Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2021;46(4):1632-1639
DOI: 10.17826/cumj.982873

ARAŞTIRMA / RESEARCH

IRF5 inhibits prostate cancer metastasis and drug resistance by decreasing CXCR4/CXCL12 complex

IRF5 CXCR4/CXCL12 kompleksini azaltarak prostat kanseri metastazını ve ilaç direncini inhibe eder

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Cukurova Medical Journal 2021;46(4):1632-1639

Abstract

Purpose: Prostate cancer is the most common type of cancer in men, and drug resistance is typical in its treatment. Chemokines especially play a role in tumor growth and drug resistance mechanisms. IRF5 is a critical transcription factor in immune response, and its relationship with the chemokine family CXCR4 and CXCL12 was investigated in this study.

Materials and Methods: The pIRF5 plasmid was transfected in an androgen-independent human prostate cancer cell line (PC3), and the IRF5 protein was overexpressed. CXCR4 and CXCL12 protein expression levels were determined by western blot and gene expression levels by the qPCR method. In addition, colony formation was examined in cells after IRF5 transfection, and CXCL12 secretion was measured in cell media.

Results: Cell viability and colony formation were found to be significantly reduced in IRF5 transfected PC3 cells. In addition, CXCR4 and CXCL12 protein expression and gene expression levels of IRF5 transfected cells were found to be significantly decreased.

Conclusion: This study shows that IRF5, a transcription factor, affects CXCR4/CXCL12, which is involved in microenvironment-mediated metastasis developing in prostate cancer. Thus, in the treatment of prostate cancer, IRF5 gene therapy can prevent metastasis and offer essential contributions to newly developed treatment methods in this regard.

Keywords: IRF5, prostate cancer, CXCR4, CXCL12, chemokines

Öz

Amaç: Prostat kanseri erkeklerde en sık görülen kanser türüdür ve tedavisinde ilaç direnci tipiktir. Kemokinler özellikle tümör büyümesinde ve ilaç direnç mekanizmalarında rol oynarlar. IRF5, immün yanıtta kritik bir transkripsiyon faktörüdür ve bu çalışmada kemokin ailesi CXCR4 ve CXCL12 ile ilişkisi araştırılmıştır.

Gereç ve Yöntem: pIRF5 plazmidi, androjenden bağımsız bir insan prostat kanseri hücre hattında (PC3) transfekte edildi ve IRF5 proteini aşırı eksprese edildi. CXCR4 ve CXCL12 protein ekspresyon seviyeleri western blot ve gen ekspresyon seviyeleri ile qPCR yöntemi ile belirlendi. Ek olarak, IRF5 transfeksiyonundan sonra hücrelerde koloni oluşumu incelendi ve hücre ortamında CXCL12 sekresyonu ölçüldü.

Bulgular: IRF5 ile transfekte edilmiş PC3 hücrelerinde hücre canlılığı ve koloni oluşumunun önemli ölçüde azaldığı bulundu. Ek olarak, IRF5 ile transfekte edilmiş hücrelerin CXCR4 ve CXCL12 protein ekspresyonu ve gen ekspresyon seviyelerinin önemli ölçüde azaldığı tespit edildi.

Sonuç: Bu çalışma, bir transkripsiyon faktörü olan IRF5'in prostat kanserinde gelişen mikroçevre aracılı metastazda yer alan CXCR4/CXCL12'yi etkilediğini göstermektedir. Böylece prostat kanseri tedavisinde IRF5 gen tedavisi metastazı önleyebilir ve bu konuda yeni geliştirilen tedavi yöntemlerine önemli katkılar sunabilir.

Anahtar kelimeler: IRF5, prostat kanseri, CXCR4, CXCL12, kemokinler

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Geliş tarihi/Received: 16.08.2021 Kabul tarihi/Accepted: 29.10.2021 Çevrimiçi yayın/Published online: 22.11.2021

INTRODUCTION

Prostate cancer is the most common type of cancer in men, caused by abnormal cell division in the prostate gland and impaired apoptosis balance[1]. Since they are androgen-dependent and independent cells in the prostate tissue, treatments differ during the cancer process. Androgen ablation therapy is applied to hormone-dependent cells, but these cells become resistant to the hormone over time. Androgen-independent cells are metastatic cells and not affected by hormone treatment. Most prostate cancer deaths are not the result of primary tumor growth but are caused by cancer's metastasis to other organs[2]. Many factors affect the metastasis of cancer, one of which is chemokines.

