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Effect of Angiogenesis Related Growth Factors VEGF-a and FGF-1 on Osteosarcoma Cell Proliferation

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Abstract

Osteosarcoma is the most common bone tumor in children and adolescents. Alterations in the expression of some genes, cytokines and growth factors are responsible for the development of the malignant phenotype in osteosarcoma. Some members of the VEGF and FGF families have been associated with poor prognosis and the metastasis in various tumor types including osteosarcoma. Among the members of the family, information about effects of VEGF-a and FGF-1 on osteosarcoma cell proliferation is limited.

In the present study, it was aimed to elucidate the effects of VEGF-a and FGF-1 on osteosarcoma cell proliferation. Saos-2 cells, having osteoblastic features, were used for osteosarcoma model. Cells were treated 20 ng/mL VEGF-a and FGF-1 after 24 hours of serum starvation. MTT assay was applied to measure cell viability after incubation for 1-72 hours. Results indicated that VEGF-a promoted cell proliferation for all incubation times. Maximum increase was observed after 48 hours of incubation (1.7 fold) with a statistically important manner. FGF-1 led to very slight increase in Saos-2 cell proliferation. Consequently, these findings can contribute development of new therapy strategies for osteosarcoma.

Keywords: Osteosarcoma; Saos-2; MTT; VEGF-a; FGF-1.

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Anjiyogenez Bağlantılı Büyüme Faktörleri VEGF-a ve FGF-1'in Osteosarkoma Hücre Proliferasyonu Üzerine Etkileri

Öz

Osteosarkoma çocuklar ve ergenlerde en sık görülen kemik tümörüdür. Bazı genlerin, sitokinlerin ve büyüme faktörlerinin ekspresyonundaki değişiklikler osteosarkomada malign fenotipin gelişiminden sorumludur. VEGF ve FGF ailelerinin bazı üyeleri, osteosarkomayı da içeren çeşitli tümör tiplerinde kötü prognoz ve metastaz ile ilişkilendirilmiştir. Ailenin üyelerinden VEGF-a ve FGF-1'in osteosarcoma hücre proliferasyonuna olan etkileri hakkında bilgiler sınırlıdır.

Bu çalışmada VEGF-a ve FGF-1'in osteosarkoma hücre proliferasyonu üzerindeki etkilerinin aydınlatılması amaçlanmıştır. Osteosarkoma modeli olarak osteoblastik özelliklere sahip Saos-2 hücreleri kullanıldı. Hücreler, 24 saatlik serum açlığından sonra 20 ng/mL VEGF-a ve FGF-1 ile muamele edildi. 1-72 saat inkübasyon süresinden sonra hücre canlılığını ölçmek için MTT testi uygulandı. Sonuçlar VEGF-a'nın tüm inkübasyon süreleri için hücre proliferasyonunu arttırdığını gösterdi. Maksimum artış, istatistiksel olarak anlamlı bir şekilde, 48 saatlik inkübasyon (1.7 kat) süresinden sonra gözlendi. FGF-1, Saos-2 hücre proliferasyonunda çok küçük bir artışa yol açtı. Sonuç olarak, bu bulgular osteosarkom için yeni tedavi stratejilerinin geliştirilmesine katkıda bulunabilir.

Anahtar Kelimeler: Osteosarkoma; Saos-2; MTT; VEGF-a; FGF-1.

1. Introduction

Osteosarcoma is a highly malignant primary bone tumor characterized by aggressive growth and early metastatic potential, which mainly affects children and adolescents [1-5]. Immature bone or osteoid tissue formation are characteristic features of the osteosarcoma. Lung and bone are the most common metastasis sites [6, 7]. Metastasis leads to poor prognosis for tumor progression. Current therapies have quite a few effects on metastatic osteosarcoma patients [8, 9]. Therefore, elucidation of the molecular mechanisms underlying osteosarcoma pathogenesis and metastasis may be beneficial for developing more effective treatment strategies [10]. Cells with different levels of differentiation lead to heterogeneous tissue formation in bone. Tissue heterogeneity is closely related to the success of the response to treatment. Cytokines can affect cancer formation, progression, and metastasis at different stages [1, 11, 12]. While some cytokines have been used for cancer treatment, some of them contribute to advanced cancer development

[13-16]. Understanding of the cytokine networks in oncogenesis has prognostic importance for the course and progression of osteosarcoma [17].

