Journal of Cellular and Molecular Medicine

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Sex-Dependent Paracrine Effect of Conditioned Media From Adipose Tissue Derived Mesenchymal Stem Cells on Prostate Cancer Cells

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Received: 24 February 2025 | **Revised:** 8 April 2025 | **Accepted:** 17 April 2025

Funding: The authors received no specific funding for this work.

Keywords: gene expression | mesenchymal stem cells | prostate cancer | sex-dependent paracrine

ABSTRACT

Considering the different behaviour of cells in response to diseases in different conditions and sex hormone-dependent cancers, we addressed the possible effect of the sex of the source of these cells from adipose tissue on prostate cancer cells. In this in vitro study, we evaluated the effects of male and female MSC Conditioned Media (MCM, FCM) on prostate cancer cells. The assessment included Hoechst dye staining, a scratch-wound assay, a colony formation assay, and a flow cytometric analysis of apoptosis and the cell cycle. We also performed real-time PCR to examine various genes, including apoptosis-related genes, epithelial-mesenchymal transition (*EMT*) genes, angiogenesis-related genes, and cell growth and survival biomarkers. Our results indicated that the IC_{50} values were 50% and 75% media in MCM and FCM in each of the three prostate cancer cell lines, respectively. An evaluation of gene expression revealed that in all three prostate cancer cell lines, treatment with MCM was more effective than FCM in reducing the expression of *N-Cadherin* and *Vimentin*, *EGFR* and *BCL2* genes ($p < 0.001$). Furthermore, the MCM significantly increased the expression of *BAX* and *E-Cadherin* genes ($p < 0.001$) in the PC3 cell line. MCM proved to be more effective than FCM in reducing the expression of the epithelial-mesenchymal transition pathway, *EGFR* gene, and Apoptosis Regulator (*BCL2*) in the PC3 cell line. Due to its potential in regenerative medicine and cell therapy, this approach may serve as an effective treatment option for advanced prostate cancer.

1 | Introduction

Prostate cancer (PCa) impacts over 90% of men aged 80 and older, posing a significant risk to patient survival. It ranks among the five most common cancers, representing a major public health concern due to its prevalence and associated mortality rates. As the global population ages rapidly, projections indicate that by 2030, more than 1.7 million men will receive a PCa diagnosis, leading to approximately 500,000 new fatalities attributed to this disease. The underlying causes of PCa remain largely unknown, and its biological diversity reflects variations among individuals, tumours, and genetic factors throughout treatment [1]. A range of treatment options exists, including surgery, androgen deprivation therapy, chemotherapy, radiotherapy, and active

Abbreviations: BAX, Bcl-2-associated protein x; BCL2, B-cell leukaemia/lymphoma 2; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; FCM, female conditioned media; IC_{50}, half-maximal inhibitory concentration; MCM, male conditioned media; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; PCa, prostate cancer; *VEGFA*, vascular endothelial growth factor A.

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surveillance through prostate-specific antigen monitoring. However, determining the most effective treatment combination remains challenging, with radical prostatectomy being the most frequently used approach. Factors such as the complexity of the tumour microenvironment, the presence of radiation-resistant stromal cells, elevated levels of inflammatory cytokines and growth factors, and the overexpression of certain receptors contribute to cancer recurrence and the emergence of resistant tumours. Given the limitations of current therapies for preventing disease progression and effectively treating PCa, it is essential to develop targeted, less toxic, and more effective therapeutic strategies, particularly to address the disease's metastatic potential [1, 2].

In androgen-dependent conditions, prostate cancer cells proliferate in response to sex hormones. In advanced stages, prostate tumours metastasize to lymph nodes and bones. Understanding the sex-specific differences in gene expression of human adipose-derived stromal cells (hADSCs) is crucial, as these cells have significant potential in the field of regenerative medicine. hADSCs are multipotent stromal cells that can be readily obtained from adipose tissue during plastic surgery procedures, and they exhibit properties such as anti-inflammatory, immunomodulatory, immunotolerance, and anti-angiogenic effects [3–5].

The existence of differences in the response of MSCs isolated from males and females to prostate cancer could be a turning point in studies in this field. Investigating the difference in the effect of these cells in tumour tissues can be important not only in revealing therapeutic or oncogenic properties but also in producing engineered mesenchymal stromal cells in cell therapy to combat tumours and other diseases. One of the best methods to investigate the effects of mesenchymal stromal cells on cancer cells is to use Conditioned Media containing the secretome (secreted substances) of mesenchymal stromal cells. Exposing prostate cancer cells to MSC Conditioned Media can assess its paracrine effect.

This study aims to identify the differences between MSC Conditioned Media derived from male and female donors, as well as changes in tumour cell growth and proliferation, expression of genes involved in apoptosis, and epithelial-mesenchymal transition between groups and the control group.

2 | Material and Method

2.1 | Cell Lines and Cell Culture

The LNCaP-FGC-10 (ATCC Number "CRL-10995", NCBI Code "C439"), DU145 (ATCC Number "CRL-HTB-81", NCBI Code "C428"), and PC3 (ATCC Number "CRL-1435", NCBI Code "C427") cell lines were obtained from the National Cell Bank of the Pasteur Institute of Iran. The original source (ATCC) has confirmed that there was initial ethical approval for collecting human cells and that the donors had signed informed consent. These cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco 11965092), supplemented with 10% fetal bovine serum (FBS, Gibco16000044), along with 1000 units/mL of Penicillin and 100 μg/mL of Streptomycin (Gibco 15140122). The cultures were incubated at 37°C in a humidified environment containing 5% CO_2.

2.2 | Preparation of Adipose Tissue Sample

All experimental procedures adhered to the ethical standards established by the Tehran University of Medical Sciences in Iran (Title of the approved project: A Comparative Study on Gender-Dependent Paracrine Impacts of Adipose Derived Mesenchymal Stem Cell Condition Media on Prostate Cancer) (IR. TUMS. SINAHOSPITAL. REC. 1401. 112), approved on 2023/02/05. The written consent form is taken from patients. Human samples were collected as discarded materials from patients undergoing cosmetic abdominal liposuction. Following collection, oil droplets were eliminated, and the samples were thoroughly rinsed with phosphate-buffered saline (PBS). Subsequently, the oil-free tissue slices were utilised to isolate cells.

2.3 | Isolation of MSC With Non-Enzymatic Method

Four small samples, each approximately 5 mg, were thoroughly rinsed with fetal bovine serum (FBS). After washing, the tissue fragments were placed in the corners of two distinct tissue culture flasks. Subsequently, each specimen's surface was covered with 50 μL of pre-warmed FBS, ensuring the specimens did not float. The explants were then incubated for 24 h under standard conditions of 37°C and 5% CO_2. Upon completion of the incubation, the FBS was substituted with DMEM supplemented with antibiotics and 20% FBS, and the cultures were monitored daily under a microscope until fibroblast-like cells emerged around the tissue fragments, typically within 3–5 days post-seeding. At this stage, 5 mL of DMEM containing antibiotics and 20% FBS was added to the flasks, and the cells were incubated under standard conditions until they reached confluence. The cells from subconfluent cultures were subsequently harvested and expanded through three passages.

2.4 | Preparation of Condition Media (CM) From Adipose Mesenchymal Stromal Cells

Male and female adipose-derived mesenchymal stromal cells (MCM and FCM) from non-smokers aged 40–43 years were cultured in two separate flasks. The cells were incubated at 37°C with 5% CO_2 until they reached a confluency of 70%–80%. For this purpose, DMEM medium with 10% FBS serum is used, then penicillin/streptomycin is added to prevent contamination of the cell culture medium. When the desired level is reached, the medium on the cells is collected and replaced with a serum-free medium. This medium is left on the mesenchymal stromal cells for 48 h, after which it is collected and centrifuged at 1000 *g* for 10 min to remove dead cells and debris. The supernatants are then stored in a refrigerator at 4°C in designated falcons.