Chemokines are chemotactic cytokines that control cell migration and cell positioning throughout development, homeostasis, and inflammation. They can also coordinate the interactions between cells and immune system cells[3]. Chemokines are composed by small protein ligands weighing 8-12 kDa and their receptors. The receptors of chemokines are seven-transmembrane G-protein-coupled receptors, GPCRs[4,5]. CXCR4 is an evolutionarily conserved protein containing 352 amino acids with two exons, with 89% similarity between humans and mice. The chemokine receptor is expressed in multiple cell types, including stromal fibroblasts, lymphocytes, cancer cells, epithelial cells, and endothelial cells[6]. CXCL12, also known as stromal cell-derived factor 1 (SDF-1), is an inducible chemokine. CXCL12 contains 68 amino acids with nine exons and is expressed in many normal tissues and serums. Although CXCL12 is a chemokine ligand, it functions as a modulator of biological processes with its receptor, CXCR4[7,8]. CXCL12 is involved in the metastasis of organs in which CXCR4 is expressed, allowing tumor cells to access cellular niches such as bone marrow that support tumor cell survival and growth. CXCL12 can stimulate the survival and growth of neoplastic cells and promote tumor angiogenesis by attracting endothelial cells to the tumor microenvironment[9-11]. The interaction of CXCR4 and CXCL12 contributes to events such as tumor invasion, metastasis, drug resistance, and maintenance of stemness[12].

Interferon regulatory factors (IRFs) are a family of transcription factors that regulate innate and adaptive immune responses. IRF5 was first identified and characterized as a transcriptional regulator of type I interferon (IFN) expression after virus infection[13]. IRF5 plays a role in the antiviral immune response due to IFN genes in transcriptional activation of genes encoding proinflammatory cytokines. IRF5 can also bind to DNA and encode proteins that regulates cell growth regulation and apoptosis. The IRF5 protein is usually found in the cell's cytoplasm and is activated through post-translational modification after induction, resulting in nuclear translocation and binding to promoters of target genes[14]. It has been shown that IRF5 is also expressed in prostate cancer cells and has post-translational modification[15]. In this study, we focused on the relationship between IRF5 and chemokines in metastatic prostate cells. Therefore, we aimed to determine the extent to which the CXCR4/CXCL12 is affected after possible IRF5 transfection. In addition, we aimed to evaluate IRF5 protein upregulation would be more effective against interaction of CXCR4 and CXCL12, both gene and protein expression. Thus, the molecular changes that occur in the metastatic prostate cancer cells after IRF5 transfection will be revealed and role of chemokine receptor CXCR4 and its ligand CXCL12 the possible metastasis will be mentioned. This study aimed to investigate the effect of IRF5 protein on CXCR4 and CXCL12.

MATERIALS AND METHODS

Cell culture

In the study, PC3 cell, an androgen-independent human prostate cancer cell line, was commercially obtained from ATCC (CRL-1435). Ethics committee approval is not required as we use a commercial cell line. PC3 cells were grown using RPMI-1640 (Sigma) medium containing 10% FBS, Penicillin-Streptomycin (Sigma) (100 units), L-Glutamine, and $NaHCO_3$ as cell culture medium, it is allowed to incubate at 37 °C, in an environment containing 95% humidity and 5% CO_2.

IRF5 transfection

The IRF5 expression plasmid was used in our previous studies[15,16]. pIRF5 and pV (control plasmid) plasmids were transformed using the *E.coli* DH5α bacteria strain with the electroporation technique at 1.8 kV and 25 μF condition. Competent bacteria containing pIRF5 and pV plasmid were inoculated in LB broth medium and grown in a shaker incubator at 37 °C. Growth bacteria were used in plasmid isolation with the commercial kit (QIAGEN Plasmid

Mini Kit 12123). Isolated plasmid DNA concentration were measured in nanodrop (Nabi UV/Vis NANO). pIRF5 and pV expression plasmids were transfected with 1 µg plasmid DNA with liposome (Lipofectamine 2000) in 6-well plates to entry into PC3 cells.

Cell survival and colony formation

To cell survival, PC3 cells were grown for 0-72 hours after transfection. After the incubation, the MTT test was performed on the cells with the kit (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium broth) (Vybrant, Invitrogen). MTT reagent was added to each well and incubated for 4 hours in a humidified atmosphere in a 37 °C incubator with 5% CO_2 in the air. After incubation, 100 µL of SDS buffer was added to each well to dissolve the formazan precipitate. Then absorbance was measured at 570 nm with a microplate reader and performed in triplicate of each test (Epoch, Biotek). Each measurement was repeated three times.

For the colony formation experiment, transfected PC3 cells (500 cells/well) were seeded in 12-well plates and kept at 37 °C for seven days. The medium was changed every three days. At the end of the incubation, each well was washed with PBS, fixed with cold methanol / acetic acid, washed for 15 minutes with 0.5% crystal violet staining solution, and sequentially ddH_2O. The number of colonies in each well was counted and then analyzed.

