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The Effect of Interferon-Inducible T Cell Alpha Chemoattractant (I-TAC) on Transplanted Mice with Breast Cancer

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Abstract

Background Interferon-inducible T-cell alpha chemoattractant (I-TAC, CXCL11) is a novel chemokine that used as immunotherapy. CXCL11 develops an effective anti-tumor immune response that relies on the coordinated interactions between immunocompetent cells, suppress angiogenesis and leading to an anti-tumor effect.

Objective To investigate the anti-tumor effect of CXCL11 on breast cancer transplanted in an animal's model.

Methods CXCL11 adoptive immunotherapy inducible, was intratumorally injected in mice breast cancer model one dose weekly for three weeks, each dose concentration 20 ng/100µl. Weight of mouse and size of tumor were recorded every 48 hr until 25 days. Parameters examined on mice transplanted with murine mammary adenocarcinoma (AMN3) cells (to study the therapeutic of CXCL11 in vivo) were included growth inhibition (GI%), relative tumor volume (RTV), relative mice weight (RMW) and survival time in mice.

Results In-vivo CXCL11 treatment showed to induce GI% from day 3 and continued to day 25 with significant increase in mice weight in comparison to non-treated control group during 25 days ($P<0.0001$). Also, the CXCL11 treatment exhibited a significant reduction in RTV and improving survival time in mice.

Conclusion CXCL11 showed an anti-cancer effect on breast cancer AMN3 implanted in mice compared to control. CXCL11 may have immunotherapeutic effect on breast cancer by triggering the activation of immunity against cancer.

Keywords Breast cancer, CXCL11, immunotherapy, AMN3

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List of abbreviations: AMN3 = Murine mammary adenocarcinoma cell line, CCR3 = Cysteine-cysteine chemokine receptor-3, CTLs = Cytotoxic T lymphocytes, CXCL11 = C-X-C motif chemokine ligand 11, CXCR3 = C-X-C motif chemokine receptor 3, GI% = Growth inhibition, IFN-α = Interferon alpha, IFN-β= Interferon beta, IFN-γ = Interferon gamma, IL-2 = Interleukin-2, I-TAC = Interferon-inducible T-cell alpha chemoattractant, NK= Natural killer cells, NKT = Natural killer T, RTV = Reducing relative tumor volume, Th= T helper, TNF-α = Tumor necrosis factor alpha

Introduction

Cancer immunotherapy is considered a new cornerstone in cancer treatment using the patient's immune system to fight cancer [(1)]. The success of immunotherapy can be explained by the enormous complexity of interactions between tumor cells and the immune system [(2-4)]. Migration of the immune cells to specific organs is controlled in part by small proteins called chemokines (i.e.,

chemotactic cytokines) [5,6]. The C-X-C motif (CXC) chemokine have two N-terminal cysteines separated by one amino acid "X". There have been 17 different CXC chemokines described in mammals. CXC chemokines are classified into two groups; with and without Glu-Leu-Arg (ELR) motif [7]. Those with the ELR motif can allow neutrophils to migrate and have an angiogenic effect, whereas those without the ELR motif primarily allow lymphocytic migration and inhibit angiogenesis [8].

C-X-C motif chemokine 11 (CXCL11) is ELR-negative CXC chemokines that attenuate angiogenesis, leading to an anti-tumor effect. However, some reports show that CXCL11 increases tumor proliferation and metastases [8]. CXCL11 is mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells in response to interferon gamma (IFN-γ), synergistically enhanced by tumor necrosis factor alpha (TNF-α) [9,10] bind to C-X-C motif chemokine receptor 3 (CXCR3), which is a receptor preferentially expressed on the surface of monocytes, T cells, Natural killer (NK) cells, dendritic cells and cancer cells [11,12].