2.5 | Cell Survival

The Microculture Tetrazolium Test (MTT, [3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) assay was utilised to evaluate the half-maximum inhibitory concentration (IC_{50}) of dimethyl fumarate and bicalutamide in both time-dependent and dose-dependent contexts. Initially, 5000 cells were seeded in each well of 96-well plates for each cell line, followed by treatment with varying drug concentrations over 24, 48, and 72 h. Subsequently, the optical densities of the plates were measured at a wavelength of 545 nm using an ELISA microplate reader. Dose–response curves were generated, and IC_{50} values were calculated using GraphPad Prism software version 9.0.2. The percentage of cell survival was determined using the formula: Cell survival (%) = (Average absorbance of treated wells/Average absorbance of control wells) × 100.

2.6 | Assessment of Cellular Morphology Through Crystal Violet Staining

In six-well plates, 5×10^4 cells of LNCaP, DU145, and PC3 were exposed to the identified IC_{50} concentrations of dimethyl fumarate and bicalutamide. After two washes with PBS and fixation with 4% paraformaldehyde in PBS, the cells were stained using a 0.5% *w/v* crystal violet solution and examined under a reverse microscope.

2.7 | Cell Clonogenicity

The invasiveness of cell lines under both untreated and treated conditions was assessed using a colony formation assay. In six-well plates, 2000 cells were seeded in each well and exposed to either the IC_{50} of MCM or FCM for a specified duration. Following this treatment, the plates were incubated at 37°C for 2 weeks to promote the development of visible colonies. The colonies were then analysed using crystal violet staining (0.5%) and observed under a reverse microscope. A colony was defined as a cluster of 50 cells, and the quantification of colonies was conducted using ImageJ software.

2.8 | Cell Migration

To assess the migratory capacity of the cells, they were grown in six-well plates until they reached approximately 85% confluency. A vertical scratch was then introduced in each well using pipette tips, and the detached cells were carefully rinsed twice with serum-free medium. After a 24-h period of serum deprivation, the experimental cells received treatment with the specified reagents, while the control groups received PBS. The cells' migration rates were evaluated after 48 h by measuring the area between the two edges of the scratch using ImageJ software.

2.9 | Hoechst Dye (33342) Staining

The Hoechst dye assay was utilised to assess apoptosis in various cancer cell lines. Prostate cancer cell lines were cultured in a 96-well plate format, with a seeding density of 5000 cells per well. Following this, the cells underwent treatment with MCM and FCM. After a 48 h of incubation, the cells were fixed using a 4% paraformaldehyde solution in PBS and washed twice with PBS. Next, 2 μL of Hoechst dye was added to the cell pellet and incubated at 25°C in a dark environment. The samples were then examined and photographed under a fluorescent microscope at a magnification of 400×. This method enabled the visualisation of nuclear condensation and the evaluation of fragmented nuclei within the viable cells. The presence of fragmented nuclei in certain cells serves as an indicator of apoptosis.

2.10 | Flow Cytometric Measurement of Apoptosis

Flow cytometric analysis to assess apoptosis was performed using the Annexin-V and propidium iodide (PI) kit (Bio Legend; CAT Number: 640914), adhering to the manufacturer's instructions to determine cell viability, apoptosis, and necrosis. After incubating cell lines overnight in DMEM with 10% FBS at 37°C, the cells were treated with specific concentrations of FCM and MCM for 48 h.

Post-treatment, the cells were incubated in the dark at 37°C for 20 min after adding Annexin-V and PI. Subsequently, the cells were analysed with a flow cytometry instrument, producing scatter plots that were categorised into four distinct regions corresponding to various cell populations. The viable cell population, characterised by being annexin-V negative and PI negative, was identified as Q4 in the lower left quadrant. The early apoptotic cells, which were positive for annexin-V and negative for PI, were classified in quadrant Q3 in the lower right. Late apoptotic cells, marked as Q2, were located in the upper right quadrant (annexin-V positive and PI positive). In contrast, necrotic cells, designated as Q1, were found in the upper left quadrant (annexin-V negative and PI positive). The percentage of apoptosis was determined by calculating the ratio of annexin V+/PI− cells using a flow cytometer. The resulting data were analysed with Flow Jo software (Tree Star Inc., version 9.6.3, USA).

2.11 | DNA Cell Cycle Analysis

Cells underwent treatment as outlined, while untreated samples were fixed by soaking in 70% cold ethanol for 24 h. Following the fixation process, the cells were rinsed twice with PBS and then treated with RNase I and 500 μL of propidium iodide (PI) for 30 min at 37°C. The cells were analysed using a flow cytometer, and the resulting data were processed with Flow Jo software (Tree Star Inc., version 9.6.3, USA). The presence of cell arrest in the sub-G0/G1 phase served as an indicator of apoptosis.

2.12 | Gene Expression Analysis by Real-Time PCR

The Tri-Pure Isolation Reagent was used to extract total RNA. The concentration of RNA was determined with the Colibri Micro Volume Spectrometer. The Easy-TM cDNA Synthesis Kit from Pars Toos Co. in Iran was utilised for cDNA synthesis. Real-time polymerase chain reaction (PCR) was conducted

TABLE 1 | Primer sequences of target and normaliser genes.

Gene	Forward primer (5′-3′)	Reverse primer (3′-5′)	Product size (bp)
B_2M	TGTCTTTCAGCAAGGACTGGT	TGCTTACATGTCTCGATCCCAC	143
BCL2	CCCCGCGACTCCTGATTCAT	CAGTCTACTTCCTCTGTGATGTTGT	168
BAX	CGGGTTGTCGCCCTTTTCTAC	AGTCCAATGTCCAGCCCATGA	103
E-Cadherin	TCGTAACGACGTTGCACCAA	TTCGGAACCGCTTCCTTCAT	175
N-Cadherin	GCCATCAAGCCTGTGGGAAT	GGAGCCACTGCCTTCATAGT	198
Vimentin	TGGACCAGCTAACCAACGAC	AAGGTCAAGACGTGCCAGAG	179
VEGFA	AGGAGGAGGGCAGAATCATCAC	GGATGGCTTGAAGATGTACTCG	136
EGFR	CTTGCCGCAAAGTGTGTAAC	GAAGGAGTCACCCCTAAATGC	149
SRY	GCGAAGTGCAACTGGACAAC	CTGCGTTGATGGGCGGTAAG	102

using a QIAGEN thermocycler with a sample volume of 20 μL. The specificity of the PCR reactions was verified through melting curve analysis. To evaluate fold changes in expression levels, the $2^{-\Delta\Delta CT}$ method was applied, with B_2M mRNA levels acting as the internal control. SRY gene expression was used to confirm gender. The nucleotide sequences of the primers are detailed in Table 1.

2.13 | Statistical Analysis

The experiments were carried out in triplicate, and the findings are expressed as means ± standard deviation (SD). Variance analysis (ANOVA) and the student's *t*-test were utilised for statistical evaluation. Statistical significance was established with the following thresholds: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, compared to the control group.

3 | Result

3.1 | Findings of Cell Survival Tests

The MTT assay was utilised to investigate the cytotoxic effects of MCM and FCM on three prostate cancer cell lines: LNCaP, DU145, and PC3. This examination was conducted at 24, 48, and 72 h after exposure to the treatments. The introduction of varying concentrations of conditioned media, ranging from 0% to 100%, revealed that the half maximal inhibitory concentrations (IC_{50}s) for the media from both genders were 50% and 75% for the LNCaP, DU145, and PC3 cell lines at the 48-h mark. Furthermore, as depicted in Figure 1, the anti-proliferative effects of the treatments exhibited a clear dose- and time-dependent relationship throughout the study (Figure 1).