Western blot studies

After the transfection, the cells were collected and kept in ice using RIPA blasting buffer, they were centrifuged at 12,000 xg at 4 °C for 10 minutes and Western Blot studies were performed on the upper phase. Protein concentration in the samples was measured according to the BCA kit (Santa Cruz, sc-202389). For the samples, 30 µg of protein was loaded into the wells of 4-12% SDS PAGE gels. After the samples were run with the help of electric current, they were transferred to the PVDF membrane with a semi-dry system.

After the membrane was blocked with 3% BSA for 1 hour, it was incubated with primary antibody ((1:1000): GAPDH (sc-25778), IRF5 (sc-390364), CXCR4 (sc-9046), CXCL12 (sc-28876)) overnight at 4°C. After incubation, the membrane washed with TBST and incubated with HRP-conjugated secondary antibody for 2 hours, then the bands were analyzed in the imaging system using ECL reagent. GAPDH was used as the control protein and the results were calculated.

QPCR

Total RNA was isolated with the RNA extraction kit according to the manufacturer's instructions (PureLink, Life Technologies). A total of 1 µg of RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosytem) following the manufacturer's protocols. Primers (CXCR4 Forward 5'-TGACGGACAAGTACAGGCTGC -3', CXCR4 Reverse 5'-CCAGAAGGGAAGCGTGATGA-3'; CXCL12 Forward 5'-TGCCAGAGCCAACGTCAAG-3', CXCL12 Reverse 5'-CAGCCGGGCTACAATCTGAA-3'; GAPDH Forward 5'-AGGGCTGCTTTTAACTCTGGT-3', Reverse 5'-CCCCACTTGATTTTGGAGGGA-3) were used for amplification. One hundred nanograms of cDNA were programmed at 95 °C for 5 minutes, then 40 cycles at 95 °C for 15 seconds, 60 °C for 1 minute using Sybr Green PCR Master Mix (Applied Biosytems) in ABI StepOne Plus detection system. Results were analyzed using StepOne Software v2.3 (Applied Biosystems) and normalized to GAPDH gene. Data was expressed as fold induction relative to control.

Statistical analysis

Statistical analysis was conducted using the SPSS 15.00 analysis program. To compare groups, One way ANOVA and Tukey test as post-hoc tests were used. The significance level was accepted as $p < 0.05$.

RESULTS

After IRF5 transfection, the morphology of PC3 cells were examined under a microscope, and survival was measured during 72 hours. It has been observed that the IRF5 protein negatively affects cell proliferation. Cell morphologies at 36 hours in Figure 1A shows that the PC3 cells are separated from each other and become sparse, and their tendency to approach each other decreased. It also appears that between 36 and 48 hours, the cell death rate of PC3 cells were about 50% (Figure 1B). In the study, experiments continued with 36h incubations to see the better effect of the microenvironment. When colony formation was examined in cells, it was observed that IRF5

expression decreased colony-forming potential of PC3 cells during seven days (Figure 1C) and significantly decreased by 52% compared to the control pV group (Figure 1D, p<0.001). After IRF5 expression in PC3 cells, CXCR4 and CXCL12 protein expression levels were analyzed by western blotting (Figure 2A). Increasing IRF5 protein levels more than 20-fold (Figure 2B, p<0.001) resulted in a statistically significant decrease in CXCR4 protein levels compared to the control pV group (Figure 2C, p<0.001).

There was no significant change in CXCL12 protein expression levels in PC3 cells and could not be detected in cells (Figure 2D). Similar to protein expression levels, changes were observed in gene expression levels. As a result of the IRF5 gene expression of approximately 60% by transfection (Figure 3A), CXCR4 levels were significantly decreased compared to the control pV group (Figure 3B, p<0.001). When CXCL12 levels were examined, IRF5 significantly decreased after overexpression in PC3 cells (Figure 3C, p<0.001).

