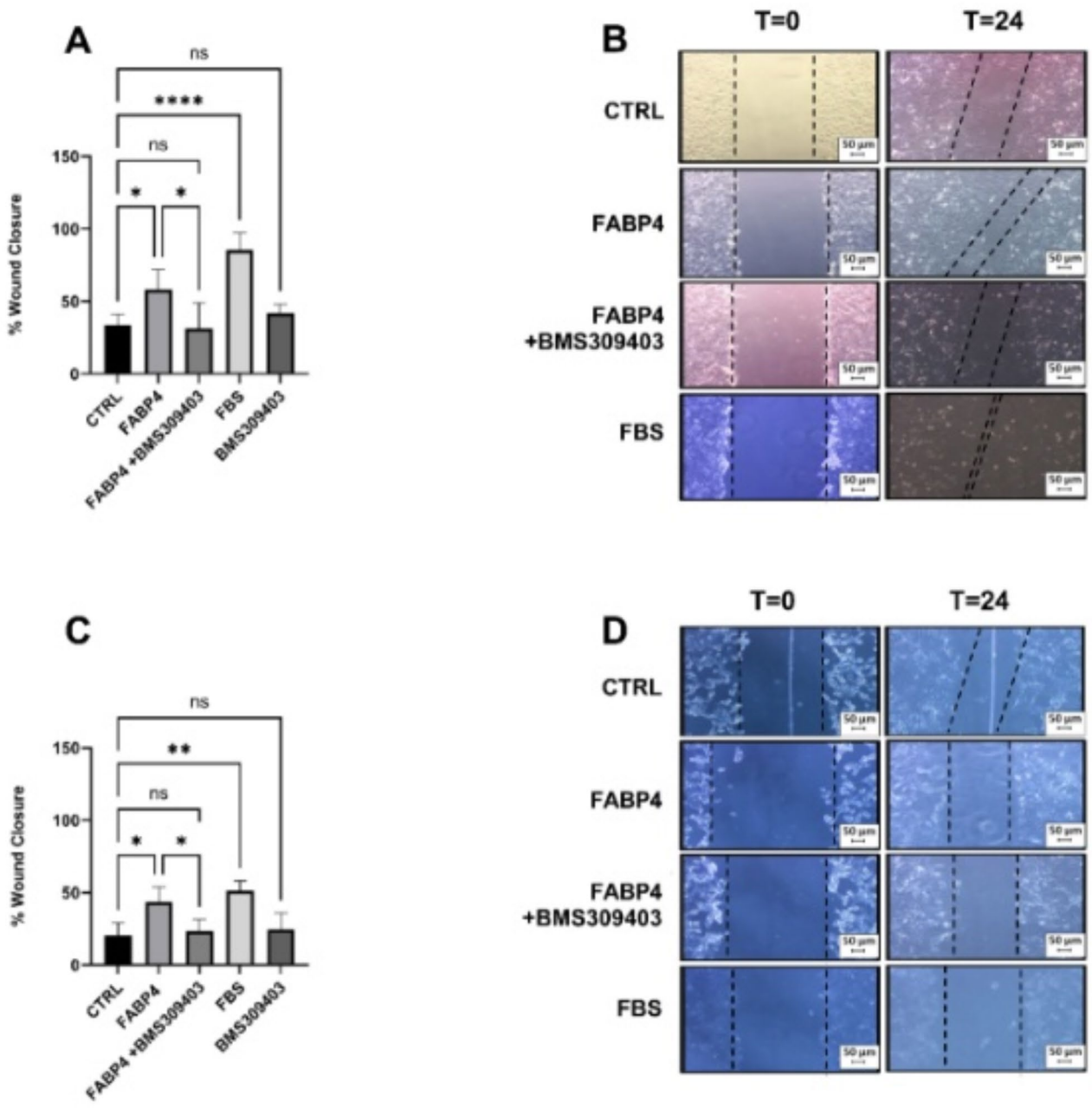


**Fig. 3** Ad-CM ccRCC enhances migration in 786-O and ACHN cells through FABP4 release. 786-O (**A**) and ACHN (**B**) cells were seeded ($1 \times 10^5$cells/ well) in 12-well. In vitro "wound" was created by a straight-line scratch across the cell monolayer and cells were incubated with medium containing 10% FBS, 0.25% BSA, and human recombinant FABP4 10000 pg/mL and BMS309403 40 μM. The images were acquired at 0 and 24 h using a camera connected to the microscope. The distance toward the wound edges was photographed and measured (magnification 10x). Cell motility was calculated as percentage of wound healing rate. Data represent the mean ± SD of 6 independent experiments for 786-O and 6 independent experiments for ACHN. The *p*-value was calculated using the one-way ANOVA test. Ns indicates a *p*-value > 0.05, *Indicates a *p* value < 0.05, **indicates a *p*-value ≤ 0.01, and **** a *p*-value ≤ 0.0001