CXCL11, also known as an interferon-inducible T-cell alpha chemoattractant (I-TAC) or interferon-gamma-inducible protein 9 (IP-9), is induced by IFN-γ and interferon beta (IFN-β), and weakly induced by interferon alpha (IFN-α) [13]. The binding domain of CXCL11 on CXCR3 is located at a different site from that of CXCL9 and CXCL10 [14]. CXCL11 can bind to CXCR7, which is associated with invasiveness and reduces apoptosis of tumor cells [15]. For immune cell migration, each of the CXCR3 ligands is equally effective on activated T helper-1 (Th1) cells, cytotoxic T lymphocytes (CTLs) and NK cells in vivo models of cell recruitment [16,17].

CXCL11 attract Th1 cells and block the migration of Th2 cells in response to cysteine-cysteine chemokine receptor-3 (CCR3) ligands due to their ability to serve as antagonists for CCR3 [18]. On the other hand, NK cell subsets, the anti-tumor effectors that express CXCR3, are also recruited to the site in a CXCR3-dependent manner [17]. For immune cell activation, CXCL11 stimulates immune cells through Th1 polarization and activation. Th1 cells produce IFN-γ, TNF-α, interleukin-2 (IL-2) and enhance anti-tumor immunity by stimulating CTLs, NK cells, natural killer T (NKT) cells and macrophages [19,20]. The IFN-γ-dependent immune activation loop also promotes CXCL11 release. Importantly, NK cells can display immune activity by modulating dendritic cell function and also provide an early source for IFN-γ production [17]. Naturally, immune cells, mainly Th1, CTLs, NK cells and NKT cells, show an anti-tumor effect against cancer cells through paracrine CXCL9, CXCL10, CXCL11, and their receptor (CXCR3) axis in tumor models [21-23].

The research objective was to characterize the in-vivo antitumor effect induced by CXCL11 on breast cancer transplanted mice.

Methods

Animals

Ten female healthy mice, three weeks old, weighed between 15-26 g, obtained from the animal house of Iraqi Center for Cancer and Medical Genetics Research (ICCMGR). All animals were housed in individual cages designed for changing sawdust and feed. The temperature-controlled at 20-30°C with relative humidity ranging from 40-65%, the light:dark period of 12 hr. The adult female mice back were injected with sterile murine mammary adenocarcinoma cell line (AMN3) suspension according to Al-Shammari [24], where was supplied from ICCMGR, the tumor cells were taken from a tumor-bearing mouse used to obtain tumor-cell suspension that transplanted into other mice. The animals were distributed randomly into two groups, one control group and one treated group with CXCL11, each consisting of five mice.

Mouse C-X-C Motif Chemokine 11/I-TAC (CXCL11)

Interferon-inducible T cell alpha - chemoattractant (I-TAC; CXCL11), adoptive

immunotherapy inducible. This chemokine produced by Abbexa, UK. (Catalogue No. abx261542). The origin of chemokine from mouse and it expressed as recombinant from *Escherichia coli*. It was reconstituted in sterile 18 MΩ · cm water to a concentration of 200 µg/ml. After reconstitution, it was stored at below -18°C for long-term storage, and at 4°C for up to 7 days, for immediate use.

Experimental design

Mice were intratumorally injected by CXCL11 one dose weekly for three weeks, each dose concentration 20 ng/100 µl. Via intratumoral injection, with a recorded weight of mouse and size of tumor every 48 hr until 25 days, parameters examined on mice transplanted with AMN3 cells to study the therapeutic effect of CXCL11 in vivo by investigation growth inhibition (GI%), relative tumor volume (RTV), relative mice weight (RMW) and survival time in mice.

Statistical analysis

Graph Pad Prism 7.0 software was analyzed using the unpaired T-test method. P-value of <0.05 was considered to be significant.

Results

Effect of CXCL11 on the GI% of breast cancer in experimental animals

Tumor volume was calculated using vernier to measure tumor length and width, and by using the following equation: tumor volume = (Length)*(Width)²/2. Figure (1) shows an increase in GI% during the period of the experiment. GI% started with 40.67% and continue to increase to reach 70% on day 17, from day 17 until day 25 there was a slight increase to reach approximately 77% on day 25. The highest GI% was reached in the fourth week of experiment as shown in figure (1, A and B).