3.2 | Findings of Cell Morphology Evaluations

Utilising an inverted microscope and applying MCM and FCM to prostate cancer cells demonstrated that the conditioned media induced notable alterations in cell morphology, resulting in characteristics such as rounding, shrinkage, and wrinkling of the cells. The extent of these morphological changes is significant, as illustrated in Figure 2. Morphologically, it was observed that MCM had a more pronounced impact on the morphological changes in prostate cancer cell lines compared to FCM. After the cells were fixed, stained, and washed, significant alterations were observed in the PC3 cell line treated with MCM. These changes included cell loss, shrinkage, fragmentation, the disappearance of cell appendages, and rounding. As a result, there was a notable reduction in cell count. In contrast, MCM treatment of the DU145 cell line resulted in a decrease in cell numbers, accompanied by elongation and extension of cell appendages, which appeared to be a mechanism for resisting treatment and enhancing survival. This elongation and the reduction in cell count were less pronounced in the DU145 line treated with FCM.

Regarding the LNCaP cell line, MCM treatment led to smaller cell sizes, increased debris within the culture medium, and decreased cell numbers. In contrast, the morphological characteristics of the LNCaP cells remained largely unchanged, except for the reduction in cell count observed with FCM.

3.3 | Findings of Cell Apoptosis Changes by Flow Cytometry

A flow cytometric assay was performed to assess the effects of the IC_{50} values of MCM and FCM on LNCaP, DU145, and PC3 prostate cancer cell lines. Figure 3 illustrates the results of the apoptosis evaluations after a 48-h treatment period. The introduction of conditioned media from MCM significantly enhanced apoptosis in the prostate cancer cells. Notably, treatment with MCM media was the most effective among all three cell lines compared to the FCM in each group.

3.4 | Findings of Cell Cycle Changes by Flow Cytometry

Cell cycle flow cytometry analysis was performed to evaluate the impact of the MCM and FCMs' IC_{50} values on the cell cycle arrests in LNCaP, DU145, and PC3 cell lines. The results indicated that all three cell lines exhibited cell cycle arrest in the G1/S phase (Figure 4).

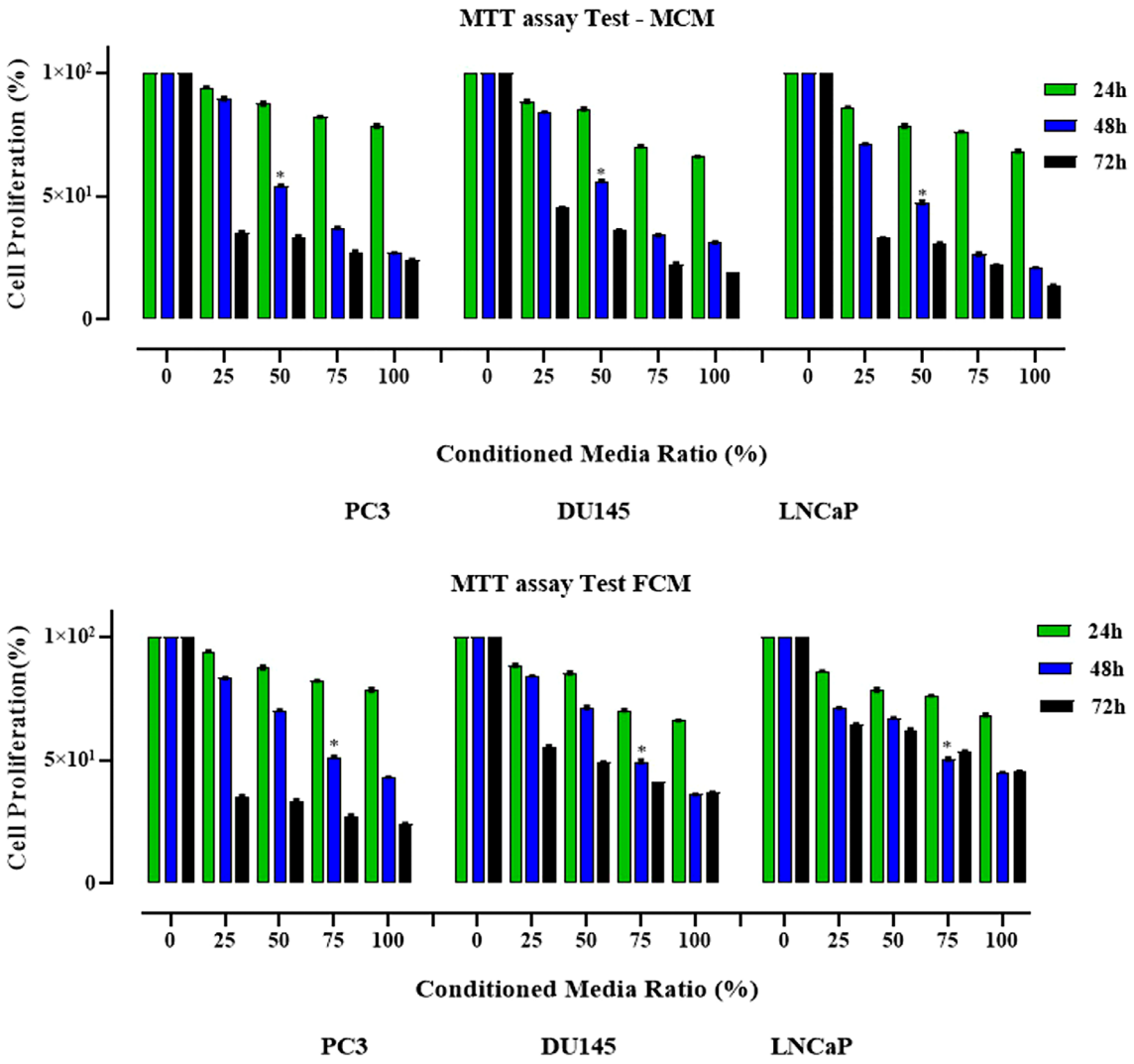

FIGURE 1 | The viability of LNCaP, DU145, and PC3 prostate cancer cells at 24-, 48-, and 72-h post-treatment with MCM and FCM (ranging from 0% to 100% of culture media). The treatment resulted in a substantial inhibition of cell growth, with the IC_{50} values marked by stars on the graph.

3.5 | Findings of Cell Nucleus Examinations

Figure 5 illustrates the outcomes of Hoechst dye staining across the nine groups. Using a fluorescent microscope, we observed changes such as fragmented and disintegrated nuclei in the MCM and FCM treatment groups. The presence of dispersed nuclei is indicative of apoptotic cell death.

3.6 | Findings of Cell Migration Ability Tests

Migration assays demonstrated that the application of MCM and FCM's IC_{50}s to three prostate cancer cell lines led to a notable reduction in cell migration.

In contrast, untreated cells were significantly able to occupy the available space after a 48-h period. Figure 6 shows the results of the experiment in LNCaP, DU145, and PC3 cell lines, respectively. When comparing the various cell lines and treatment groups, it was observed that the MCM demonstrated greater efficacy than its FCM counterpart, particularly showing enhanced effectiveness on the PC3 cell line compared to the other two cell lines.

3.7 | Findings of Cell Clonogenicity Tests

Figure 7 illustrates the clonogenic potential of prostate cancer cells in both treated and untreated groups. After exposure to IC_{50s} MCM and FCM, a significant reduction in the cells' proliferative capacity was observed, leading to a decrease in colony formation.

The lowest colony count was observed in the MCM-treated group, specifically in the PC3, DU145, and LNCaP cell lines. Colony numbers were analysed using ImageJ Software; the results are shown in Figure 7.