[22]. Campo-Verde-Arbocco and colleagues evaluated the effects of CM from human AT explants from renal cell carcinoma near or far from the tumor, demonstrating that AT educated by ccRCC acquired the ability to promote cell migration and invasion [23]. In a second study, the same authors investigated the intracellular pathway involved in the enhanced motility of ccRCC, identifying pERK and pPI3K as downstream mediators [24]. More recently, it has been demonstrated that PRAT explants from ccRCC patients underwent browning process that may partially explain the different secretory profile of ccRCC PRAT compared to HD PRAT [25, 26]. Although these studies collectively showed an established cross-talk between PRAT and ccRCC, adipocyte-released

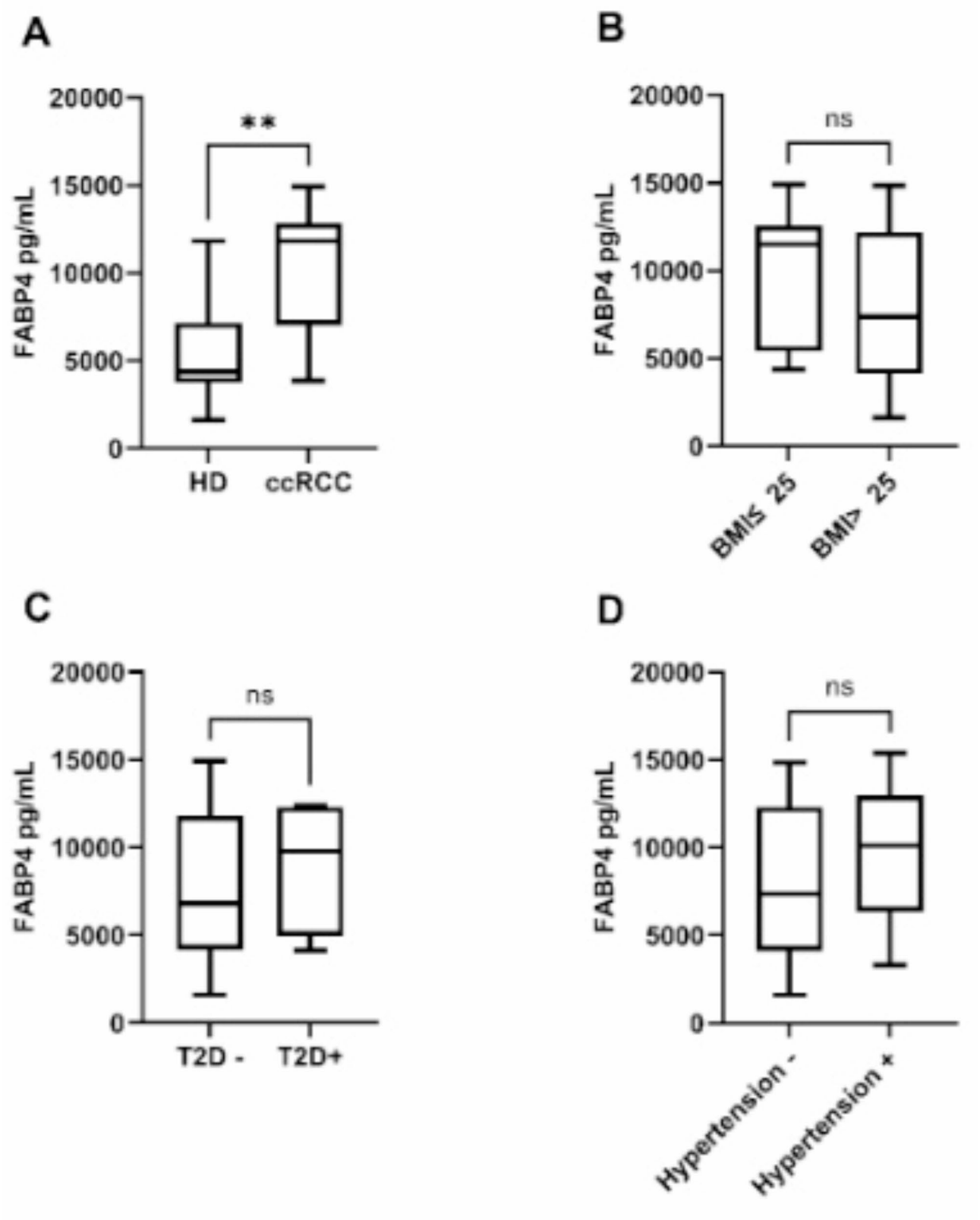


**Fig. 4** (See legend on next page.)

(See figure on previous page.)