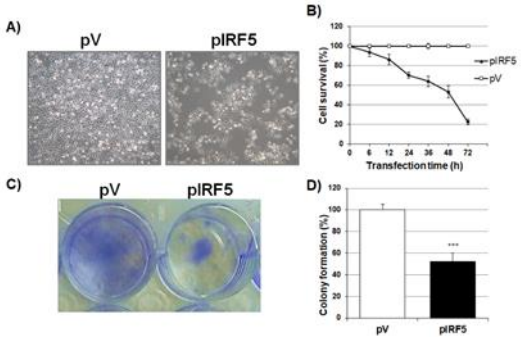

Figure 1. Effect of IRF5 transfection on cell growth and colony formation A) IRF5 transfection microscope images in PC3 cells (10X) at 36h B) Time dependent IRF5 transfection cell survival percentages in PC3 cells C) After seven-days IRF5 transfection in PC3 cells colony formation images D) Colony formation percentages in IRF5 transfected PC3 cells on 7th days (* p<0.001 compared to pV).**

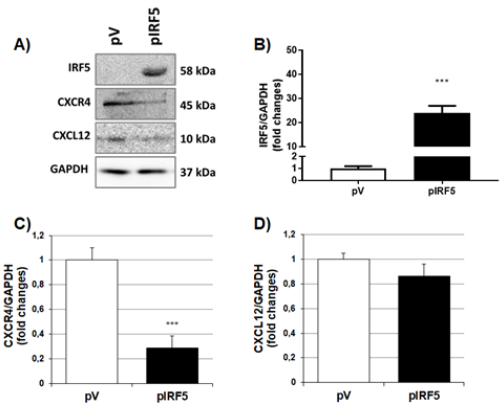

Figure 2. A) IRF5, CXCR4, CXCL12 western blotting bands of IRF5 transfected PC3 cells B) Densitometric analysis of IRF5/GAPDH on PC3 cells C) Densitometric analysis of CXCR4/GAPDH on PC3 cells D) Densitometric analysis of CXCL12/GAPDH on PC3 cells (* p<0.001 compare to pV).**

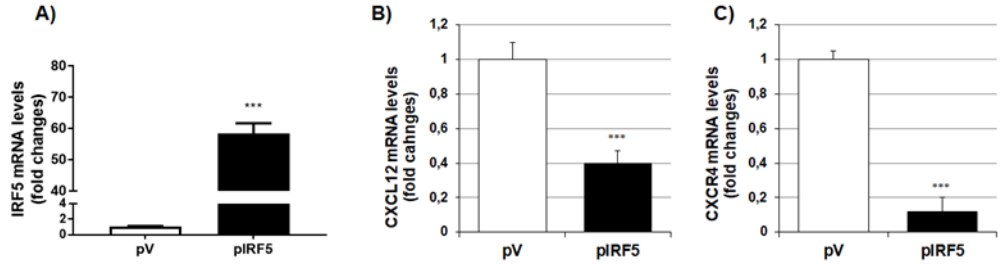

Figure 3. A) IRF5 mRNA levels of transfected PC3 cells B) CXCL12 mRNA levels of IRF5 transfected PC3 cells. C) CXCR4 mRNA levels of IRF5 transfected PC3 cells (* p<0.001 compare to pV).**

However, while there was no change in CXCL12 protein levels in cells, changes in gene expression levels were detected. When CXCL12 levels were examined in the collected cell media, it was found that CXCL12 passed into the cell medium and did not remain in the cell after expression (Figure 4A). When CXCL12 levels were examined in PC3 cell media, it was found that CXCL12 secretion was suppressed in IRF5 overexpressed cells and significantly decreased against control cells (Figure 4B, $p<0.001$).

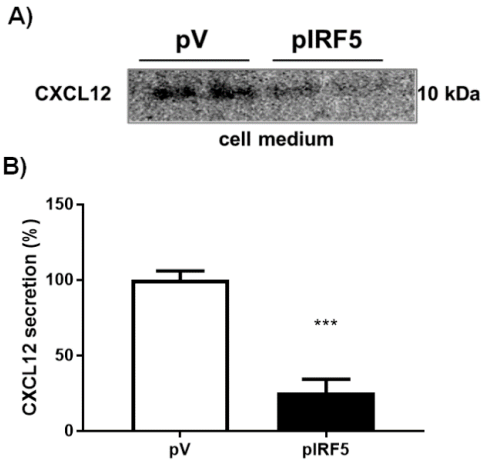

Figure 4. A) CXCL12 western blotting bands of PC3 cell media B) Densitometric analysis of CXCL12 secretion on PC3 cells (* p<0.001 compared to pV).**