3.8 | The Impact of MCM and FCM on Gene Expression in LNCaP, DU145, and PC3 Prostate Cancer Cell Lines

After 48 h of treatment with IC_{50}s of MCM and FCM, the cells were analysed for the expression levels of apoptosis-related genes (*BAX* and *BCL2*), components of the epithelial-mesenchymal transition (*EMT*) pathway (*Vimentin*, *E-Cadherin*, and *N-Cadherin*), markers associated with

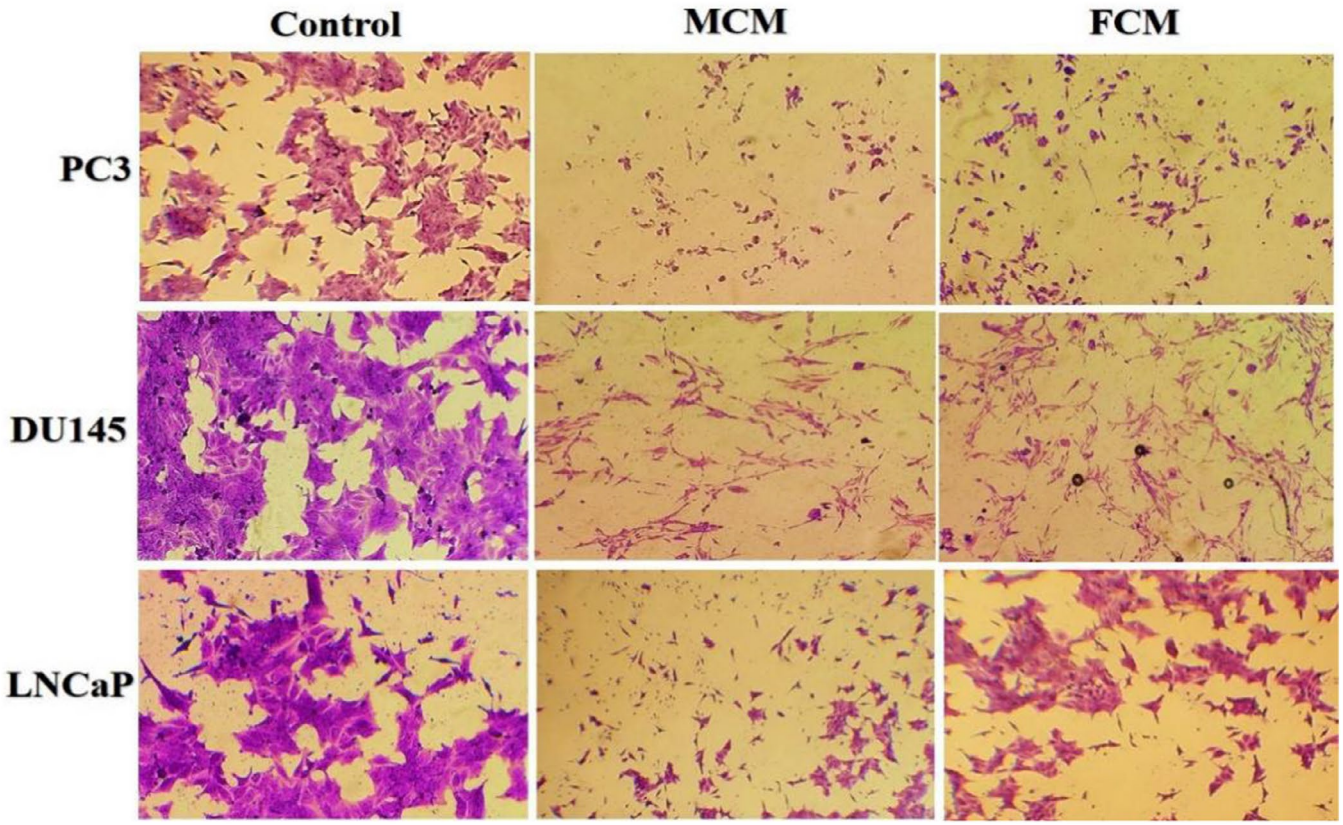

FIGURE 2 | The morphology of LNCaP, DU145, and PC3 cell lines was examined under various conditions, including control and exposure to MCM and FCM at IC_{50} concentrations. The treatments with conditioned media led to notable alterations in cell morphology. Significant changes, such as the loss of cellular appendages, were observed in the treated groups, highlighting the impact of the media on cellular structure.

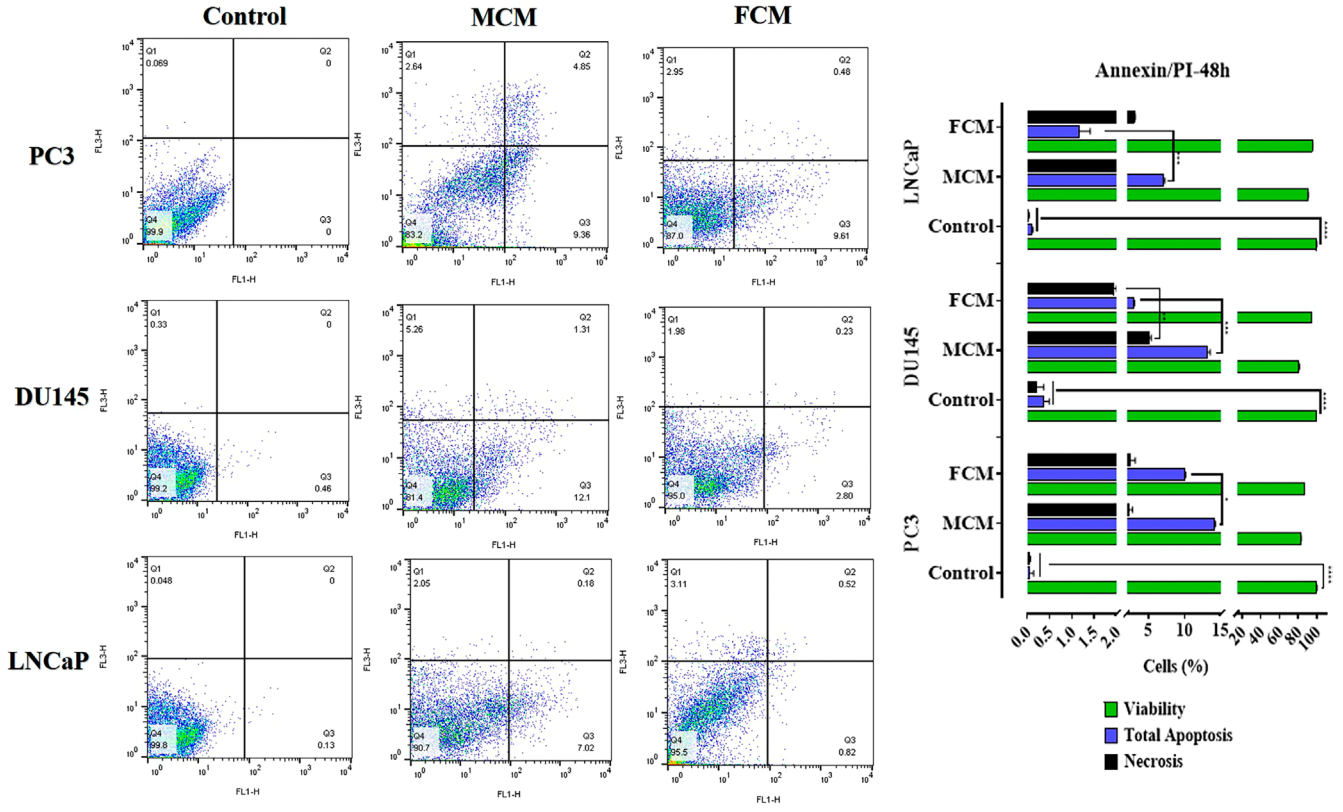

FIGURE 3 | Flow cytometric assessment of necrosis, apoptosis, and viability in LNCaP, DU145, and PC3 cells utilising Annexin-V-Fluos.

