ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
October 2025; 24(5):653-663.
DOI: 10.18502/ijaai.v24i5.19748

Exploring the Therapeutic Potential of Fluorinated CXCR4 Inhibitor A1: Insights from Breast Cancer In Vitro Investigations

Ali Rahimi[1], Hossein Khorramdelazad[2], Ali Darehkordi[3], Gholamhossein Hassanshahi[2], Majid Khoshmirsafa[1], Milad Karimi[1], Reza Falak[1], and Elaheh Safari[1,4,5]

[1] Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[2] Department of Immunology, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[3] Department of Chemistry, Faculty of Science, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran
[4] Breast Health and Cancer Research Center, Iran University of Medical Sciences, Tehran, Iran
[5] Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran

Received: 9 November 2024; Received in revised form: 5 January 2025; Accepted: 11 January 2025

ABSTRACT

The impacts of the CXC motif chemokine 12 (CXCL12)/ C-X-C chemokine receptor type 4 (CXCR4) axis on the infiltration of anti-tumor and pro-tumor immune cells in the tumor microenvironment (TME) of breast cancer (BCa) have been noted in previous studies. Accordingly, regulating the downstream signals of this axis can effectively increase $CD8^+$ cytotoxic T cells and decrease the frequency of immunosuppressive cells in the TME. This study investigated the anti-tumor effects of N, N''-thiocarbonylbis (N'-(3,4-dimethylphenyl)-2,2,2 trifluoroacetimidamide) (A1), a novel fluorinated CXCR4 inhibitor on a BCa cell line.

In this study, the impacts of A1 on cell viability, proliferation, apoptosis, and cell cycle were examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and flow cytometry assays. Moreover, the effect of A1 on the number of $CXCR4^+$ 4T1 cells was measured by flow cytometry.

A1 treatment exhibited cytotoxic effects on 4T1 cells, promoting cell apoptosis and G_2/M cell cycle arrest. In addition, A1-treated cells showed a reduced cell proliferation than CXCL12 treated cells. Furthermore, treatment with A1 alongside CXCL12 significantly decreased the number of $CXCR4^+$ cells compared to the control group treated with only CXCL12 as a proliferator factor.

These results indicate that A1 exerts potential anti-tumor effects and may serve as a possible therapeutic agent for BCa treatment; however, further studies are required.

Keywords: Breast cancer; CXCR4; Fluorine; Small molecule

Corresponding Authors: Elaheh Safari, PhD;
Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 912) 5975 038, Fax: (+98 21) 8670 3280), Email: el.safari@yahoo.com

Reza Falak, PhD;
Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 912) 2753 942, Fax: (+98 21) 8862 2652, Email: rezafalak@gmail.com

INTRODUCTION

Breast cancer (BCa) is one of the most common malignancies, representing an enormous concern for women worldwide.[1] According to the molecular subtypes of BCa, triple-negative breast cancer (TNBC) exhibits lack of expression of three steroid hormone receptors (SHRs), including estrogen-receptor (ER), progesterone-receptor (PR), and human epidermal growth factor receptor 2 (HER2), which are observed in about 15-20% of BC patients.[2] Nevertheless, to date, there is no targeted therapeutic approach for TNBC treatment. TNBC represents a remarkable recurrence/metastasis rate, resulting in short recurrence interval and high mortality.[3] Previous studies have demonstrated that dysregulated immune system and its components contribute to the pathogenesis of TNBC and are associated with poor prognosis.[4]

Cytokines and chemokines, as essential immune system mediators, can be produced by various immune and non-immune cells and are responsible for multiple pro-tumor and anti-tumor immune responses within the breast tumor microenvironment (TME).[5] Evidence shows the potent contribution of chemokine ligands and receptor profiles in several cancers based on the location of tumor cells.[6,7] Therefore, the imbalance in chemokine production and their relative receptors is a critical factor in enhancing the pathological features of TNBC.[8] Furthermore, to improve overall survival (OS) and further reduce the recurrence/metastasis rate from TNBC patients, the development of targeted therapies is necessary.[3] In this regard, chemokines and their receptors have been identified as potential targets for cancer therapy.[9] Among these chemokine receptors, CXC chemokine receptor 4 (CXCR4) is a seven trans-membrane and G protein-coupled receptor that can activate various downstream signaling pathways like mitogen-activated protein kinases (MAPKs), phosphoinositide 3-kinases (PI3Ks), and Wnt following the binding of its ligand CXC motif chemokine 12 (CXCL12). This binding ultimately regulate migration, adhesion, and survival.[10,11] Furthermore, the CXCL12/CXCR4 axis is pivotal in tumor progression by inducing angiogenesis, cell proliferation, immune evasion, and metastasis in various malignancies, including leukemia, multiple myeloma, and BCa,[12,13] Previous studies have indicated an association between CXCR4 overexpression, poor prognosis, and multiple organ metastasis in BCa patients.[14,15] In recent years, several attempts have been made to develop effective anti-tumor agents focused on targeting chemokine receptors, although only a few drugs have been clinically approved.[16] AMD3100 (plerixafor) is a well-studied small molecule drug that can pharmacologically block the CXCR4 binding site and reduce the interaction betweentumor and stroma cells.[17] In this way, pharmacological inhibition of CXCR4 via AMD3100 administration has provided promising outcomes in BCa trials.[17,18]

Interestingly, previous studies have demonstrated that involving a single fluorine atom or trifluoromethyl group (-CF_3) within the structure of active compounds leads to higher membrane permeability and enhanced metabolic stability, resulting in greater bioavailability and pharmacological activity.[19] Due to the controversial effects of AMD3100 in TNBC, we designed and synthesized A1, a trifluoromethyl CXCR4 inhibitor, to enhance lipophilicity, bioavailability, and anti-tumor activity. According to our recently published study on the CT26 colorectal cancer cell line, A1 demonstrated more promising anti-tumor effects than AMD3100 both *in silico* and *in vitro*.[20] These findings suggest that A1 may provide beneficial effects through various malignancies. Accordingly, this study aims to assesses the anti-tumor effects of A1 on the 4T1 mouse TNBC cell line.