DISCUSSION

IFNs are pleiotropic cytokines with antiviral, antitumor, and immunomodulatory properties central coordinators of the immune response. The IFN regulatory factor (IRF) family enables the regulation of IFN responses. IRF5, one of the IRF proteins in mammals, is involved in Toll-like receptors (TLRs) dependent induction of type I IFNs. The prominent role of IRF5 is to induce proinflammatory cytokine genes such as IL-12β, IL-6, IL-10, IL-12, IL-23, MIP-1-alpha, IP10, and TNF-α by activating type 1 interferons[16-18]. Little is known about the function of IRF5 in cancer and its tumor suppressor function[14]. IRF5 expression is absent or significantly reduced in immortalized tumor cell lines and primary samples from patients with hematological malignancies, it has suggested the role of IRF5 in the tumor suppressor gene. Similar findings showed that embryonic fibroblasts in IRF5 deficient mice are resistant to DNA damage and exposure to virus-induced apoptosis. It has been reported that the effects of IRF5 on cell cycle regulation and apoptosis are independent of p53. IRF5 has been shown to function as a tumor suppressor by acting in a pathway that may differ from that of p53[16,19]. The studies reported that IRF5 also provides IFN-γ induction[20-22]. Besides, studies on the role of IRF5 in prostate cancer effectively regulate cytokines and the control of stem cell factors with androgen. It is known that IRF5 has essential effects on various signaling pathways and different transcription factors in prostate cancer cells. It has been shown in a previous study that it can control the behavior of prostate cancer cells by making modifications such as acetylation methylation in transcription factors that control cytokines in these processes[15].

CXCR4 is overexpressed in more than 23 different human cancers, including kidney, lung, brain, prostate, breast, pancreas, ovaries, and melanomas, and contributes to tumor growth, angiogenesis, metastasis, and therapeutic resistance[23,24]. Cancer cells are thought to take over the CXCR4 / CXCL12 axis to create distant organ metastasis in drug resistance[25]. CXCL12 expression levels are the highest in areas of common metastasis, such as the brain, bone marrow, lungs, and liver[26,27]. Growth factors such as hypoxia-inducible factor (HIF) -1α[28], essential fibroblast growth factor, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF)[29] and transcription factors such as nuclear respiratory factor-1 also increase and thus drug resistance develops[30,31]. Increased activation of the CXCL12 / CXCR4 axis has been associated with prostate cancer metastasis to bone. While prostate cancer cells express CXCR4, bone marrow stromal cells produce CXCL12, and prostate cancer cells migrate partially to the bone marrow through interaction with CXCR4[32]. Therefore, drug therapy in prostate cancer treatment may lose its effect, and develop drug resistance can contribute. Currently, it is not an effective treatment for prostate cancer, but the number of studies on the interaction between cancer cells and bone microenvironment for the development of new therapeutic agents are rapidly increasing[33,34].

This study showed the relationship between IRF5 and chemokine family members CXCL12 and CXCR4 on prostate cancer cells. Previous studies have investigated prostate cancer and the CXCL12 /

CXCR4 complex in different aspects. In this context, increased activation of CXCL12 / CXCR4 has been associated with bone metastasis of prostate cancer. The interaction of prostate cancer cells and bone marrow stromal cells resulting from the CXCL12 / CXCR4 axis has become a potential therapeutic target in treating metastatic prostate cancer. Therefore, blocking the CXCL12 / CXCR4 axis by treating prostate cancer cells with a CXCR4 antagonist AMD3100 or antibody reduces tumor size and progenitor cell population and can effectively inhibit prostate cancer cell metastasis to bone. AMD3100 (plerixafor, Mozobil) is known as a small molecular weight CXCR4 inhibitor and approved by the FDA. In studies conducted, it was determined by transcriptional analysis that inhibition of CXCR4 with a 1-week continuous infusion of AMD3100 induced immune response in metastasis biopsies from patients with colorectal and pancreatic cancer.

Moreover, blocking CXCL12/CXCR4 the interaction may be beneficial for chemotherapy; some studies have shown that inhibition of the interaction between cancer cells and bone stromal cells increases the sensitivity of cancer cells to chemotherapy[33]. There are also different CXCR4 inhibitors such as MSX-122, LY2510924, Motixafortide (BL-8040), WZ811. It is crucial to develop agents that provide CXCR4 inhibition or be suppressed by some signal pathways in the cell to prevent metastases in cancer treatments.

Various evidence has been presented regarding the association with various signaling pathways between CXCR4 and interferons. Stimulation of IFN / IFN receptors has been reported to be controlled by IFN regulatory factors via Janus-activated kinase / STAT signaling, resulting in suppressing SP1, one of the transcription factors that bind to the CXCR4 promoter region[35,36]. The IFN / IFN receptor response induces the Janus-activated kinase / STAT signaling pathway. IFN / IFN receptor stimulation can also be controlled by cyclic AMP-mediated intracellular messengers acting on the CXCR4 promoter. It has been demonstrated in human T cells[37], human glial cells[38], and dendritic cells[39] that the CXCR4 promoter is mediated by a cyclic AMP that enhances CXCR4 expression. It has also been shown that IFN-γ significantly inhibits cyclic AMP-mediated gene expression in human endothelial cells in a dose-dependent manner[40]. These studies have shown that IRFs inducing interferon types affect CXCR4 promoters through intracellular signals.