**Fig. 4** Ad-CM ccRCC displayed a higher concentration of e-FABP4 (**A**) while no significant differences were observed by stratifying patient according to BMI (**B**), hypertension (**C**) and type 2 diabetes (**D**). Peri-renal adipocytes were seeded (200 µL of adipocytes suspension/well) and incubated with 500 µL of medium containing 0,25% BSA to isolate the Ad-CM. After 16 h the Ad-CMs were collected below 80 °C until the assay. Box plots denote median and 25th to 75th percentiles (boxes) and Tukey whiskers. The *p*-value was evaluated using Mann-Whitney test. Ns indicates a *p*-value > 0.05 and **indicates a *p*-value ≤ 0.01

factors affecting cancer aggressiveness remain to be clarified. To address this issue, we focused our attention on FABP4, a small protein highly expressed in adipocytes, greatly induced during adipocyte differentiation and able to activate lipolysis [27]. In adipocytes and macrophages, FABP4 is involved in metabolic and inflammatory pathways of insulin resistance, diabetes mellitus, and atherosclerosis [28]. Guaita-Esteruelas and colleagues demonstrated an association between serum FABP4 levels and breast cancer independent of BMI, it could be speculated that tumor cells may influence FABP4 secretion in surrounding adipocytes, suggesting a role for this protein in the cross-talk between cancer cells and adipocytes [29].

We showed that FABP4 significantly increased cell migration, suggesting that it could be a driver of the AT cancer-promoting effect.

Differently from previous studies based on ex-vivo AT explant, we isolated adipocytes from PRAT. This model allows us to isolate the role of factors specifically released by adipocytes.

Using a CM approach, we demonstrated for the first time that adipocyte-released FABP4 increases ccRCC cancer cell motility. Accordingly, it has been reported that FABP4 is involved in the motility-promoting effect of Ad-CM on colon cancer cell [14] and in prostate cancer invasiveness [30]. Interestingly, these tumors were highly affected by lipid accumulation, suggesting that FABP4, as lipid chaperone, could act as free fatty acids transporter from tumor microenvironment to cancer cells [31, 32]. In addition, e-FABP4 effect on ccRCC cells can be driven by ERK phosphorylation regardless of free fatty acid transport. Chen and coworkers, in fact, reported that FABP4 alone affects MCF7 cell proliferation by binding Desmoglein 2 and inducing the Erbin release and ERK phosphorylation [33]. Here, we showed that the inhibition of FABP4 by BMS309403 decreased the effect of Ad-CM on migration, indicating that FABP4 is a pivotal factor in the reculation of ccRCC cell motility by adipocytes. We further demonstrated that e-FABP4 effect on ccRCC cell migration was mediated by pERK in 786-O and ACHN cell line. Several authors reported that ERK is a crucial transductor of extracellular signal regulating cell shape and motility. In this regard, it was demonstrated that during collective cell migration occurring on the wound edge, waves of ERK activity are propagating across cells, coordinating cells movement in response to extracellular stimulation by serum or growth factors [34, 35].

Despite our data obtained only on in vitro models and needing in vivo validation, we provided insights into the molecular basis underlying the correlation between PRAT thickness and poor PFS, suggesting that cancer-associated adipocytes may drive ccRCC spread through the release of molecules with endocrine and paracrine effect. In addition, our findings support the clinical relevance of measuring PRAT thickness to assess patient's recurrence risk. Notably, novel molecular imaging opportunities for the clinical management of renal cell carcinoma are now available [36].

As previously demonstrated in other cancers [8, 29, 37], we found that circulating FABP4 levels were significantly higher in ccRCC patients compared to HD. Accordingly, Yang and coworkers previously demonstrated that FABP4 concentration was higher in urine of ccRCC patients compared to HD, whereas no difference was detected in FABP4 expression between cancer and benign renal tissue [38].

Collectively, these data suggest that FABP4 could be used as noninvasive prognostic biomarker for ccRCC, as showed in breast, cervical and gastro-intestinal cancers [29, 39, 40].

Further studies on a larger population are needed to assess the benefit of FABP4 assessment in clinical management of ccRCC patients.

## Conclusion

Cancer cell migration is a key step in the metastatic spread. To the best of our knowledge, our study showed for the first time that FABP4 released by peri-tumoral adipocytes promotes renal cancer cell migration, increasing ERK phosphorylation (Fig. 8). Our findings indicated that FABP4 could represent a therapeutic target in ccRCC patients.