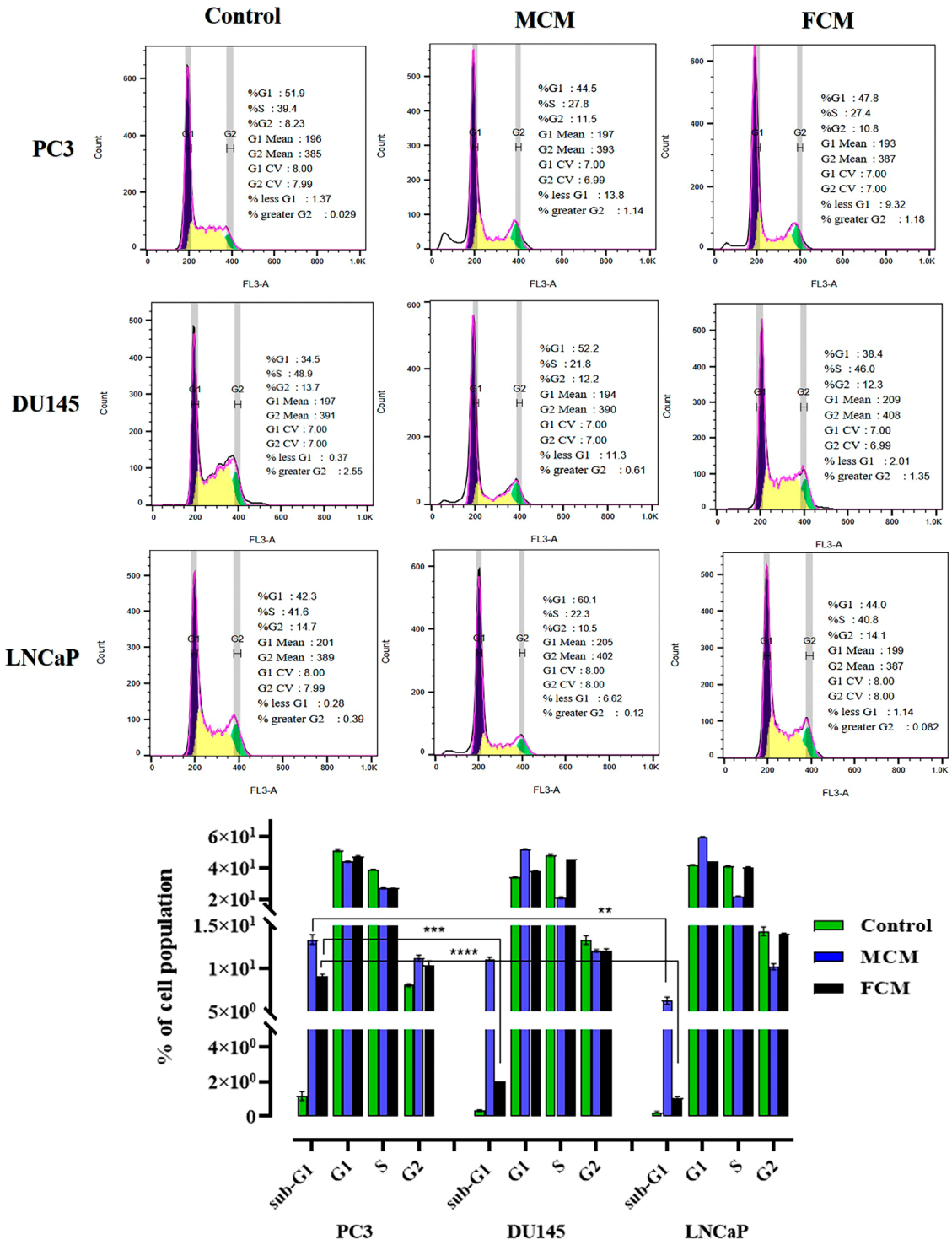

FIGURE 4 | Cell cycle analysis of LNCaP, DU145, and PC3. Cells accumulation in sub-G1 phase indicates the increased rate of apoptosis.

angiogenesis (*VEGFA*) and cell growth and cell survival biomarker (*EGFR*) using reverse transcription polymerase chain reaction (RT-PCR).

As illustrated in Figure 8, in the PC3 cell line, treatment with MCM was more effective than FCM in reducing the expression of genes associated with the epithelial-mesenchymal transition (*EMT*: *N-Cadherin* and *Vimentin*) pathway, *EGFR* gene, and Apoptosis Regulator (*BCL2*) ($p < 0.001$). Furthermore, the MCM significantly increased the expression of *BAX* and *E-Cadherin* genes ($p < 0.001$).

In the DU145 cell line, the MCM resulted in a significant reduction in the expression of *BCL2*, *N-Cadherin*, *Vimentin*, and *EGFR* genes ($p < 0.01$), which suggests an inhibition of the epithelial-mesenchymal transition (*EMT*) pathway.

In LNCaP cell line demonstrated a significant decrease in the expression of *BCL2* ($p < 0.05$), *N-Cadherin*, *Vimentin*, and *EGFR* ($p < 0.01$) after treatment with MCM and FCM. Additionally, there was no significant increase in the expression levels of *BAX* and *E-Cadherin* following MCM and FCM treatments.

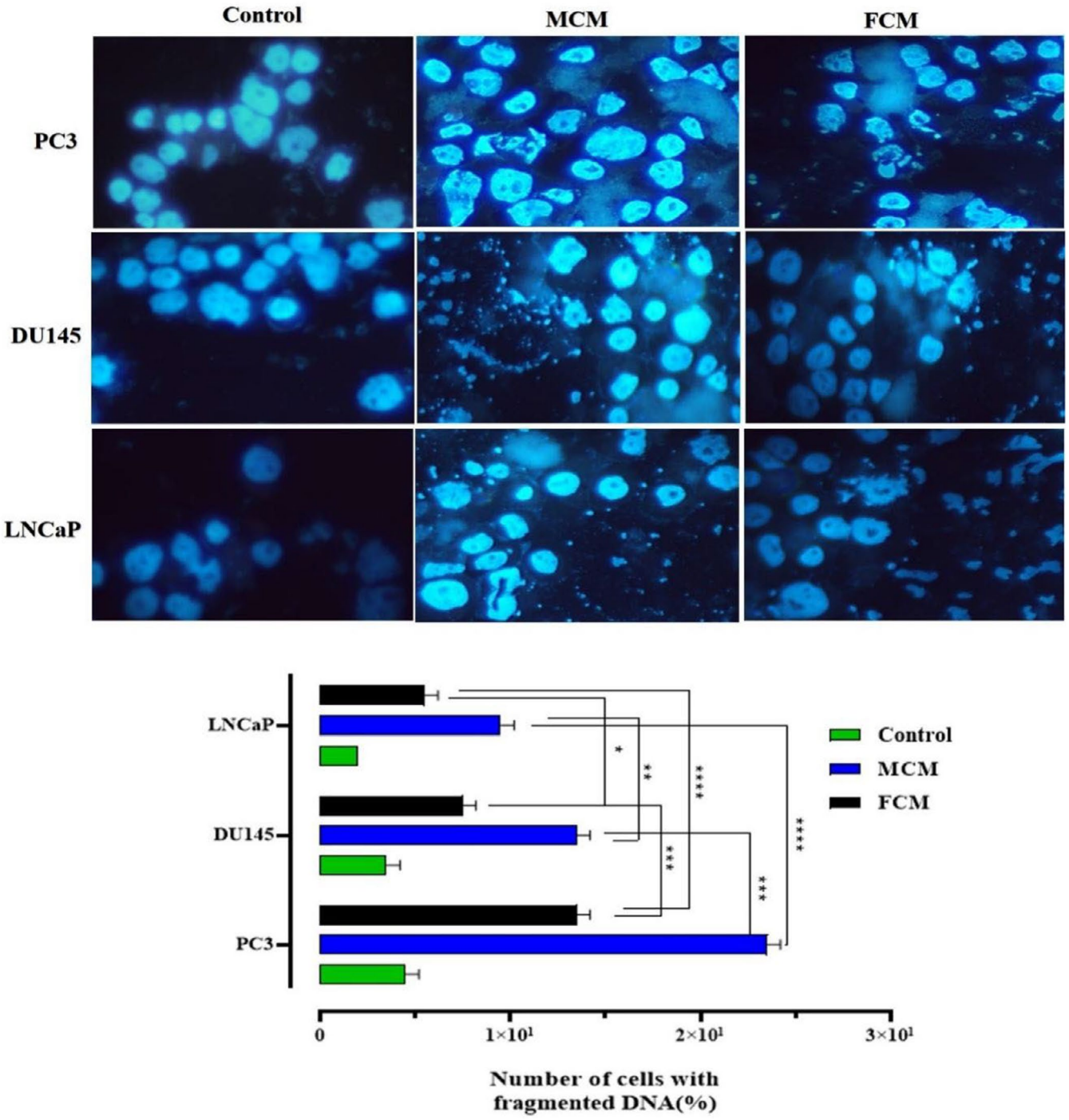

FIGURE 5 | Hoechst dye staining (33342) of LNCaP, DU145, and PC3 cells after 48 h.