MATERIALS AND METHODS

Fluorinated CXCR4 Inhibitor A1

Our previous *in silico* studies and molecular docking confirm the binding ability of A1 to CXCR4 compared to AMD3100.[20] Furthermore, following *in silico* studies, A1 was synthesized based on chemical processes, and the drug was characterized by Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) presented in Khorramdelazad et al study.[20]

Cell Culture

Mouse TNBC cell line 4T1 was purchased from the Pasteur Institute of Iran (Tehran, Iran) and cultured in DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) (Gibco, USA) supplemented with 10% FBS and 1% antibiotics at 37°C in a 5% CO_2 environment. The viability of cultured 4T1 cells was>98%, as examined by the trypan blue staining procedure.

Cell Viability and Proliferation Assays

To analyze the effect of A1 on cell viability, determine the 50% inhibitory concentration (IC_{50}) value, and define the cell proliferation, the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] (Sigma, USA) was performed.[21] In this assay, 4T1 cells (5,000 cells/well) were seeded in a 96-well plate and incubated overnight in serum-free DMEM. Subsequently, cells were treated with varying concentrations of A1 (0-200 µg/mL) for 24, 48, and 72 hours. After treatment, 20 µL of MTT solution was added, followed by 100 µL of DMSO. The absorbance was measured at 570 nm using an ELISA microplate reader (BioTech Ltd, USA). Also, DMSO was served as a negative control. The optimal results for determining IC50 were obtained at the 72 hours time point and for proliferation assessment, the analysis was conducted at a concentration of 10 µg/mL for 72 hours. Moreover, CXCL12 served as a positive control, and untreated cells were used as a negative control in proliferation assay.

Apoptosis Evaluation

4T1 cells (2 x 10^5 cells/well) were seeded in a 12-well plate and treated with A1 at its IC_{50} concentration (102 µg/mL) for 72 hours to assess A1-induced apoptosis. Cells were stained with Annexin-V-FLUOS and propidium iodide (PI) to differentiate apoptotic and necrotic populations. Moreover, untreated cells were included as a negative control. Flow cytometry analysis (BD Bioscience FACScaliber, USA) was conducted in triplicate.

Analysis of Cell Cycle Arrest

For cell cycle analysis, 1×10^5 4T1 cells/well were cultured into six-well plates containing 102 µg/mL of A1 and incubated for 72 hours. After the incubation, the supernatant was removed, the cells were washed twice with PBS, and trypsinized for detachment from the bottom of each well. The suspended cells were then fixed with cold methanol for 24 hours and treated with a mixture of RNase and propidium iodide (2 µg/mL). Finally, cell cycle arrest was measured by BD Bioscience FACScaliber flow cytometer (BD, USA).

Flow cytometric analysis of CXCR4 expression

To determine the number of $CXCR4^+$ 4T1 cells by flow cytometry, various treatments were conducted, including 102 µg/mL of A1, 100 ng/mL of CXCL12 (R&D Systems), and a combination of CXCL12 (100 ng/mL) with A1 (102 µg/mL). After 72h incubation, cells were stained with PE-conjugated anti-CXCR4 monoclonal antibodies (R&D Systems) and incubated at 4°C for 30 minutes. Subsequently, 4T1 cells were assessed on a BD FACScaliber flow cytometer (BD, USA). Mouse IgG1 isotype control antibodies (R&D, USA) were included to confirm result accuracy.

Statistical Analysis

All experiments were conducted in triplicate, with each experiment repeated three times independently. The normality of data distribution was assessed using the Shapiro-Wilk test, which is appropriate for smaller sample sizes. For normally distributed data, comparisons between two groups were performed using Student's t-tests (unpaired). For comparisons involving three or more groups, ANOVA was employed, followed by Tukey's post-hoc test for group-wise comparisons. Data are presented as mean ± SD, and a p value of less than 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 9 (GraphPad Prism Inc., USA).

RESULTS

Cytotoxicity and Proliferation

MTT assay was performed to determine the cytotoxicity of A1 on 4T1 cells at 24, 48, and 72h. Results showed that 4T1 cells were sensitive to the cytotoxic effect of A1. A1 from 12.5 to 200 µg/mL could increase cell death in the 4T1 cell line. Notably, 50% of 4T1 cells were eliminated *in vitro* at 102 µg/mL as IC_{50} concentration, as shown in Figure 1a after 72h. The proliferation assay results revealed a significant decrease in cell proliferation by treating cells with A1 (10 µg/mL) plus 100 ng/mL CXCL12 compared to cells treated with CXCL12 alone (p=0.0037) and A1 alone (p<0.0001) after 72 hours. However, treating cells with either CXCL12 or A1 significantly increased cell proliferation compared to the untreated control group (p=0.0182 and p=0.0002 respectively). Moreover, treatment with A1 elevated cell proliferation compared to CXCL12 (p=0.0167) in 72hours (Figure 1b).

Cell Apoptosis

The effect of A1 on cell apoptosis was assessed by Annexin V/ Propidium iodide (PI) staining on 4T1. Our analysis demonstrated a significant increase in the percentage of apoptotic A1-treated 4T1 cells compared

to the untreated group as a negative control. A1 treatment resulted in a 30.83% increase in late apoptosis, while untreated cells show 13.98% late apoptosis (p=0.044). Moreover, treating cells with A1 led to remaining 29.7% live cells compared to 54.16% in the untreated cells (p<0.0001) (Figure 2a, b, and c). Additionally, the percentage of necrosis was 31.1% in treated cells and 25.1% in untreated cells. The percentage of early apoptosis was 8.36% in treated cells and 8.09% in untreated cells. These findings suggest increased cell apoptosis following treatment of 4T1 cells with 102 µg/mL of A1 after 72 h.