The frequency of CXCL12 rs1801157 polymorphism in peripheral blood of breast cancer patients and expression of CXCL12, CXCR4, and IFN-γ mRNA in normal and mammary gland tumor tissues were investigated in a study. The study claims that the GG and A transporters CXCR4 and IFN-γ mRNA expressions are different in CXCL12 and that the microenvironment may affect metastatic cell migration[41]. Various IRF5 polymorphisms detected in humans play a role in autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease[13]. The results of CXCL12 polymorphism with CXCR4 and IFN-γ suggest that it may be related to IRF5 polymorphisms in different cancers.

There are limited studies on the relation of IFN-γ induced by IRF5 with CXCR4 on cancer cells. Cytokines such as interleukin-1β, tumor necrosis factor-α, and IFN-were added to cells in a study investigating the role of CXCR4 expression and interferons in head and neck squamous cell carcinomas (HNSCC) using HSQ-89, IMC-3, and Nakamura cells. CXCR4 expression and CXCL12 levels were measured by cell proliferation, migration with matrigel, qPCR, flow cytometry, and western blot after incubation in the cells. In CXCR4-positive IMC-3 and Nakamura cells, IFN-γ decreased CXCR4 expression in a dose and time-dependent manner at both mRNA and surface protein levels, and IFN-γ also inhibited CXCL12-mediated cell migration and cell proliferation in these cells. CXCR4 negative HSQ-89 cells have been shown IFN-γ did not affect CXCR4 expression. At the same time, biopsy samples were examined in patients with HNSCC, and it was reported that CXCR4 expression was suppressed by interferon-gamma and inhibited tumor metastasis[42].

In a study with human ductal carcinoma MDA-MB-231 cells, it has been shown that CXCR4 cell surface expression is reduced by IRF5 overexpression, and cells with high CXCL12 expression could not migrate in response. These data confirm the negative regulation of CXCR4 expression by IRF5 in breast cancer cells[19]. CXCR4 overexpression; when evaluated as an essential factor in the migration, invasiveness, proliferation, and bone metastasis of breast cancer cells, it suggests that CXCR4 silencing by IRF5 may also be effective in preventing bone metastasis of prostate cancers in which CXCR4 is overexpressed.

The limitations of our study are that only the interaction of CXCR4/CXCL12 was demonstrated

by IRF5 upregulation. Interaction with IRF5 and other chemokines has not been demonstrated. According to the results of this study, which was carried out in in-vitro cells, it is thought to show the molecular effects in in-vivo tumor models by different studies by examining the interaction of other chemokines.

In conclusion, in this study, overexpression of IRF5 levels in prostate cancer PC3 cells caused the change of chemokine receptor CXCR4 and its ligand CXCL12. In our study, when IRF5 expression increased in prostate cancer cells, it significantly reduced CXCR4 mRNA and protein expression levels, which have an essential role in migration and drug resistance in cancer, and decreased the secretion of CXCL12, which provides tumor growth in the microenvironment. IRF5 can be an effective treatment option in prostate cancer to prevent metastasis and against drug resistance and may be a crucial therapeutic protein for new drugs to be developed.

Yazar Katkıları: Çalışma konsepti/Tasarımı: BIA, NB, ÖÇ; Veri toplama: BIA, NB, ÖÇ; Veri analizi ve yorumlama: BIA, NB, ÖÇ; Yazı taslağı: ÖÇ; İçeriğin eleştirel incelenmesi: ÖÇ; Son onay ve sorumluluk: BIA, NB, ÖÇ; Teknik ve malzeme desteği: ÖÇ; Süpervizyon: ÖÇ; Fon sağlama (mevcut ise): yok.
Etik Onay: Ticari bir hücre hattı kullanıldığı için Etik kurul onayı gerekli değildir.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Tüm yazarlar çıkar çatışması olmadığını beyan etmişlerdir..
Finansal Destek: Bu çalışma Adnan Menderes Üniversitesi Araştırma Hibe Projeleri (TPF-19021) ve Türkiye Bilimsel ve Teknolojik Araştırma Konseyi'nden (TÜBİTAK) Dr. Özge Çevik'e hibe (214Z057) ile desteklenmiştir.
Yazarın Notu: PC3 hücre hatlarını ve rekombinant proteinleri sağladığı için ODC Araştırma ve Geliştirme A.Ş.'ye minnettarız.