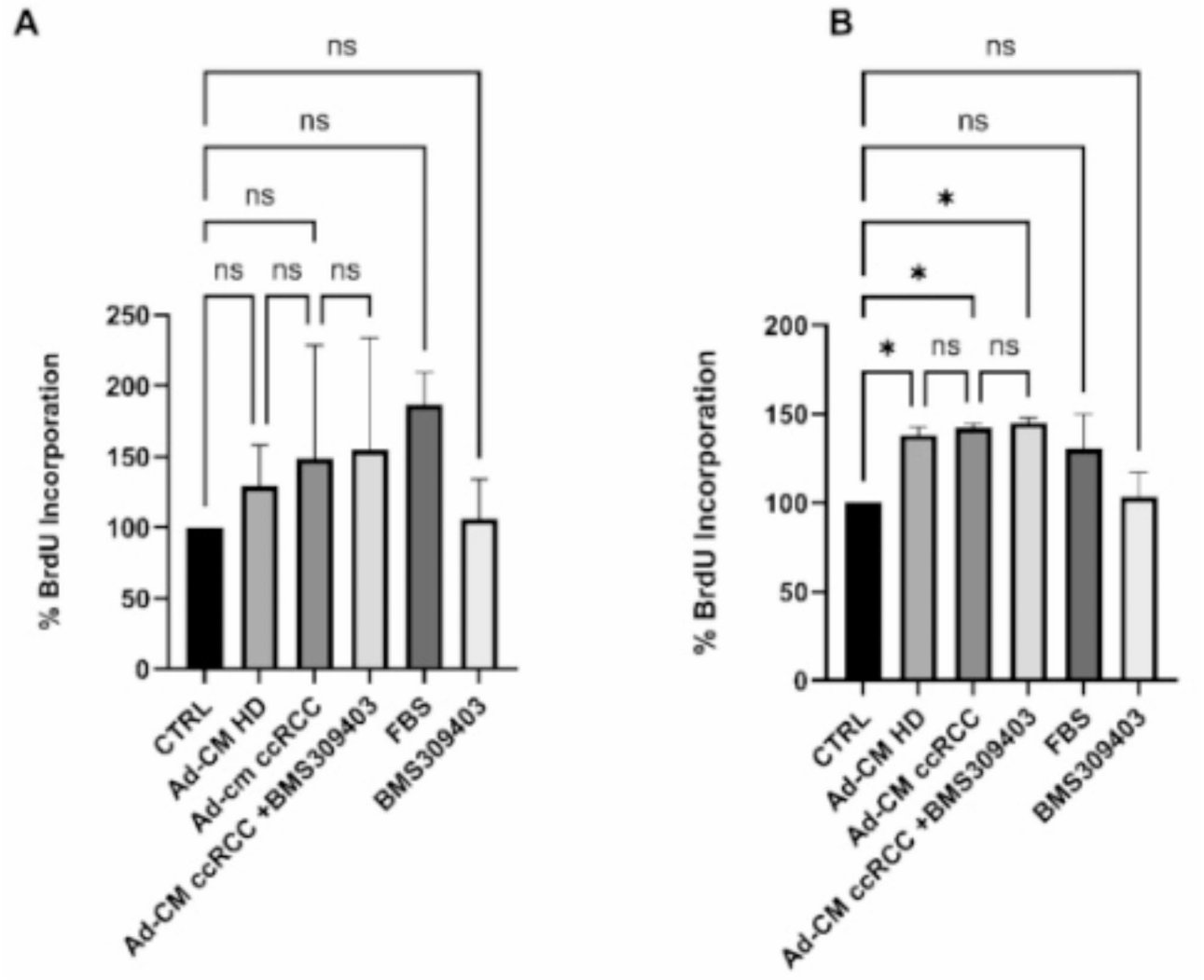


**Fig. 5** Ad-CM affect cell proliferation in ACHN, whereas no effect was observed in 786-O cell line. 786-O (**A**) and ACHN (**B**) cells were seeded ($1 \times 10^3$cells/well) in 96-well. and incubated with medium containing 10% FBS, 0.25% BSA and recombinant FABP4 10000 pg/mL alone or in combination with BMS309403 40 µM for 24 h. Cell proliferation was calculated as percentage of BrdU incorporation compared to control. Data represent the mean ± SD of 5 independent experiments for 786-O and 3 independent experiments for ACHN. The *p*-value was calculated using the one-way ANOVA test. Ns indicates a *p*-value > 0.05, * Indicates a *p* value < 0.05