Additionally, In the three cell lines, treatment with MCM and FCM resulted in a significant increase in the ratio of *BAX* to *BCL2* and *E-Cadherin* to *N-Cadherin*. This increase was particularly pronounced in the PC3 prostate cancer line compared to the other two lines ($p < 0.001$).

4 | Discussion

In the last two decades, there have been remarkable developments in the treatment and management of prostate cancer. Approximately 5%–10% of men with prostate cancer are diagnosed with metastatic disease [6]. Nevertheless, the challenge of controlling its progression remains a concern for researchers and healthcare practitioners [7]. Even with hormonal therapies, advanced stages of prostate cancer often develop resistance to treatment, making them incurable. Therefore, it is crucial to continue exploring new strategies and therapies to effectively address the challenges of resistance and progression associated with prostate cancer [8, 9].

The results of our study demonstrate that treatment with MCM and FCM mesenchymal stromal cells led to an increase in fragmented nuclei, a decrease in cell migration, and a reduction in

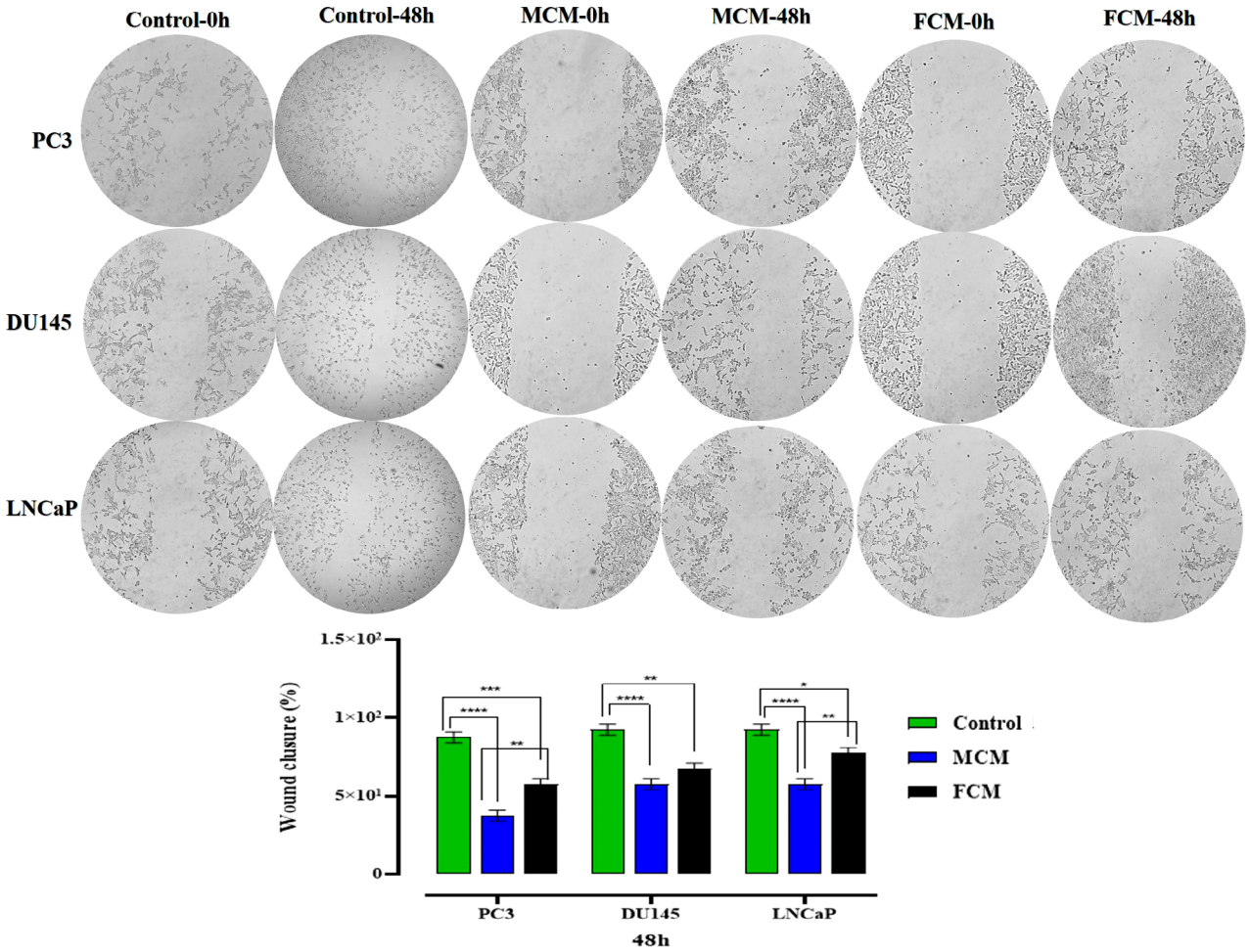

FIGURE 6 | Migration assay in three prostate cancer cell lines (LNCaP, DU145 and PC3). Comparing to the corresponding control groups, MCM and FCM are able to weaken prostate cancer cells' migration ability following 48 h.

colony formation. Analysis of gene expression in three prostate cancer cell lines presented that MCM was more effective than FCM in downregulating genes related to the epithelial-mesenchymal transition (*EMT*), specifically *N-Cadherin* and *Vimentin*, as well as the epidermal growth factor receptor gene (*EGFR*) and the apoptosis regulator *BCL2*, with statistical significance ($p < 0.001$). Additionally, in the PC3 cell line, MCM notably enhanced the expression of *BAX* and *E-Cadherin* genes, with significant results ($p < 0.001$).

In this regard, Mirfendereski et al. (2025) showed that adipose-derived MSCs (adMSCs) reduced the viability of gastric adenocarcinoma cells in co-culture, suggesting cytotoxic effects of the adMSC secretome. This secretome downregulated *Jak2*, *STAT3*, *PI3k*, and *mTOR* gene expression in both co-cultured cell lines, though the effects varied between the A431 and AGS cells. This highlights the importance of these pathways in the growth and proliferation of each cancer cell line [10].

In 2020, Nastały P et al. confirmed that Epithelial-mesenchymal transition (*EMT*) and cellular plasticity play crucial roles in the metastatic advancement of prostate cancer. Furthermore, the epidermal growth factor receptor (*EGFR*) has been implicated in prostate tumour development and progression processes. Research indicates that *EGFR* expression correlates with higher tumour grades, more advanced stages, and an increased risk of bone metastases. Moreover, *EGFR* has been shown to influence bone development. As a key regulator of *EMT*, differentiation, proliferation, and angiogenesis, *EGFR* may facilitate tumour spread and metastasis, positioning it as a potential surrogate marker for elevated metastatic capability [11, 12].

In 2020, Bianconi and colleagues conducted a comprehensive meta-analysis of human mesenchymal stem cell (hMSC) microarrays utilising the Transcriptome Mapper (TRAM) software. This bioinformatics tool facilitated the integration and normalisation of datasets from various sources, enabling the researchers to identify chromosomal regions and genes that exhibited differential expression in hMSCs derived from adipose tissue (hADSCs) of both male and female donors. The analysis revealed that the chromosomal regions and differentially expressed genes in male and female hADSCs were associated with several biological processes, including inflammation, adipogenic and neurogenic differentiation, as well as cell communication. These findings prompted the researchers to propose that the sex of the donor from whom hADSCs are derived may significantly influence a broad spectrum of biological processes in stem cells [5].