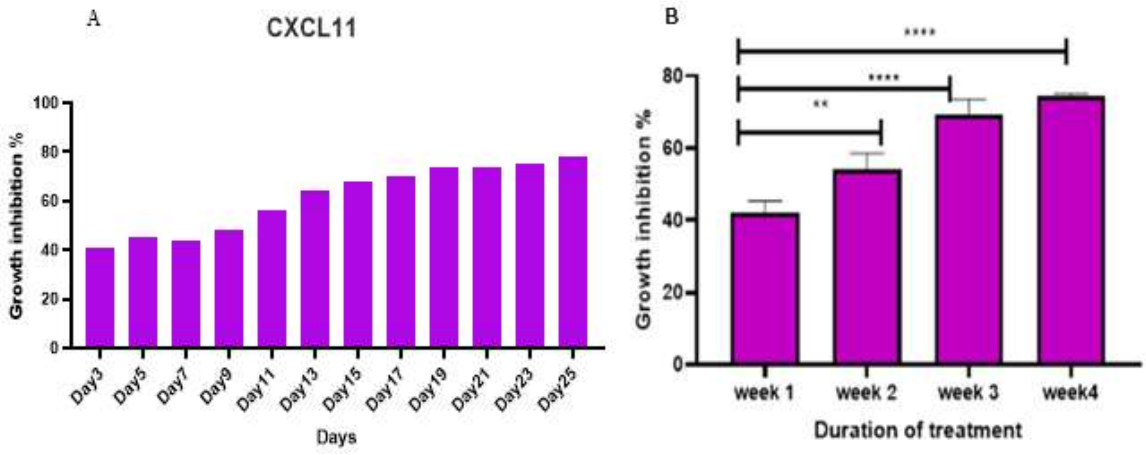

Figure 1. A) The growth inhibition of CXCL11 treatment started on day 3 and continued to day 25. B) Comparison between growth inhibition of CXCL11 treatment group for four weeks. Values represent the (mean±SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.0001

Effect of CXCL11 on the weight of experimental animals

Mice weight was used as parameter for toxicity of CXCL11 treatment in this study. Evaluation of the RMW of experimental animals throughout the experimental period after intratumoral injection with CXCL11 and control groups was achieved by using electronic balance and the equation: RMW (day x) = mouse weight in (day x) / mouse weight in (day 0) X 100. RMW value was recorded for each group and CXCL11 treatment appeared to have no cytotoxic effect on mice compared to non-treated

control group during the period of experiment (Figure 2, A and B).

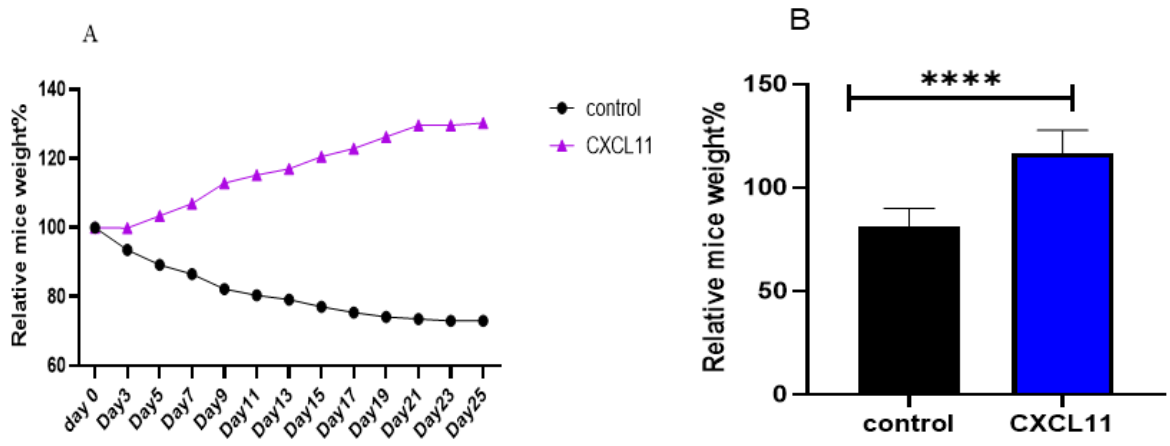

Figure 2. A) The relative mice weight to control and CXCL11 for 25 days. B) Comparison between non treated control and in-vivo CXCL11 treatment group depending on accumulative relative mice weight percentage. Values represent the (mean±SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.000