Cell Cycle Arrest

Cell cycle distribution in 4T1 cells was also determined using flow cytometry analysis after 72h treatment with A1 at a 102 µg/mL concentration. A1 treatment led to a 13% decrease in S phase frequency, whereas untreated cells increase 14.57% S phase frequency (p=0.011). Moreover, treating cells with A1 resulted in an increase of 6.47% frequency of G2 phase compared to 3.90% in untreated cells (p=0.028) (Figure 3a, b, and c). These results suggest that A1 administration may induce cell cycle arrest at the G2/M phase in 4T1 cells.

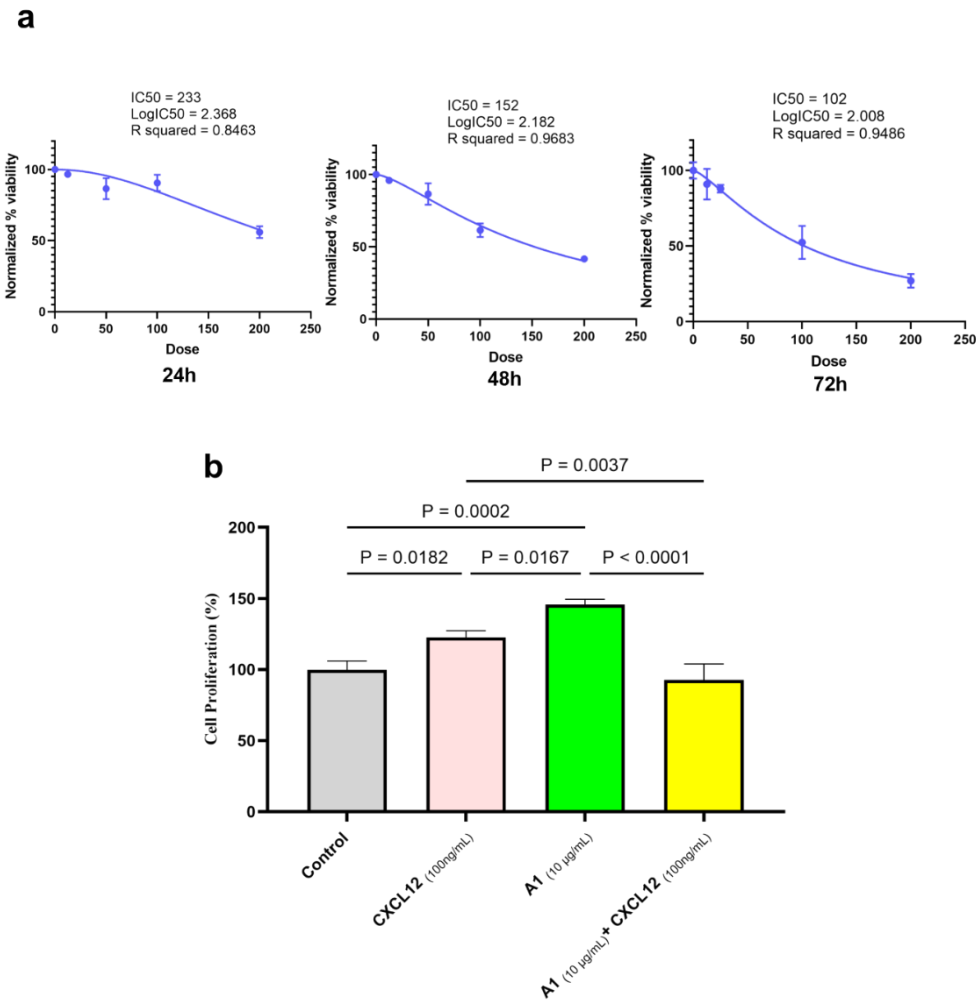

Figure 1. Effects of A1 on viability and proliferation of 4T1 cells. After 24 h incubation, cells growing in 96-well plates were treated with A1 for 24, 48, and 72h. 50% inhibitory concentration (IC_{50}) value, log IC_{50}, and R squared of A1 treatment for 4T1 cells are depicted in Figure (a). A1 at 10 µg/mL plus 100 ng/mL of CXC motif chemokine 12 (CXCL12) remarkably inhibited 4T1 cell proliferation compared to the CXCL12-only (p=0.0037) and A1-only (p<0.0001) treating cells, while treatment with CXCL12 (p=0.0182) or A1 (p=0.0002) also showed a significant increase in cell proliferation compared to the untreated control group. In addition, treatment with A1 augments cell proliferation in comparison with CXCL12 (p=0.0167) after 72h (b). Values represent mean±SD (n=3).