Among stromal cell studies, mesenchymal stromal cells are among the most widely used cells due to the availability and

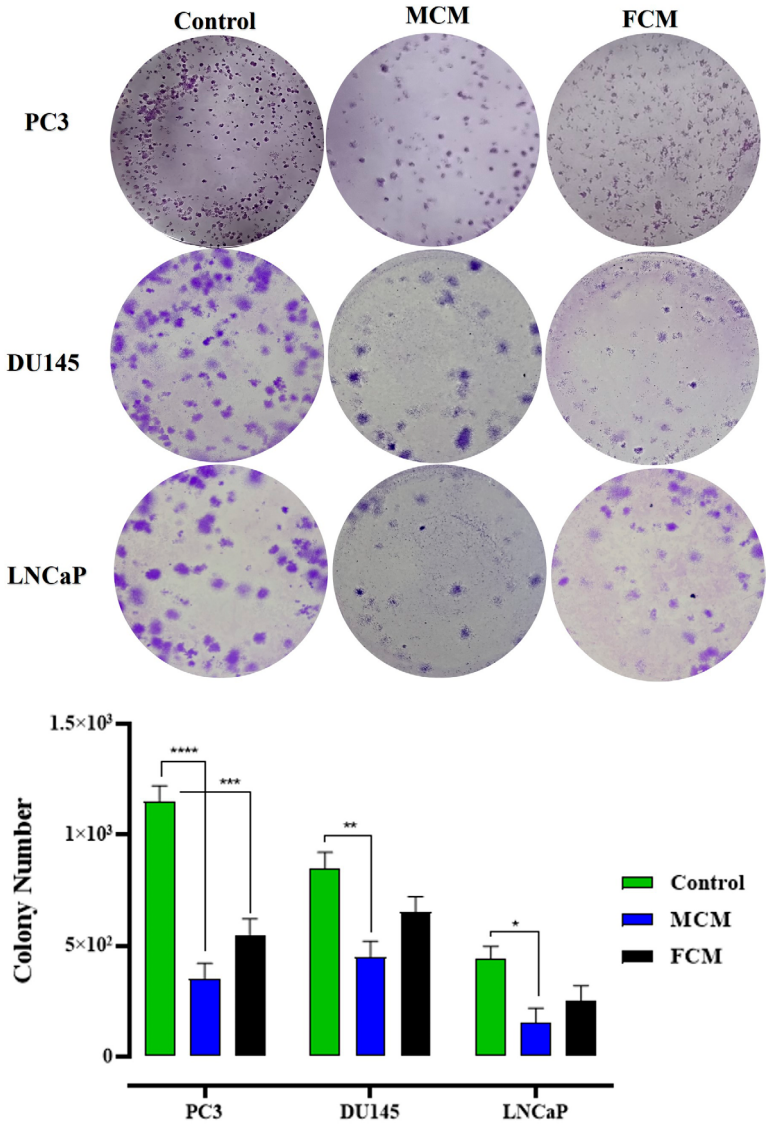

FIGURE 7 | Prostate cancer clonogenicity test.

diversity of primary tissue for isolation. This has led to increased attention towards their use for clinical studies. Compared to embryonic stromal cells, these cells have fewer ethical problems when isolated from primary tissue, and their worrying tumorigenic properties have also been reduced. For this reason, mesenchymal stromal cells are commonly used in cancer research and other disease studies. In a new perspective, mesenchymal stromal cells have been introduced as a natural vector candidate due to their inherent ability to migrate to damaged or tumour areas [13].

In studies, the relationship and effect of mesenchymal stromal cells with cancer cells seems controversial. In addition to their ability to differentiate into adipose, bone, and cartilage cells, the approach to using them has also expanded due to paracrine properties such as modulation of immune system responses [14], anti-inflammatory, and regenerative capabilities [15].

A group of studies shows inductive effects on tumour growth and angiogenesis and causes it to spread further. In contrast, in line with the results obtained in this research, other studies indicate suppressor properties of these cells on cancer cells. In these studies, mesenchymal stromal cells can inhibit tumour growth and proliferation [16] or directly cause tumour cell death by secreting substances [17].

These controversial effects have also been reported in prostate cancer. While many articles have reported on the oncogenic effects of mesenchymal stromal cells on prostate cancer [18], others reveal their tumoricidal effects [19, 20].

In studies examining the effects of mesenchymal stromal cells, the primary tissue of the mesenchymal cells and the donor's age are of greater interest. However, another factor that can differentiate mesenchymal stromal cells is the donor's gender. Many studies have examined the behavioural differences between these cells in the two sexes. These differences include changes in the characteristics of immune system modulation reactions as well as anti-inflammatory and angiogenic properties [21].

These characteristics can be influential in the cancer discussion, on which the effect of mesenchymal stromal cells is in a halo of ambiguity. Despite many studies that explore the

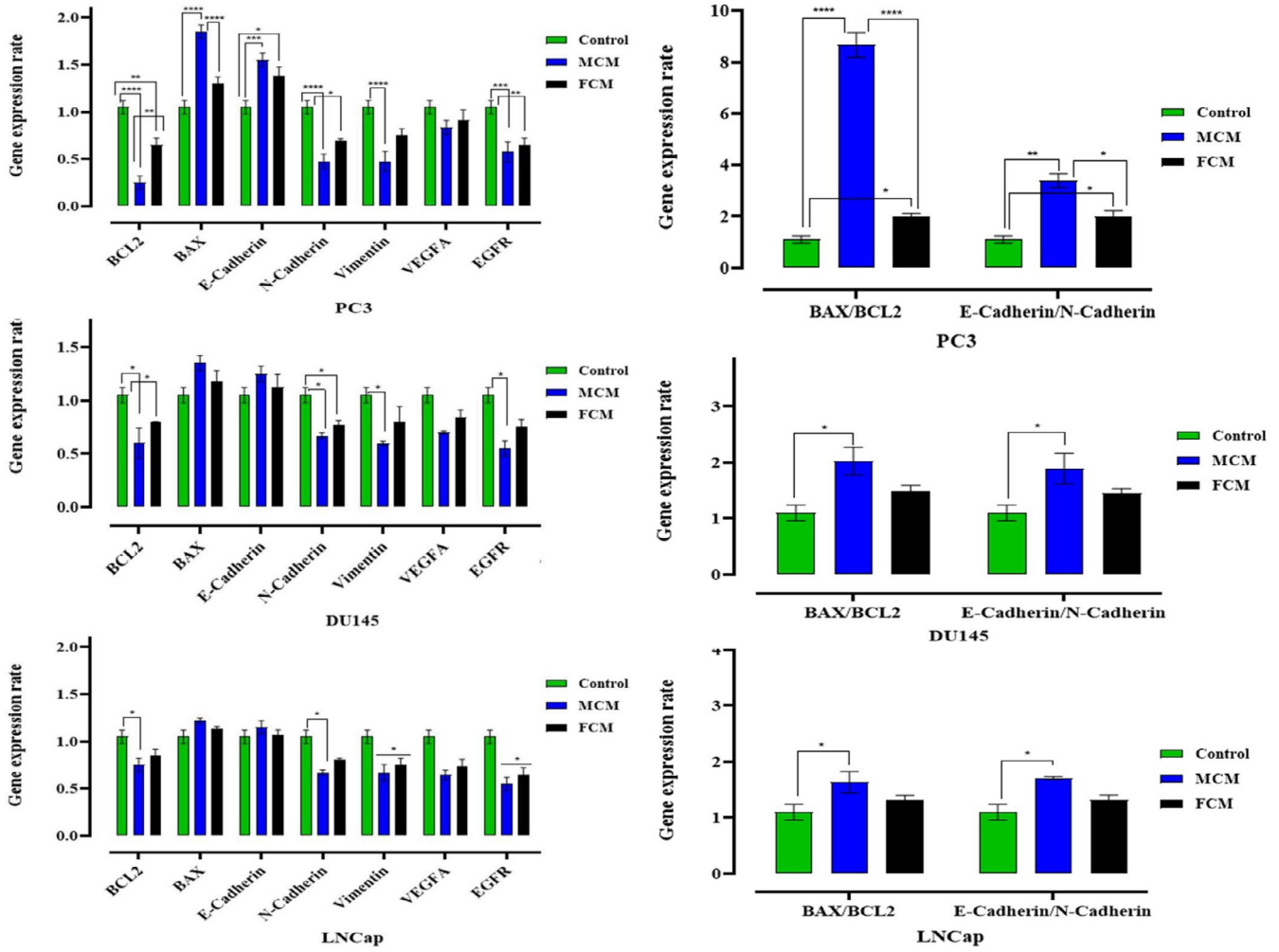

FIGURE 8 | Gene expression levels by real-time PCR. Gene expression levels were assessed using Real-time PCR to evaluate the fold change in prostate cancer cell lines (LNCaP, DU145, and PC3) following treatment with MCM and FCM over a duration of 48 h. The results indicated statistical significance at $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ when compared with the control group.