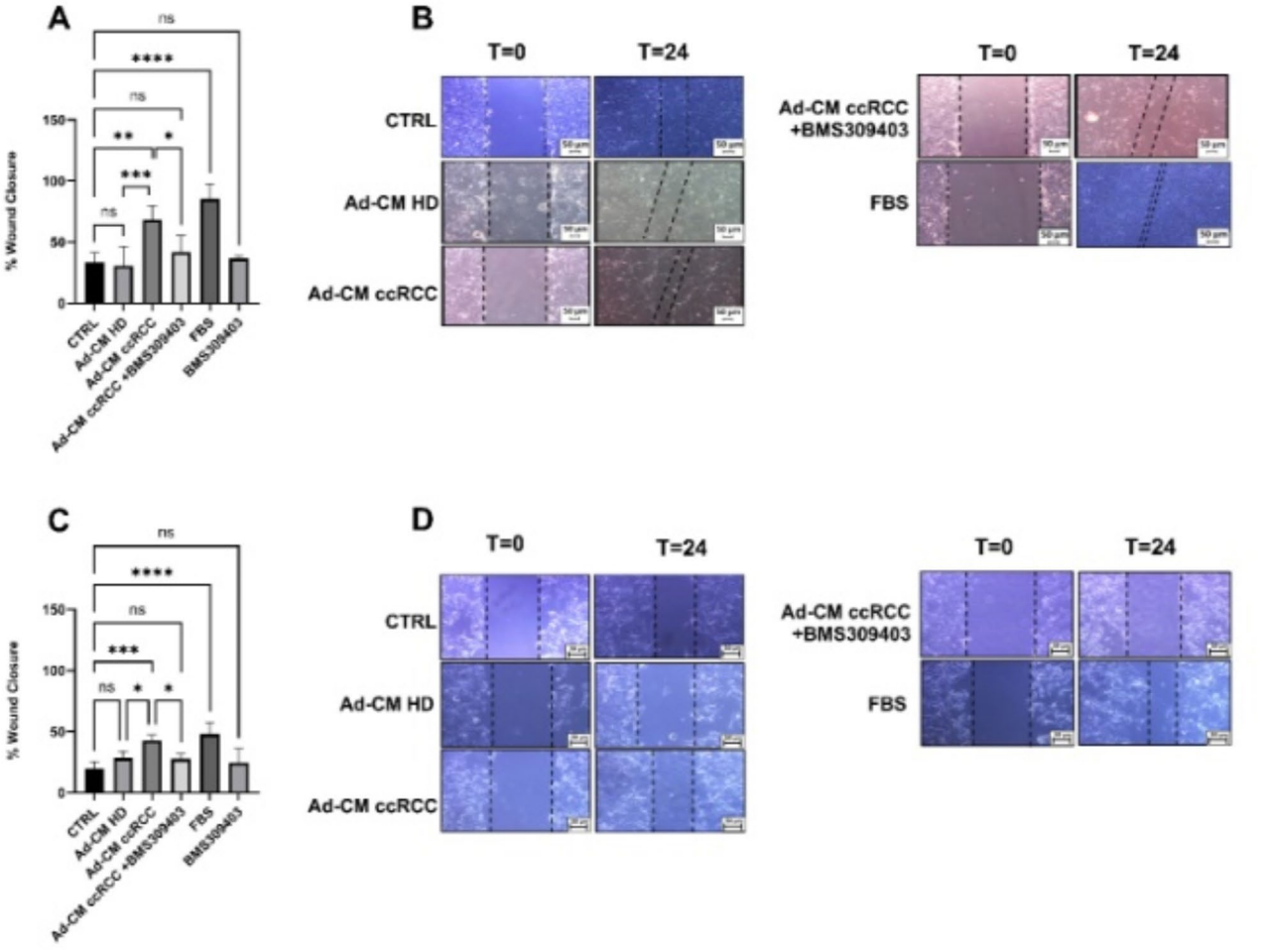


**Fig. 6** Ad-CM ccRCC enhances migration in 786-O and ACHN cells through FABP4 release. 786-O (**A**) and ACHN (**B**) cells were seeded ($1 \times 10^5$cells/ well) in 12-well. In vitro "wound" was created by a straight-line scratch across the cell monolayer and cells were incubated with medium containing 10% FBS, 0.25% BSA, Ad-CM obtained from mature adipocytes both from HD (Ad-CM) and from ccRCC patients (Ad-CM ccRCC), and BMS309403 40 μM. The images were acquired at 0 and 24 h using a camera connected to the microscope. The distance toward the wound edges was photographed and measured (magnification 10x). Cell motility was calculated as percentage of wound healing rate. Data represent the mean ± SD of 6 independent experiments for 786-O and 6 independent experiments for ACHN. The *p*-value was calculated using the one-way ANOVA test. Ns indicates a *p*-value > 0.05, *Indicates a *p* value < 0.05, **indicates a *p*-value ≤ 0.01, and **** a *p*-value ≤ 0.0001, Ns indicates a *p*-value > 0.05, *Indicates a *p* value < 0.05, **indicates a *p*-value ≤ 0.01, ***indicates a *p*-value < 0.001 and **** a *p*-value ≤ 0.0001