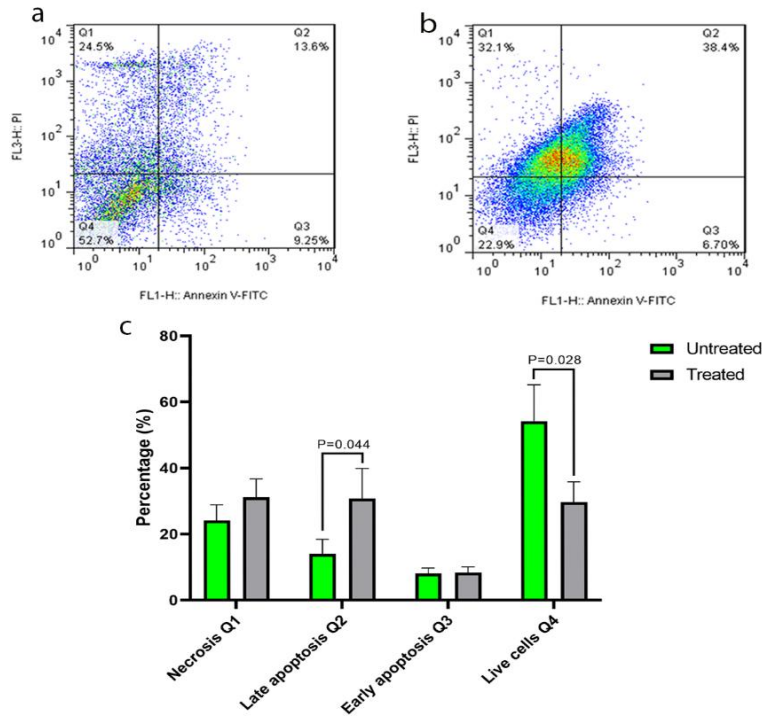

Figure 2. 4T1 cells were treated with the IC_{50} concentration of A1, and after 72 h, annexin-V- Fluorescein isothiocyanate (FITC) and Propidium iodide (PI) staining were performed to evaluate the percentage of cell apoptosis using flow cytometry analysis. The percentage of necrotic (Q1), late apoptotic (Q2), early apoptotic (Q3), and normal cells (Q4) in untreated (a) and treated (b) 4T1 cells. The differences in cell death percentages between untreated and treated cells in each quadrant are illustrated in (c).

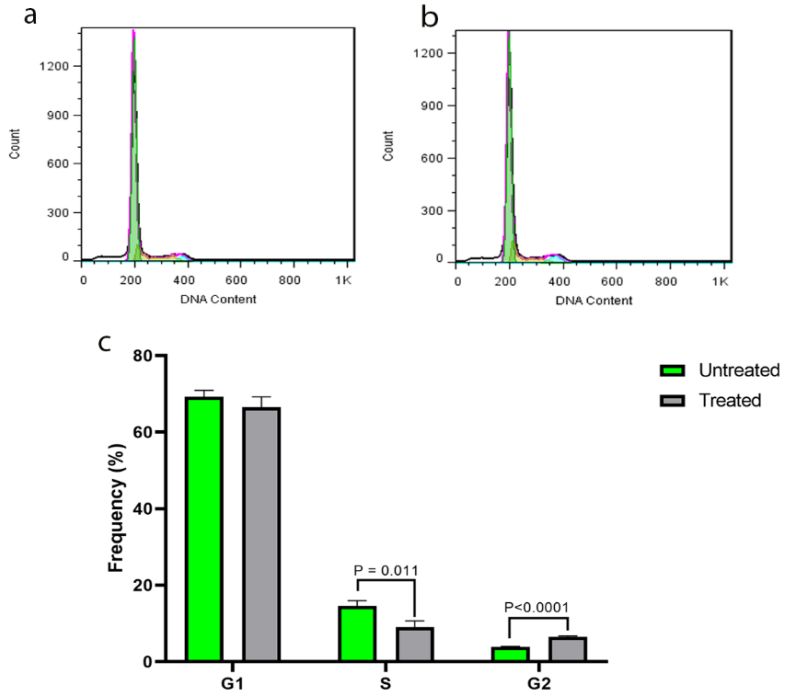

Figure 3. To evaluate the impact of A1 on cell cycle arrest, 4T1 cells were treated with IC50 concentrations of A1 and after 72 h of incubation, DNA content was analyzed using PI staining (a and b). The differences in DNA content across each cell cycle phase between untreated and treated cells are depicted in Figure (c). The results are presented as mean±SD (n = 3).

A1 effect on CXCR4 Expression

Treatment of 4T1 cells with 102 µg/mL of A1 plus 100 ng/mL of CXCL12 for 72 hours significantly reduced the number of CXCR4^{+} cells compared to the group treated CXCL12 alone (p=0.0005) and A1alone (p=0.0096). Treatment with A1 also decreased the expression of CXCR4 in 4T1 cells in comparison to CXCL12 treated cells as a positive control (Figure 4).