Effect of CXCL11 on the relative tumor volume

To analyze the effects of CXCL11 treatment, RTV for each group from day zero before treatment (considered as 100%) and for each treatment day was calculated by the following equation: RTV (day x) = tumor volume (day x) / tumor volume (day 0) X 100. The result showed a decrease in RTV during experiment compared to control (Figure 3A), and highly significant difference between two groups at the end of the overall experiment (Figure 3B).

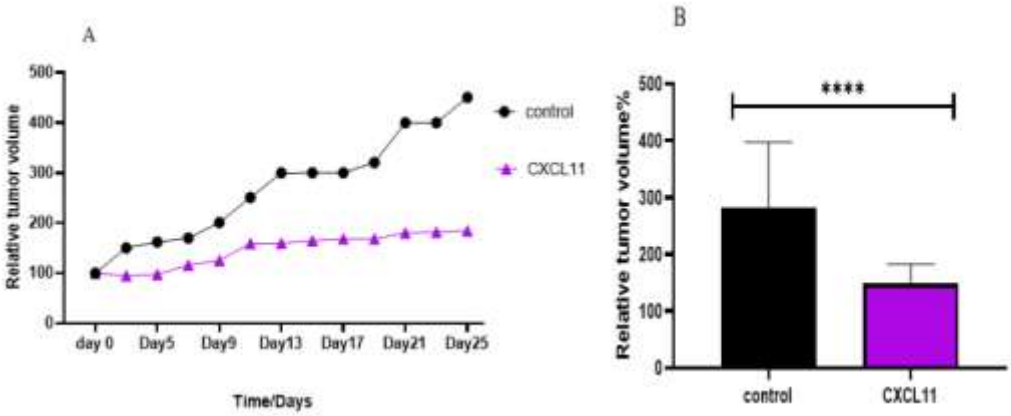

Figure 3. A) The relative tumor volume during treatment period. B): The comparison among groups depending on accumulative relative tumor volume percentage. Values represent the (mean ± SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.0001

Survival time in mice model

The survival time between treatment group throughout the experiment explained in Figure (4), which showed a difference in the number of dead mice and prolong survival. In the control group, one mouse was died in the second week, and one was dead at the end of the third week, and three mice remained to live until the end of the experiment. For CXCL11 treated group one mice died at the end of the third week, and one mouse showed complete healing from cancer in the middle of the second week.

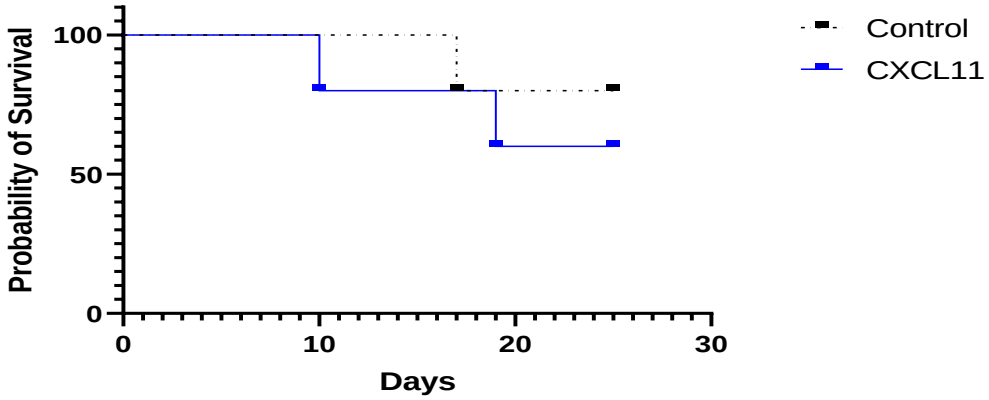

Figure 4. Prolong surviving in mice model

Discussion

In this study, a comparison between the non-treated control group and the CXCL11 treated group showed an anti-tumor effect of CXCL11, and this may be attributed to the presence of CXCL11 in the tumors. A previous study found that high concentration of CXCL11 production in tumor tissue compared to normal adjacent give good prove of healing [25].