Author Contributions: Concept/Design : BIA, NB, ÖÇ; Data acquisition: BIA, NB, ÖÇ; Data analysis and interpretation: BIA, NB, ÖÇ; Drafting manuscript: ÖÇ; Critical revision of manuscript: ÖÇ; Final approval and accountability: BIA, NB, ÖÇ; Technical or material support: ÖÇ; Supervision: ÖÇ; Securing funding (if available): n/a.
Ethical Approval: Ethics committee approval is not required as a commercial cell line was used.
Peer-review: Externally peer-reviewed.
Conflict of Interest: All authors declared that there are no conflicts of interest..
Financial Disclosure: This work was supported by the Adnan Menderes University Research Grant Projects (TPF-19021) and by a grant (214Z057) from the Scientific and Technological Research Council of Turkey (TUBITAK) to Dr. Ozge Cevik. Authors declared no financial support
Acknowledgement: We are grateful to ODC Research and Development Inc for providing the PC3 cell lines and recombinant proteins.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Dubrovska A, Elliott J, Salamone RJ, Telegeev GD, Stakhovsky AE, Schepotin IB et al. CXCR4 expression in prostate cancer progenitor cells. PLoS One. 2012;7:e31226.
3. Sokol CL, Luster AD. The chemokine system in innate immunity. Cold Spring Harb Perspect Biol. 2015;7:a016303.
4. Balkwill FR. The chemokine system and cancer. J Pathol. 2012;226:148-157.
5. Nomiyama H, Hieshima K, Osada N, Kato-Unoki Y, Otsuka-Ono K, Takegawa S et al. Extensive expansion and diversification of the chemokine gene family in zebrafish: identification of a novel chemokine subfamily CX. BMC Genomics. 2008;9:222.
6. Werner L, Guzner-Gur H, Dotan I. Involvement of CXCR4/CXCR7/CXCL12 interactions in Inflammatory bowel disease. Theranostics. 2013;3:40-6.
7. Secchiero P, Celeghini C, Cutroneo G, Di Baldassarre A, Rana R, Zauli G. Differential effects of stromal derived factor-1α (SDF-1α) on early and late stages of human megakaryocytic development. Anat Rec. 2000;260:141-7.
8. Wright LM, Maloney W, Yu X, Kindle L, Collin-Osdoby P, Osdoby P. Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. Bone. 2005;36:840-853.
9. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. Blood. 2006;107:1761-7.
10. Balestrieri ML, Balestrieri A, Mancini FP, Napoli C. Understanding the immunoangiostatic CXC chemokine network. Cardiovasc Res. 2008;78:250-6.
11. Vilgelm AE, Richmond A. Chemokines modulate immune surveillance in tumorigenesis, metastasis, and response to immunotherapy. Front Immunol. 2019;10:333.
12. Yin H, Wang Y, Chen W, Zhong S, Liu Z, Zhao J. Drug-resistant CXCR4-positive cells have the molecular characteristics of EMT in NSCLC. Gene. 2016;594:23-9.
13. Jefferies CA. Regulating IRFs in IFN driven disease. Front Immunol. 2019;10:325.
14. Li D, De S, Li D, Song S, Matta B, Barnes BJ. Specific detection of interferon regulatory factor 5 (IRF5): A case of antibody inequality. Sci Rep. 2016;6:31002.
15. Acidereli H, Turut FA, Cevik O. Acetylation of interferon regulatory factor-5 suppresses androgen receptor and downregulates expression of Sox2. Cell Biochem Funct. 2021;39:667-678.
16. Cevik O, Li D, Baljinnyam E, Manvar D, Pimenta EM, Waris G et al. Interferon regulatory factor 5 (IRF5) suppresses hepatitis C virus (HCV) replication