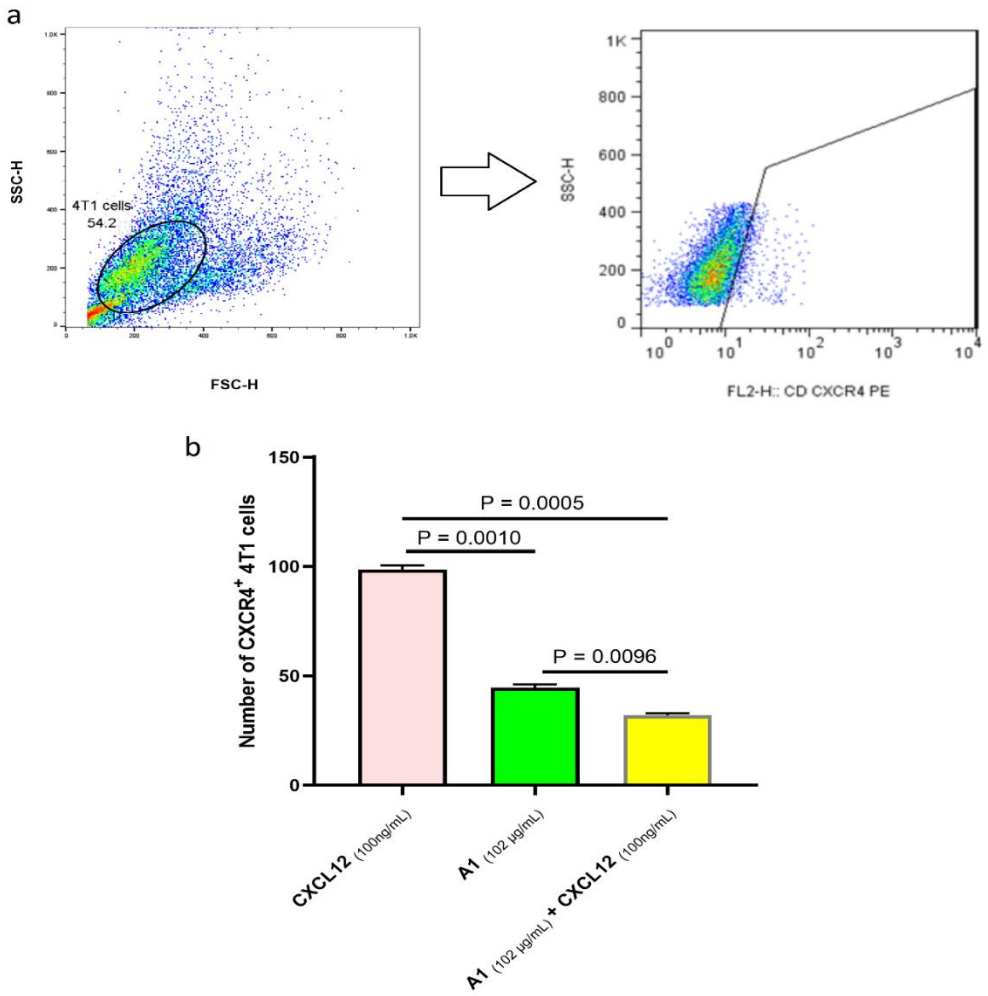

Figure 4. Flow cytometry gating strategy (a). Flow cytometry analysis indicates a remarkable reduction in C-X-C chemokine receptor type 4 (CXCR4) receptor expression in 4T1 cells treated with 102 µg/mL A1 plus 100 ng/mL of CXCL12 for 72 h, compared to the CXCL12-only (p=0.0005) and A1-only (p=0.0096) treated group. In addition, treating cells with A1 decreases the number of CXCR4^{+} 4T1 cells in comparison with CXCL12 treatment (p=0.0010) (b). The results are presented as mean±SD (n = 3).

DISCUSSION

Preclinical investigations have demonstrated that small molecules can restrict tumor growth and induce the apoptosis of different BCa cell lines.[22,23] Since the CXCR4/CXCL12 axis is considered a crucial mediator in various aspects of BCa tumorigenesis, treatments aiming to suppress this pathway might represent valuable therapeutic outcomes.[24] Activating the CXCR4 downstream signals facilitates cell survival, proliferation, and migration, thereby inducing invasion and metastasis.[25] The CXCL12/CXCR4 axis contributes to the chemotaxis of cancer cells towards CXCL12-enriched sites such as lymph nodes, liver, lungs, and bone marrow.[26] This chemotactic activity is facilitated by downstream signaling pathways, including mitogen-

activated protein kinases (MAPK) and Phosphoinositide 3-kinases (PI3K)/Akt, which regulate cytoskeletal remodeling, focal adhesion turnover, and matrix degradation via upregulation of metalloproteinases.[28,29] These mechanisms promote tumor cell invasion and metastasis, contributing to the high metastatic potential of cancers with elevated CXCR4 expression.[30] Moreover, activating CXCR4 fosters immune evasion within the TME by recruiting immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).[31] Several targeted therapies using small molecules that inhibit the CXCL12/CXCR4 axis have been developed in BCa, including AMD3100, MSX122, WZ811, TN140, and POL5551.[32-34] It is reported that T140, a peptide CXCR4 antagonist as an anti-HIV mediator, inhibited SDF-1-induced proliferation, migration, and metastasis in MDA-MB-231 human breast cancer cell line.[35] In addition, Both WZ811 and MSX-122 were effective in inhibiting lung metastases of breast cancer. However, MSX-122 provided a distinct advantage due to its improved pharmacokinetic profile, selective targeting of the Gαi-signaling pathway, and modulating cAMP levels without affecting the Gq-signaling.[34,36] Furthermore, Since AMD3100 has shown promising clinical results in hematological malignancies such as leukemia, it is under investigation for clinical applications in various cancers.[25] Previous clinical trials demonstrated preliminary promising outcomes in patients with BCa treated with AMD3100.[37] Although CXCR4 inhibitors, including AMD3100 may also potentially suppress bone marrow metastasis in BCa, side effects related to hematopoietic stem cells must be considered.[38] In this regard, previous studies have reported that AMD3100 induced hematopoiesis, particularly by mobilization hematopoietic stem cells and affecting leukocyte dynamics, which can lead to transient leukocytosis.[39] In addition to CXCR4 inhibition, other CXCR4 inhibitors (mentioned above) also exhibit cytotoxic effect on tumor cells.[40] Recently, we introduced A1 as a CXCR4 inhibitor with anti-tumor properties against the CT26 colorectal cancer cell line.[20] Notably, our previous in silico docking studies and prior characterization demonstrated that A1 effectively binds to CXCR4, likely at or near the receptor's ligand-binding site. This interaction blocks the ability of CXCL12 to bind and stimulate CXCR4, which may lead to receptor downregulation through feedback mechanisms.[20] Moreover, the fluorinated structure of A1 contributed to its enhanced bioavailability and metabolic stability, reducing the need for higher doses and minimizing systemic side effects observed with other inhibitors. However, this study is still in the early stages, and further research is needed to assess drug's safety.

In the present study, we initially measured the cytotoxicity effects of A1 on 4T1 cells. Our data demonstrated that A1 induces cytotoxic effects across concentrations from 12.5 to 200 µg/mL. In previous studies, no significant reduction in overall cell viability was observed in human cholangiocarcinoma cancer cell lines treated with AMD3100.[41,42] Our data revealed that treatment with 102 µg/mL of A1 for 72 hours increases tumor cell apoptosis in 4T1 cells compared to untreated cells. Notably, the cytotoxic effects of A1 depend on the cell line type and the doses used.