relationship between cancer cells and mesenchymal stromal cells, the effect of the gender of the source of these cells on cancer remains unknown. This is especially significant for cancers that are sexually transmitted and that are influenced by sex hormones. Although initial studies in this area date back to 15 years ago [22–24], more attention seems to have been paid to them in recent years. Many studies have investigated and reported gender-related behavioural differences in MSCs in different areas [22, 25]. For example, research has shown that female MSCs exhibit more immunomodulatory properties than male MSCs [26].

Aksu et al. (2008) described that the human adipose-derived stromal cells (hADSCs) isolated from males were more osteogenic than those from females [27]. In addition, a 2021 study on the use of mesenchymal stromal cells in bone repair highlighted the need to consider the gender of the source of these cells due to their different behaviours in this regard [28, 29].

Furthermore, in MSCs, these cells have been observed to have different transcriptomic profiles between male and female groups [20] and, for example, to show directional patterns in the expression of germ cell-specific genes [23, 30].

Crisostomo et al. (2007) confirmed that the expression of growth factors also differs in mesenchymal stromal cells between males and females [31].

In alignment with the findings of this study, which indicated that MCM had a more pronounced impact on prostate cancer cell lines [32–34], interesting research by Xiaojing Cui et al. (2025) revealed that male C57BL/6 mice had a greater presence of leptin-receptor-expressing mesenchymal stromal cells compared to their female counterparts. When cocultured with mismatched sexes, the male bone marrow niche demonstrated a superior capacity to support both in vitro colony formation and in vivo haematopoietic engraftment. Furthermore, the co-transplantation of male stromal cells significantly improved engraftment outcomes in female recipients. In a mouse model with MSC-specific Kdm5c knockout, a decrease in KDM5C levels in female MSCs led to an increase in both the quantity and functionality of MSCs, ultimately enhancing engraftment to levels comparable to those observed in males [35].

Moreover, Shaker and colleagues (2024) proposed that administering human adipose-derived stem cell-conditioned media through the intranasal route enhances cognitive function in a

male Wistar rats' model of Alzheimer's disease. Additionally, there was a reduction in amyloid plaques and dark cells within the CA1 region of the hippocampus. Furthermore, an increase in the expression level of *ERβ* was observed [36].

Also, Hui-Lan Tan and colleagues (2021) demonstrated that conditioned media from human adipose-derived stem cells (hAMSCs-CM) effectively mitigated obesity induced by a high-fat diet in mice. This effect was achieved through the suppression of adipogenesis and lipogenesis, an increase in energy expenditure, and a decrease in inflammation. The mechanisms behind the anti-obesity effects of hAMSCs-CM may involve the inhibition of lipid synthesis and adipogenesis mediated by *PPARγ* and *C/EBPα*, the enhancement of glucose metabolism through *GLUT4*, the elevation of energy expenditure regulated by *UCP1/PPARα/PGC1α*, and the promotion of M2-type macrophage polarisation via *STAT3-ARG1* signalling [37].

Our findings indicated that MCM exhibited notably greater efficacy than FCM in suppressing the expression of genes associated with *EMT*, *EGFR*, and *BCL2* ($p < 0.001$). Furthermore, MCM led to a significant upregulation of *BAX* and *E-Cadherin* genes ($p < 0.001$).

Other studies, such as Bianconi et al. (2020), have shown differences in the molecular profile of adipose-derived mesenchymal stromal cells in males and females [5]. In an experiment investigating the therapeutic properties of MSCs in improving symptoms associated with abdominal aortic aneurysms, MSCs isolated from female mice were observed to be more effective in reducing symptoms [38].

In another study, MSCs isolated from female mice exhibited enhanced anti-inflammatory and angiogenic properties in a mouse model of lung injury [3].

These studies are not limited to mice; this difference has also been observed in a study of porcine-derived mesenchymal stromal cells [39].

In cardiac injury models, MSCs isolated from female mice have also been shown to produce more growth factors during stress [40].

It seems that sex-dependent behavioural differences of mesenchymal stromal cells in sex-specific cancers, such as prostate and breast cancer, could influence cancer behaviour. This issue has not been directly investigated in any paper and is considered an innovation in research. Variations in the development, functionality, and responsiveness of adipose stromal cells regarding growth, metastasis, migration, and colonisation could impact disease outcomes. These differences may also provide insights into individual susceptibility to various diseases.

On the one hand, we have shown for the first time that MCM significantly reduces the survival, colonisation, migration, and expression of genes associated with the EMT pathway of prostate cancer cells. On the other hand, MCM increases apoptosis, especially in late-stage prostate cancer cells (PC3), more effectively than FCM.

4.1 | Limitations and Future Prospects

This study focused on in vitro outcomes. However, to obtain more reliable data, it is essential to investigate these findings in vivo; animal studies in this field can also examine gonadal and chromosomal sex models to generalise the results to clinical use. Given the small number of samples in this study, it is suggested that a wider study be conducted on more samples with age, pathological, racial, and demographic differences. Considering the heterogeneity of the conditioned media (exosomes, protein factors, miRNA, etc.), it should be investigated which factors are effective in reducing and progressing cancer. Also, RNA Sequencing can be used to identify differential genes between male and female MSCs.

4.2 | Conclusions

MCM significantly reduced the viability of prostate cancer cells, especially in the PC3, more effectively than FCM and also effectively inhibited their migration and overall progression in various cell lines, including PC3, DU145, and LNCaP, respectively. Our findings suggest that the use of MCM could be a promising therapeutic strategy for managing late-stage prostate cancer (PC3 cell line) due to its potential in regenerative medicine. Given the potential of MCM in regenerative medicine and cell therapy, further comprehensive studies are warranted to confirm and strengthen our results.

Author Contributions

Akram Mirzaei: formal analysis (lead), methodology (lead), resources (lead), writing – original draft (lead), writing – review and editing (lead). **Rahil Mashhadi:** validation (equal). **Ziba Aghsaeifard:** validation (equal). **Mehrnaz Izadi:** validation (equal). **Seyedeh Negin Hashemi Dougaheh:** methodology (equal), validation (equal). **Reza Omid:** validation (equal). **Fateme Guitynavard:** validation (equal). **Parsa Nikoofar:** validation (equal). **Seyed Mohammad Kazem Aghamir:** conceptualization (lead).

Acknowledgements

The authors would like to thank the statistics consultants of the Research Development Center of Sina Hospital for their technical assistance. The authors declare that they have not used AI-generated work in this manuscript.

Ethics Statement

All experimental procedures adhered to the ethical standards established by Tehran University of Medical Sciences in Iran (Title of the approved project: A Comparative Study on Gender-Dependent Paracrine Impacts of Adipose Derived Mesenchymal Stem Cell Condition Media on Prostate Cancer), (IR. TUMS. SINAHOSPITAL. REC. 1401. 112), approved on 2023/02/05.

Consent

Written consent form is taken from patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be provided on request. The data of this manuscript is all provided in the text.

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