and HCV-associated hepatocellular carcinoma. J Biol Chem. 2017;292:21676-21689.
17. Honda K, Taniguchi T. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol. 2006;6:644-58.
18. Negishi H, Taniguchi T, Yanai H. The interferon (IFN) class of cytokines and the IFN regulatory factor (IRF) transcription factor family. Cold Spring Harb Perspect Biol. 2018;10:a028423.
19. Bi X, Hameed M, Mirani N, Pimenta EM, Anari J, Barnes BJ. Loss of interferon regulatory factor 5 (IRF5) expression in human ductal carcinoma correlates with disease stage and contributes to metastasis. Breast Cancer Res. 2011;13:R111.
20. Yasuda K, Shukla P, Bonegio RG, Rifkin I, Section R. IFN regulatory factor-5 signaling increases IFN-gamma production and suppresses IL-2 production from CD4 + T cells , and controls IgG production in B Cells. Arthritis Rheumatol. 2016;68(Suppl 10):1121.
21. Almuttaqi H, Udalova IA. Advances and challenges in targeting IRF5, a key regulator of inflammation. FEBS J. 2019;286:1624-37.
22. Wang X, Guo J, Wang Y, Xiao Y, Wang L, Hua S. Expression levels of interferon regulatory factor 5 (IRF5) and related inflammatory cytokines associated with severity, prognosis, and causative pathogen in patients with community-acquired pneumonia. Med Sci Monit. 2018;24:3620-30.
23. Chatterjee S, Behnam Azad B, Nimmagadda S. The intricate role of CXCR4 in cancer. Adv Cancer Res. 2014;124:31-82.
24. Cojoc M, Peitzsch C, Trautmann F, Polishchuk L, Telegeev GD, Dubrovska A. Emerging targets in cancer management: role of the CXCL12/CXCR4 axis. Onco Targets Ther. 2013;6:1347-61.
25. Gatti M, Pattarozzi A, Bajetto A, Würth R, Daga A, Fiaschi P et al. Inhibition of CXCL12/CXCR4 autocrine/paracrine loop reduces viability of human glioblastoma stem-like cells affecting self-renewal activity. Toxicology. 2013;314:209-20.
26. Ho TK, Shiwen X, Abraham D, Tsui J, Baker D. Stromal-cell-derived factor-1 (SDF-1)/CXCL12 as potential target of therapeutic angiogenesis in critical leg ischaemia. Cardiol Res Pract. 2012;2012:143209.
27. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410:50-6.
28. Ishikawa T, Nakashiro K-I, Klosek KS, Goda H, Hara S, Uchida D et al. Hypoxia enhances CXCR4 expression by activating HIF-1 in oral squamous cell carcinoma. Oncol Rep. 2009;21:707-12.
29. Phillips RJ, Mestas J, Gharaee-Kermani M, Burdick MD, Sica A, Belperio JA et al. Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1α. J Biol Chem. 2005;280:22473-81.
30. Duda DG, Kozin SV, Kirkpatrick ND, Xu L, Fukumura D, Jain RK. CXCL12 (SDF1α)-CXCR4/CXCR7 pathway inhibition: an emerging sensitizer for anticancer therapies? Clin Cancer Res. 2011;17:2074 LP - 2080.
31. Teicher BA. Antiangiogenic agents and targets: A perspective. Biochem Pharmacol. 2011;81:6-12.
32. Adekoya TO, Richardson RM. Cytokines and chemokines as mediators of prostate cancer metastasis. Int J Mol Sci. 2020;21:4449.
33. Zhang X. Interactions between cancer cells and bone microenvironment promote bone metastasis in prostate cancer. Cancer Commun. 2019;39:76.
34. Lin S-C, Yu-Lee L-Y, Lin S-H. Osteoblastic factors in prostate cancer bone metastasis. Curr Osteoporos Rep. 2018;16:642-7.
35. Moriuchi M, Moriuchi H, Turner W, Fauci AS. Cloning and analysis of the promoter region of CXCR4, a coreceptor for HIV-1 entry. J Immunol. 1997;159:4322 LP-9.
36. Mamane Y, Heylbroeck C, Génin P, Algarte M, Servant MJ, LePage C et al. Interferon regulatory factors: the next generation. Gene. 1999;237:1-14.
37. Cristillo AD, Highbarger HC, Dewar RL, Dimitrov DS, Golding H, Bierer BE. Up-regulation of HIV coreceptor CXCR4 expression in human T lymphocytes is mediated in part by a cAMP-responsive element. FASEB J. 2002;16:354-64.
38. Ödemis V, Moepps B, Gierschik P, Engele J. Interleukin-6 and cAMP induce stromal cell-derived factor-1 chemotaxis in astroglia by up-regulating CXCR4 cell surface expression: implications for brain inflammation. J Biol Chem. 2002;277:39801-8.
39. Gagliardi MC, De Magistris MT. Maturation of human dendritic cells induced by the adjuvant cholera toxin: role of cAMP on chemokine receptor expression. Vaccine. 2003;21:856-61.
40. Christian M, Marangos P, Mak I, McVey J, Barker F, White J et al. Interferon-γ modulates prolactin and tissue factor expression in differentiating human endometrial stromal cells. Endocrinology. 2001;142:3142-51.
41. de Oliveira KB, Guembarovski RL, Guembarovski AMFL Herrera ACDSDA, Sobrinho WJ, Ariza CB et al. CXCL12, CXCR4 and IFNγ genes expression: implications for proinflammatory microenvironment of breast cancer. Clin Exp Med. 2013;13:211-9.
42. Katayama A, Ogino T, Bandoh N, Nonaka S, Harabuchi Y. Expression of CXCR4 and its down-regulation by IFN-γ in head and neck squamous cell carcinoma. Clin Cancer Res. 2005;11:2937 LP- 46.