Moreover, proliferation assay findings revealed that A1 treatment with CXCL12 decreased 4T1 cell proliferation compared to the CXCL12-only and A1-only treating group, demonstrating the target molecule considerably suppressed 4T1 cell growth. This finding highlights A1's potential as a promising candidate for inhibiting CXCL12-mediated proliferation in 4T1 cells, consistent with previous studies demonstrating the significant effect of CXCL12 on the growth of $CXCR4^+$ Ewing sarcoma cells under serum-starved conditions.[43,44] This proliferative influence was shown to be inhibited upon AMD3100 treatment *in vitro*.[43] Cell cycle progression through different phases has a crucial role in cell viability and proliferation, and cell cycle arrest can induce apoptosis.[45,46] This study revealed that threating 4T1 cells with IC_{50} concentration of A1 increased the DNA content in the G_2/M phase, accompanied by a remarkable decrease in the S phase, resulting in G_2/M phase arrest. A previous study demonstrated similar findings that treating the mouse colorectal cancer cell line with A1 inhibited DNA replication, leading to cell cycle arrest in the G_2/M phase.[20] The effects of A1 on apoptosis and cell cycle arrest in 4T1 cells may be attributed to its interference with crucial pathways regulated by CXCR4 signaling. Specifically, A1 binding to CXCR4 likely disrupts downstream activation of PI3K/Akt and MAPK pathways, which are well-documented mediators of cell survival and proliferation.[47,48] Inhibiting these pathways could result in mitochondrial dysfunction and activation of caspases, key events in the intrinsic apoptotic pathway.[49,50] Moreover, the induction of G2/M cell cycle arrest by A1 suggests its potential to impair DNA

replication or damage checkpoint controls.[51,52] These results highlight the beneficial impact of fluorinated anticancer compounds on cell cycle progression and apoptosis in tumor cells.

Flow cytometric analysis of 4T1 cells indicated a significant decrease in CXCR4 expression upon co-treatment with A1 and CXCL12 This finding is consistent with our previous study, in which A1 was shown to downregulate the expression of CXCR4 in CT26 colorectal cancer.[20] Previous studies have demonstrated the overexpression of CXCR4 in various types of human cancers, including kidney, brain, colon, breast, pancreas, melanomas, and ovaries, contributing in tumor growth, metastasis, and resistance to immunotherapy.[53,54] Moreover, increased CXCR4 expression observed in several cancers has been recognized as a poor prognostic biomarker.[55] Our flow cytometric data suggest that A1 may effectively inhibit CXCR4 receptor expression in 4T1 cells through exposure of CXCL12, highlighting its potential as a therapeutic strategy for modulating CXCR4-related pathways in this cell line.[56] Furthermore, chemokine receptors undergo internalization and degradation in the absence of ligand or blocking their receptors.[57] Accordingly,, our data demonstrated a significant reduction in surface $CXCR4^+$ cells, suggesting A1 accelerates receptor internalization or degradation via CXCR4 inhibition. Thus, the results of this study suggest that A1 possesses potential antitumor effects and could be considered a therapeutic option for breast cancer. To confirm these findings, further investigations in more advanced models and clinical trials are essential.

Due to the current study's limitations, further investigation will be necessary to discover the anti-tumor activity of A1 against other BCa cell lines and its possible implications in therapeutic perspectives. Moreover, the impacts of A1 therapy on the infiltration of various immune cells with different phenotypes, especially immunosuppressive cells, within the tumor milieu should be further explored to better understand the underlying mechanisms of CXCR4 inhibition following administration. Furthermore, experimental data on the pharmacokinetics and bioavailability of A1 remain unexamined in this study, yet these aspects are crucial for understanding its therapeutic potential and optimizing clinical application. Future research should include in vivo studies to evaluate these parameters and validate the current findings. While the current study focused on downstream effects like apoptosis and cell cycle arrest, our findings suggest A1 may modulate CXCR4-mediated pathways, such as MAPK and PI3K/Akt. These pathways will be investigated in future studies through Western blot and quantitative polymerase chain reaction (qPCR) analyses to elucidate the specific signaling alterations induced by A1. Although the use of the 4T1 mouse TNBC cell line is appropriate for in vitro testing, the manuscript does not explain why human TNBC cell lines (e.g., MDA-MB-231) for cross-species validation. Addressing this would enhance the relevance of A1 for human breast cancer treatment. This additional work will further support A1's therapeutic potential by defining its impact on CXCR4-mediated tumorigenesis.

In summary, the present investigation highlights the anti-tumor activity of a novel small molecule, A1, against CXCR4 through *in vitro* studies. This research reveals that A1 can promote cell apoptosis and induce cell cycle arrest at the G2/M phase in this cell line. Moreover, A1 and CXCL12 significantly reduce the proliferation of 4T1 cells compared to a control group treated with only CXCL12. Furthermore, the number of $CXCR4^+$ cells significantly decreased following the co-treatment of A1 and CXCL12. These results support the further investigation of A1 as a promising anti-tumor agent for breast cancer in preclinical and clinical settings.

STATEMENT OF ETHICS

This study was approved by the Animal Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1400.589).

FUNDING

Iran and Rafsanjan University of Medical Sciences supported this study. Grant Numbers: (1400-3-4-22145) and (31/20/1/401104)