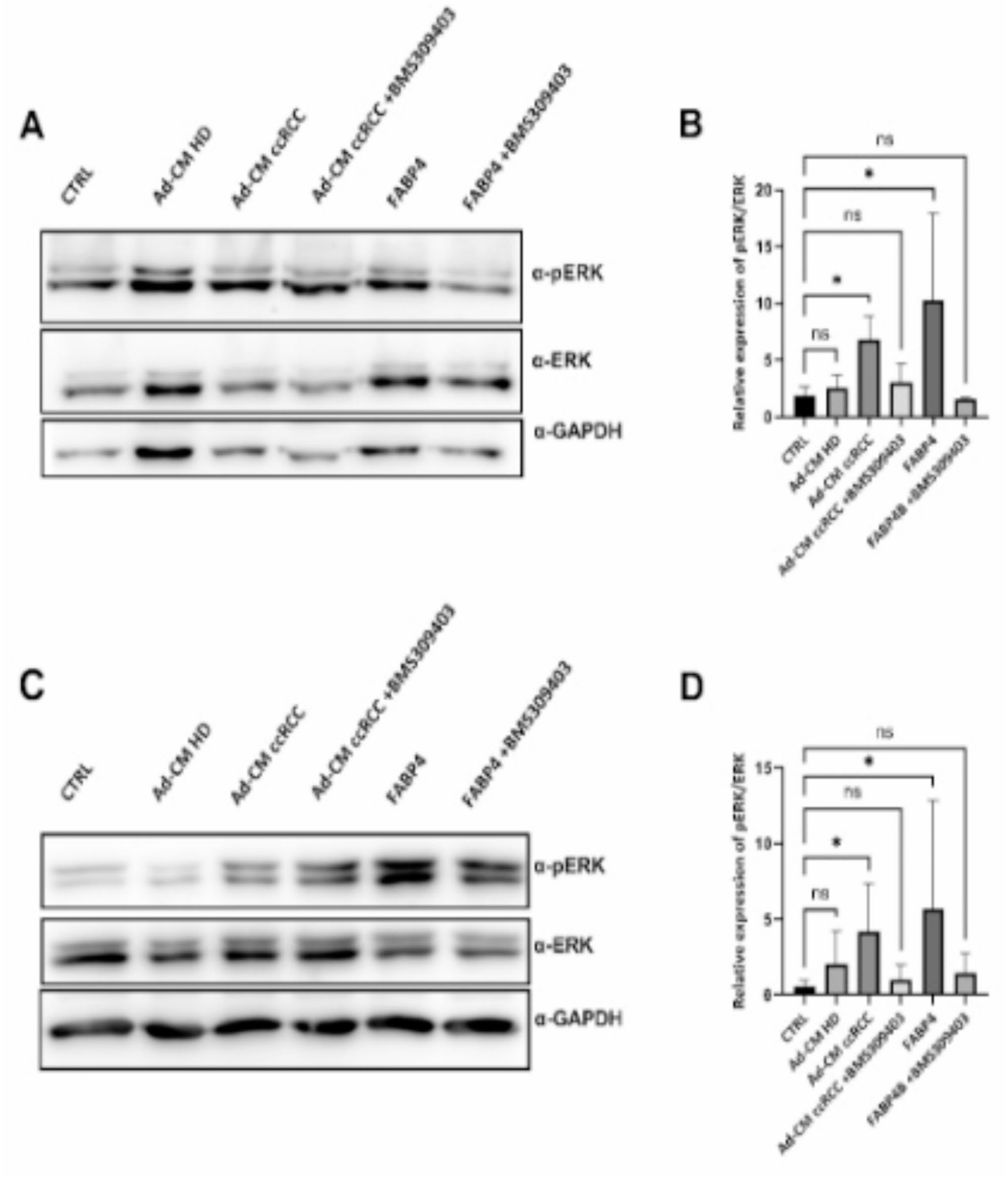


**Fig. 7** Effect of Ad-CM on the MAPK pathway in ccRCC cancer cells. 786-O and ACHN cells were seeded ($1 \times 10^5$ cells/well) and incubated with medium containing 0.25% BSA, Ad-CM from HD, Ad-CM from ccRCC, alone or in combination with BMS309403 40 μM and human recombinant FABP4 10000 pg/mL. GAPDH was used as loading control. A representative blot for 4 experiments for 786-O and 6 experiment for ACHN is presented in figure (**A**) and (**B**) respectively. Quantification of Western Blot is reported for 786-O (**C**) and ACHN (**D**). The *p value* was calculated using Kruskal-Wallis test. Ns indicates a *p*-value > 0.05, *Indicates a *p* value < 0.05. Full-length blots/gels are presented in Supplementary Fig. 1