The current study also showed that the tumor volume was enlarged in size during the treatment period of the control group. While, the CXCL11 treated group exhibited a steady ratio of tumor volume from day 10 until the end of experiment, when compared depending on accumulative tumor volume, a highly significant difference was shown between the CXCL11 treated group compared to the non-treated control group. This could be explained by the facts that CXCL11 might be able to reset the tumor microenvironment (TME) by modulating CD8+ T cell accumulation, tumor antigen-reactive T cells play a key role in eradicating tumors. In addition, CXCL11 promotes the entry of type-1 effector cells (CTLs, Th1 and NK cells) into inflamed or tumor tissues. CXCL11 is important for the T-cell attraction (effector phase) and the development of adaptive immunity (induction phase). Modulation of TME was achieved via CXCL11-mediated inhibition of suppressor factors, such as decreased transforming growth factor beta (TGF-b), cyclooxygenase-2 (COX2), and chemokine ligand 22 (CCL22). CXCL11 induces an immunotolerizing drives CD4+ T cell polarization into IL-10 high T regulatory (Tr-1) and IL-4high Th2 cells. Also, CXCL11 contributes to inhibiting angiogenesis and tumor progression. CXCL11 suppression tumor growth with subsequently increased survival rates in a therapeutic tumor model [25-31] is in consistent with current result.

Current study showed that there was decrease in mice weight in non-treated control group, while an increase in mice weight was observed in CXCL11 treated group. Also, CXCL11 treated group showed highly significant differences in mice weight in compared to non-treated control group during 25 days, this indicated that CXCL11 treatment has a little cytotoxic effect on mice throughout the entire period of experiment. This is in agreement with another

study that found that mice that received intratumoral injections of CXCL11-armed oncolytic adenoviruses gained steadily weight than mice in the control group and mice that got only oncolytic adenoviruses. The chemokine CXCL11 may cause changes in the types of immune cells within the TME. CXCL11 can increase the chemotaxis of activated T cells and NK cells and potentially suppress M2 macrophage polarization in a murine cancer model. The presence of cytotoxic CD8+ T cells, NK cells, and M1 macrophages within the TME is generally associated with regression of tumors as well as a favorable prognosis [(32)].

In addition to its beneficial effects on reducing tumor volume, CXC11 treatment resulted in a longer life span than the untreated control group. One mouse also showed fully cancer recovered by the middle of the second week after treatment (Figure 4). Another study found that mice given saline as a control developed tumors quickly and died within 30 days, but that a single injection of CXCL11-armed oncolytic adenoviruses considerably slowed tumor growth and increased survival time compared to control [(32)]. In breast cancer, high CXCL11 was determined to be positively correlated with immune response activation, increased antitumour immune cell infiltration, immune checkpoint molecule expression, and enhanced sensitivity to immunotherapy and chemotherapy [(33)].

In conclusion, growth inhibition of cancer and decrease in RTV with no toxicity effect of CXCL11 on mice weight; together these results provide important insights into the fact that CXCL11 could be used as an anti-cancer immunotherapy treatment.

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Author contribution

As part of her PhD thesis, Dr. Mohammed performed the laboratory work and wrote the draft of this study. Dr. Al-Shuwaikh and Prof. Dr. Al-Shammari designed, supervised and co-wrote this manuscript. The final version of this manuscript was read and approved by all authors.

Conflict of interest

The authors reported no potential conflict of interest.

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