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

This study is the Ph.D. thesis of the first author and was supported by Iran and Rafsanjan University of Medical Sciences.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Polyak K. On the birth of breast cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2001;1552(1):1-13.
2. Goh CY, Wyse C, Ho M, O'Beirne E, Howard J, Lindsay S, et al. Exosomes in triple negative breast cancer: Garbage disposals or Trojan horses? Cancer lett. 2020;473:90-7.
3. Ang L, Guo L, Wang J, Huang J, Lou X, Zhao M. Oncolytic virotherapy armed with an engineered interfering lncRNA exhibits antitumor activity by blocking the epithelial mesenchymal transition in triple-negative breast cancer. Cancer Lett. 2020;479:42-53.
4. Ogony JW, Radisky DC, Ruddy KJ, Goodison S, Wickland DP, Egan KM, et al. Immune responses and risk of triple-negative breast cancer: implications for higher rates among African American women. Cancer Prevent Res. 2020;13(11):901-10.
5. Standish LJ, Sweet ES, Novack J, Wenner CA, Bridge C, Nelson A, et al. Breast cancer and the immune system. J Society Integrative Oncol. 2008;6(4):158.
6. Arang N, Gutkind JS. G Protein-Coupled receptors and heterotrimeric G proteins as cancer drivers. FEBS lett. 2020;594(24):4201-32.
7. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and chemokine receptors: new targets for cancer immunotherapy. Front Immunol. 2019;10:379.
8. Newman LA, Jenkins B, Chen Y, Oppong JK, Adjei E, Jibril AS, et al. Hereditary susceptibility for triple negative breast cancer associated with western sub-saharan african ancestry: Results from an international surgical breast cancer collaborative. Ann Surgery. 2019;270(3):484-92.
9. Lai WY, Mueller A. Latest update on chemokine receptors as therapeutic targets. Biochem Society Trans. 2021;49(3):1385-95.
10. Zhou W, Guo S, Liu M, Burow ME, Wang G. Targeting CXCL12/CXCR4 axis in tumor immunotherapy. Curr Med Chemistry. 2019;26(17):3026-41.
11. Shi Y, Riese DJ, Shen J. The role of the CXCL12/CXCR4/CXCR7 chemokine axis in cancer. Front Pharmacol. 2020;11:574667.
12. Wu V, Yeerna H, Nohata N, Chiou J, Harismendy O, Raimondi F, et al. Illuminating the Onco-GPCRome: Novel G protein–coupled receptor-driven oncocrine networks and targets for cancer immunotherapy. J Biol Chemistry. 2019;294(29):11062-86.
13. Teicher BA, Fricker SP. CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res. 2010;16(11):2927-31.
14. Liang S, Peng X, Li X, Yang P, Xie L, Li Y, et al. Silencing of CXCR4 sensitizes triple-negative breast cancer cells to cisplatin. Oncotarget. 2015;6(2):1020.
15. Yang P, Liang S-X, Huang W-H, Zhang H-W, Li X-L, Xie L-H, et al. Aberrant expression of CXCR4 significantly contributes to metastasis and predicts poor clinical outcome in breast cancer. Curr Mol Med. 2014;14(1):174-84.
16. Zhao S, Wu B, Stevens RC. Advancing chemokine GPCR structure based drug discovery. Structure. 2019;27(3):405-8.
17. Zhou K, Xie L, Peng X, Guo Q, Wu Q, Wang W, et al. CXCR4 antagonist AMD3100 enhances the response of MDA-MB-231 triple-negative breast cancer cells to ionizing radiation. Cancer lett. 2018;418:196-203.
18. Hinton CV, Avraham S, Avraham HK. Role of the CXCR4/CXCL12 signaling axis in breast cancer metastasis to the brain. Clin Exp Metastasis. 2010;27:97-105.
19. Khalid M, Hussain MT, Hanif M, Qadeer G, Rama NH, Kornienko A, Khan KM. Synthesis and anti-cancer, anti-metastatic evaluation of some new fluorinated isocoumarins and 3, 4-dihydroisocoumarins. J Fluorine Chemistry. 2012;135:240-5.
20. Khorramdelazad H, Bagherzadeh K, Rahimi A, Safari E, Hassanshahi G, Khoshmirsafa M, et al. Antitumor activities of a novel fluorinated small molecule (A1) in CT26 colorectal cancer cells: molecular docking and in vitro studies. J Biomol Structure Dynamics. 2023:1-14.
21. He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, et al. The changing 50% inhibitory concentration (IC50) of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. Oncotarget. 2016;7(43):70803.
22. Ke B, Tian M, Li J, Liu B, He G. Targeting programmed cell death using small-molecule compounds to improve potential cancer therapy. Med Res Rev. 2016;36(6):983-1035.
23. Finn RS, Dering J, Ginther C, Wilson CA, Glaspy P, Tchekmedyian N, Slamon DJ. Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/“triple-negative”