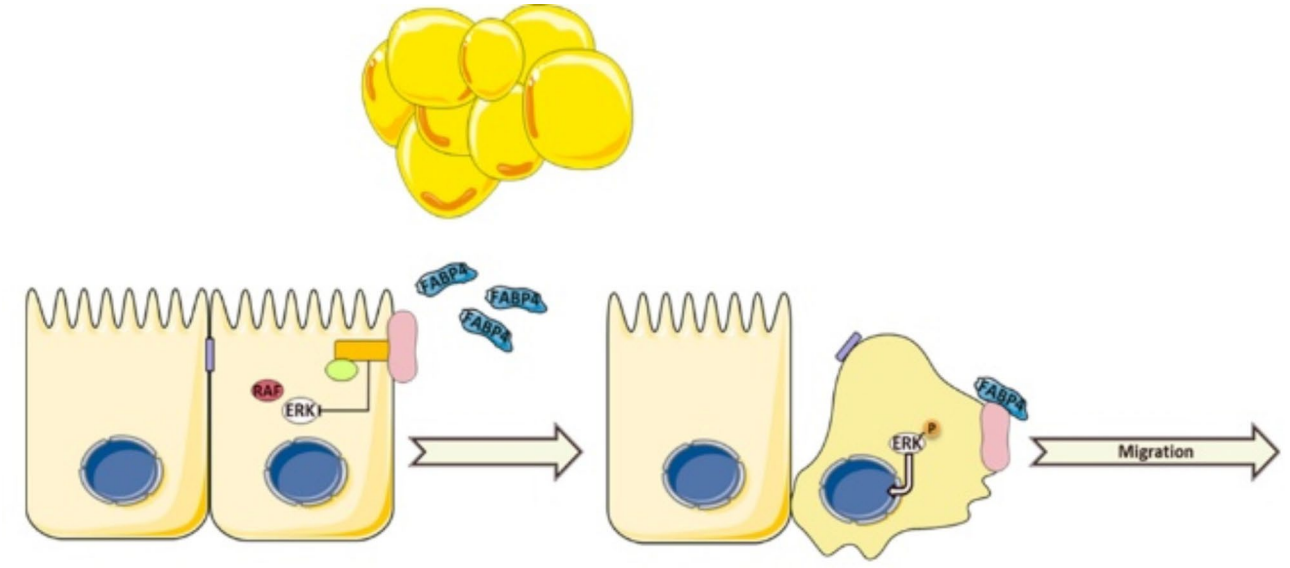


**Fig. 8** Schematic representation of the proposed role of PRAT adipocytes in promoting migration in ccRCC cells. e-FABP4 released by mature peri-renal adipocytes surrounding ccRCC stimulates ccRCC cells inducing ERK phosphorylation. Parts of the figure were drawn using pictures from Server Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License

**Abbreviations**

| | |
|---|---|
| PRAT | Peri-renal adipose tissue |
| Ad-CM | Conditioned medium of human PRAT |
| ccRCC | Clear cell renal cell carcinoma |
| AT | Adipose tissue |
| BMI | Body mass index |
| TME | Tumor microenvironment |
| DMEM | Dulbecco's Modified Eagle Medium |
| FBS | Fetal Bovine Serum |
| PBS | Phosphate-buffered saline |
| BSA | Bovine serum albumin |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase |
| TBS | Tris-Buffered Saline |

## Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13989-1.

Supplementary Material 1

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**Author contributions**
Evelina La Civita: Conceptualization, Methodology, Data Curation. Rosa Sirica: Investigation, Data curation, Visualization. Felice Crocetto: Data curation, Formal analysis. Matteo Ferro: Supervision, Writing - Review and Editing. Francesco Lasorsa: Resources, Formal analysis. Giuseppe Lucarelli: Resources, Supervision. Ciro Imbimbo: Resources, Supervision. Pietro Formisano: Supervision, Writing- Reviewing and Editing. Francesco Beguinot: Supervision, Writing - Review and Editing. Daniela Terracciano: Writing - Original Draft, Conceptualization, Funding acquisition.

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**Data availability**
Data is provided within the manuscript or supplementary information files.

## Declarations

**Ethics approval and consent to participate**
This study was conducted according to the guidelines of the Declaration of Helsinki. The study was approved by the ethical committee of University of Naples "Federico II" (protocol number 380/20) and the University of Bari "Aldo Moro" (protocol number 5648/2018). Written informed consent was obtained from all the patients.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Conflict of interest**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/ Guest Editor for *[Journal name]* and was not involved in the editorial review or the decision to publish this article.
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**References**

1. Bukavina L, et al. Epidemiology of renal cell carcinoma: 2022 update. Eur Urol. 2022;82:529–42.
2. Mohammad A, McClintock C. Renal cell carcinoma–a great mimicker. Conn Med. 2007;71:81–4.
3. Crispen PL, et al. Lymph node dissection at the time of radical nephrectomy for high-risk clear cell renal cell carcinoma: indications and recommendations for surgical templates. Eur Urol. 2011;59:18–23.