breast cancer cell lines growing in vitro. Breast cancer Res Treat. 2007;105:319-26.
24. Zielińska KA, Katanaev VL. The signaling duo CXCL12 and CXCR4: Chemokine fuel for breast cancer tumorigenesis. Cancers. 2020;12(10):3071.
25. Liu T, Li X, You S, Bhuyan SS, Dong L. Effectiveness of AMD3100 in treatment of leukemia and solid tumors: from original discovery to use in current clinical practice. Exp Hematol Oncol. 2015;5:1-11.
26. Jung H, Paust S. Chemokines in the tumor microenvironment: implications for lung cancer and immunotherapy. Front Immunol. 2024;15:1443366.
27. Martins JRdS. The role of CXCR4 in cancer exosomes and metastasis 2018.
28. Mousavi A. CXCL12/CXCR4 signal transduction in diseases and its molecular approaches in targeted-therapy. Immunol Lett. 2020;217:91-115.
29. Chinni SR, Sivalogan S, Dong Z, Filho JCT, Deng X, Bonfil RD, Cher ML. CXCL12/CXCR4 signaling activates Akt-1 and MMP-9 expression in prostate cancer cells: the role of bone microenvironment-associated CXCL12. Prostate. 2006;66(1):32-48.
30. Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M, et al. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. Cancer cell. 2004;6(5):459-69.
31. Kittang AO, Kordasti S, Sand KE, Costantini B, Kramer AM, Perezabellan P, et al. Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. Oncoimmunology. 2016;5(2):e1062208.
32. D'agostino G, Saporito A, Cecchinato V, Silvestri Y, Borgeat A, Anselmi L, Uguccioni M. Lidocaine inhibits cytoskeletal remodelling and human breast cancer cell migration. British J Anaesthesia. 2018;121(4):962-8.
33. Lefort S, Thuleau A, Kieffer Y, Sirven P, Bieche I, Marangoni E, et al. CXCR4 inhibitors could benefit to HER2 but not to triple-negative breast cancer patients. Oncogene. 2017;36(9):1211-22.
34. Xu C, Zhao H, Chen H, Yao Q. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. Drug Des Devel Ther. 2015:4953-64.
35. Tamamura H, Hori A, Kanzaki N, Hiramatsu K, Mizumoto M, Nakashima H, et al. T140 analogs as CXCR4 antagonists identified as anti-metastatic agents in the treatment of breast cancer. FEBS lett. 2003;550(1-3):79-83.
36. Dorsam RT, Gutkind JS. G-protein-coupled receptors and cancer. Nat Rev Cancer. 2007;7(2):79-94.
37. Cavnar S, Ray P, Moudgil P, Chang S, Luker K, Linderman J, et al. Microfluidic source-sink model reveals effects of biophysically distinct CXCL12 isoforms in breast cancer chemotaxis. Integrative Biol. 2014;6(5):564-76.
38. Hirbe AC, Rubin J, Uluçkan Ö, Morgan EA, Eagleton MC, Prior JL, et al. Disruption of CXCR4 enhances osteoclastogenesis and tumor growth in bone. Proc Natl Acad Sci U S A. 2007;104(35):14062-7.
39. Larochelle A, Krouse A, Metzger M, Orlic D, Donahue RE, Fricker S, et al. AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. Blood. 2006;107(9):3772-8.
40. Luo Z, Wang B, Chen Y, Liu H, Shi L. Novel CXCR4 Inhibitor CPZ1344 Inhibits the Proliferation, Migration and Angiogenesis of Glioblastoma. Pathol Oncol Res. 2020;26:2597-604.
41. Leelawat K, Keeratichamroen S, Leelawat S, Tohtong R. CD24 induces the invasion of cholangiocarcinoma cells by upregulating CXCR4 and increasing the phosphorylation of ERK1/2. Oncol lett. 2013;6(5):1439-46.
42. Mayr C, Neureiter D, Pichler M, Berr F, Wagner A, Kiesslich T, Namberger K. Cytotoxic effects of chemokine receptor 4 inhibition by AMD3100 in biliary tract cancer cells: Potential drug synergism with gemcitabine. Mol Med Rep. 2015;12(2):2247-52.
43. Berghuis D, Schilham MW, Santos SJ, Savola S, Knowles HJ, Dirksen U, et al. The CXCR4-CXCL12 axis in Ewing sarcoma: promotion of tumor growth rather than metastatic disease. Clin Sarcoma Res. 2012;2:1-9.
44. Liao Y-X, Fu Z-Z, Zhou C-H, Shan L-C, Wang ZY, Yin F, et al. AMD3100 reduces CXCR4-mediated survival and metastasis of osteosarcoma by inhibiting JNK and Akt, but not p38 or Erk1/2, pathways in in vitro and mouse experiments. Oncol Rep. 2015;34(1):33-42.
45. Qiu J, Zhao B, Shen Y, Chen W, Ma Y, Shen Y. A novel p-terphenyl derivative inducing cell-cycle arrest and apoptosis in MDA-MB-435 cells through topoisomerase inhibition. Europ J Med Chemistry. 2013;68:192-202.
46. Goodell JR, Ougolkov AV, Hiasa H, Kaur H, Remmel R, Billadeau DD, Ferguson DM. Acridine-based agents with topoisomerase II activity inhibit pancreatic cancer cell proliferation and induce apoptosis. J Med Chemistry. 2008;51(2):179-82.
47. Morein D, Erlichman N, Ben-Baruch A. Beyond cell motility: the expanding roles of chemokines and their receptors in malignancy. Front Immunol. 2020;11:952.
48. McConnell AT. Implications of MEK and VEGFR2 inhibition on melanoma CXCR4-CXCL12 chemotaxis, survival and tumour progression: Newcastle University; 2017.

49. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977-92.
50. Esmaeili MA, Farimani MM, Kiaei M. Anticancer effect of calycopterin via PI3K/Akt and MAPK signaling pathways, ROS-mediated pathway and mitochondrial dysfunction in hepatoblastoma cancer (HepG2) cells. Mol Cell Biochemistry. 2014;397:17-31.
51. Löbrich M, Jeggo PA. The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. Na Rev Cancer. 2007;7(11):861-9.
52. Wang F-Z, Fei H-r, Cui Y-J, Sun Y-K, Li Z-M, Wang X-Y, et al. The checkpoint 1 kinase inhibitor LY2603618 induces cell cycle arrest, DNA damage response and autophagy in cancer cells. Apoptosis. 2014;19:1389-98.
53. Balkwill F, editor The significance of cancer cell expression of the chemokine receptor CXCR4. Seminars Cancer Biol; 2004: Elsevier.
54. Darash-Yahana M, Pikarsky E, Abramovitch R, Zeira E, Pal B, Karplus R, et al. Role of high expression levels of CXCR4 in tumor growth, vascularization, and metastasis. FASEB J. 2004;18(11):1240-2.
55. Ishikawa T, Nakashiro K-I, Klosek SK, Goda H, Hara S, Uchida D, Hamakawa H. Hypoxia enhances CXCR4 expression by activating HIF-1 in oral squamous cell carcinoma. Oncol Reports. 2009;21(3):707-12.
56. Spinello I, Quaranta M, Riccioni R, Riti V, Pasquini L, Boe A, et al. MicroRNA-146a and AMD3100, two ways to control CXCR4 expression in acute myeloid leukemias. Blood Cancer J. 2011;1(6):e26-e.
57. Neel NF, Schutyser E, Sai J, Fan G-H, Richmond A. Chemokine receptor internalization and intracellular trafficking. Cytokine Growth Factor Rev. 2005;16(6):637-58.