4. Tsimafeyeu I, Basin MF, Bratslavsky G. Adjuvant therapy for renal cell carcinoma in 2023: hopes and disappointments. World J Urol. 2023;41:1855–9.
5. Makino T, Kadomoto S, Izumi K, Mizokami A. Epidemiology and prevention of renal cell carcinoma. Cancers (Basel). 2022;(14)(16): 4059.
6. Lowrance WT, et al. Obesity is associated with a higher risk of clear-cell renal cell carcinoma than with other histologies. BJU Int. 2010;105:16–20.
7. Huang H, Chen S, Li W, Wu X, Xing J. High perirenal fat thickness predicts a poor progression-free survival in patients with localized clear cell renal cell carcinoma. *Urol Oncol*2018;36:(157)e1-157 e6.
8. Du Y, et al. Perirenal fat as a new independent prognostic factor in patients with surgically treated clear cell renal cell carcinoma. Clin Genitourin Cancer. 2022;20:e75–80.
9. Venkatesh N, Martini A, McQuade JL, Msaouel P, Hahn AW. Obesity and renal cell carcinoma: biological mechanisms and perspectives. Semin Cancer Biol. 2023;94:21–33.
10. Huang N, et al. Novel insight into perirenal adipose tissue: A neglected adipose depot linking cardiovascular and chronic kidney disease. World J Diabetes. 2020;11:115–25.
11. Zhang K, et al. Role of dysfunctional peri-organ adipose tissue in metabolic disease. Biochimie. 2023;212:12–20.
12. Grigoras A et al. Perirenal adipose Tissue-Current knowledge and future opportunities. J Clin Med. 2021(10) (6):1291.
13. Hao J, et al. Circulating adipose fatty acid binding protein is a new link underlying Obesity-Associated breast/mammary tumor development. Cell Metab. 2018;28:689–705. e5.
14. Tian W, et al. FABP4 promotes invasion and metastasis of colon cancer by regulating fatty acid transport. Cancer Cell Int. 2020;20:512.
15. Nieman KM, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med. 2011;17:1498–503.
16. Shao N, Wang HK, Zhu Y, Ye DW. Modification of American joint committee on cancer prognostic groups for renal cell carcinoma. Cancer Med. 2018;7:5431–8.
17. Ariemma F, et al. Low-Dose Bisphenol-A impairs adipogenesis and generates dysfunctional 3T3-L1 adipocytes. PLoS ONE. 2016;11:e0150762.
18. Shuster A, Patlas M, Pinthus JH, Mourtzakis M. The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. Br J Radiol. 2012;85:1–10.
19. Otunctemur A, Dursun M, Ozer K, Horsanali O, Ozbek E. Renal cell carcinoma and visceral adipose index: a new risk parameter. Int Braz J Urol. 2016;42:955–9.
20. Celik E, et al. Does visceral adiposity have an effect on the survival outcomes of the patients with endometrial cancer? J Obstet Gynaecol Res. 2021;47:560–9.
21. van den Bosch AAS, et al. The impact of adipose tissue distribution on endometrial cancer: a systematic review. Front Oncol. 2023;13:1182479.
22. Zi X, et al. Effect of Perineoplasm perinephric adipose tissues on migration of clear cell renal cell carcinoma cells: a potential role of WNT signaling. Oncotarget. 2016;7:53277–88.
23. Campo-Verde-Arbocco F, et al. Human renal adipose tissue induces the invasion and progression of renal cell carcinoma. Oncotarget. 2017;8:94223–34.
24. Bruna FA, et al. Human renal adipose tissue from normal and tumor kidney: its influence on renal cell carcinoma. Oncotarget. 2019;10:5454–67.
25. Wei G et al. The thermogenic activity of adjacent adipocytes fuels the progression of ccRCC and compromises anti-tumor therapeutic efficacy. *Cell Metab*. 2021;33:2021–2039 e8.
26. Ferrando M, et al. Renal peritumoral adipose tissue undergoes a Browning process and stimulates the expression of epithelial-mesenchymal transition markers in human renal cells. Sci Rep. 2022;12:8687.
27. Prentice KJ, et al. Sympathetic tone dictates the impact of lipolysis on FABP4 secretion. J Lipid Res. 2023;64:100386.
28. Furuhashi M, Saitoh S, Shimamoto K, Miura T. Fatty Acid-Binding protein 4 (FABP4): pathophysiological insights and potent clinical biomarker of metabolic and cardiovascular diseases. Clin Med Insights Cardiol. 2014;8:23–33.
29. Guaita-Esteruelas S, et al. Adipose-Derived fatty Acid-Binding proteins plasma concentrations are increased in breast cancer patients. Oncologist. 2017;22:1309–15.
30. Uehara H, Takahashi T, Oha M, Ogawa H, Izumi K. Exogenous fatty acid binding protein 4 promotes human prostate cancer cell progression. Int J Cancer. 2014;135:2558–68.
31. di Meo NA et al. Renal cell carcinoma as a metabolic disease: an update on main pathways, potential biomarkers, and therapeutic targets. Int J Mol Sci. 2022;23 (22):14360.
32. Liotti A, et al. Oleic acid promotes prostate cancer malignant phenotype via the G protein-coupled receptor FFA1/GPR40. J Cell Physiol. 2018;233:7367–78.
33. Chen D, et al. Desmoglein 2 functions as a receptor for fatty acid binding protein 4 in breast cancer epithelial cells. Mol Cancer Res. 2023;21:836–48.
34. Gagliardi PA, Pertz O. The mitogen-activated protein kinase network, wired to dynamically function at multiple scales. Curr Opin Cell Biol. 2024;88:102368.
35. Fan D, et al. Prognostic significance of PI3K/AKT/ mTOR signaling pathway members in clear cell renal cell carcinoma. PeerJ. 2020;8:e9261.
36. Tataru OS et al. Molecular imaging diagnosis of renal cancer using (99m)Tc-Sestamibi SPECT/CT and Girentuximab PET-CT-Current evidence and future development of novel techniques. Diagnostics (Basel). 2023;13 (4):593.
37. Harraz AM, et al. Evaluation of serum fatty acid binding protein-4 (FABP-4) as a novel biomarker to predict biopsy outcomes in prostate biopsy Naive patients. Int Urol Nephrol. 2020;52:1483–90.
38. Yang Y, et al. Excavation of diagnostic biomarkers and construction of prognostic model for clear cell renal cell carcinoma based on urine proteomics. Front Oncol. 2023;13:1170567.
39. Li G, et al. FABP4 is an independent risk factor for lymph node metastasis and poor prognosis in patients with cervical cancer. Cancer Cell Int. 2021;21:568.
40. Zang WJ, Wang ZN, Hu YL, Huang H, Ma P. Expression of fatty acid-binding protein-4 in Gastrointestinal stromal tumors and its significance for prognosis. J Clin Lab Anal. 2021;35:e24017.


## Publisher's note