Vascular endothelial growth factor (VEGF) is a member of a large growth factor family and has a critical function for the induction of the angiogenesis. Angiogenesis is a key regulator for tumor development and metastasis. It has been shown that increased VEGF expression is related to poor prognosis in many tumor types [18-26]. It has been reported that VEGF variants and their receptors were expressed in osteosarcoma cells and promote tumor angiogenesis [27-29].

FGF-1 (Fibroblast Growth Factor) is another important factor related to tumorigenesis, epithelial-to-mesenchymal transition, as well as invasion and metastasis. Dysregulation of the FGF receptors has been reported in many cancer types such as urothelial carcinoma, hepatoma, ovarian cancer, and lung adenocarcinoma. Clinical studies have been indicated that blocking the FGF/FGFR signaling may enable the development of a new strategy for the treatment of various human cancers. Therefore, numerous FGFR inhibitors were developed for therapy [30-33]. The effect of some FGF family members on osteosarcoma progression has been investigated so far. For instance, it has been reported that upregulation of the FGF-5 induces osteosarcoma cell proliferation [34].

Although critical functions of some cytokines have been determined on the pathogenesis of the osteosarcoma, little is known about angiogenesis-related growth factors VEGF-a and FGF-1 on the contribution of osteosarcoma proliferation. The current study is aimed to investigate the antiproliferative effect of these growth factors on osteosarcoma. Saos-2 cell model, displaying osteoblastic features, was used for proliferation studies. Cells were treated 20 ng/mL of VEGF-α and FGF-1 and cell viability was measured using the MTT method. Our findings will provide new insights for the development of new therapeutic approaches for osteosarcoma.

2. Materials and Methods

2.1. Cells and reagents

Saos-2 (Human osteosarcoma) cell line was a gift from Dr. Kenneth Wann (Cardiff, School of Biosciences, Cardiff UK). Cell culture media and reagents; DMEM (Dulbecco's Modified Eagle's Medium) and PBS (Phosphate Buffer Saline) tablets were purchased from Sigma, FCS (Fetal Calf Serum) was purchased from Invitrogen, VEGF-a and FGF-1 were purchased from Peprotech, MTT (Thiazolyl Blue Tetrazolium Bromide) and other chemicals that were used in the study were purchased from Sigma.

2.2 Cell culture and MTT assay

Saos-2 cells were grown in DMEM containing 10 % FCS and 2 mM L-Glutamine and maintained in a humidified incubator at 37˚C containing 5 % (v/v) CO_2 in air. The effect of VEGF-a and FGF-1 on cell viability was assessed by the MTT method. Briefly, Saos-2 cells were plated out in 96-well plastic plates at a concentration of $5x10^4$ cells per well and stimulated with 20 ng/mL of VEGF-a and FGF-1 for 1, 24, 48, and 72 h. Untreated groups were used as control. MTT solution was added to the cells after indicated incubation time intervals. Formazan crystals were solubilized with 200 µl Isopropanol including 0.01% per well and the optical density (OD) at 550 nm was measured with a spectrophotometric microplate reader (Thermo Scientific). The mean OD value of the control groups was subtracted from the OD value of each cytokine treated groups for all incubation times. Experiments were performed for three times [35].

2.2 Statistical analysis

Statistical analyses were performed using Mini Tab 14. Standard deviations and p values were calculated using One-way Anova analysis. Statistical significance was taken as $p \leq 0.05$. Data are representative of average results from independent experiments carried out at least three times and are expressed as the mean ± standard deviation [36].

3. Results

3.1 Effect of VEGF-a and FGF-1 on Saos-2 cell proliferation

To determine the effect of VEGF-a and FGF-1 on osteosarcoma proliferation we used Saos-2 (Sarcoma osteogenic) cell line for *in vitro* model. These cells are well characterized primary osteosarcoma model that is commonly used in bone cancer research for the late osteoblastic differentiation stage [37, 38]. Cells were serum-starved for 24 h using 0.1% BSA before the growth factor treatment to observe the effect more clearly. Saos-2 cells were treated with 20 ng/mL of VEGF-a and FGF-1 for 1-72 hours. MTT analysis was performed as described in Section 2.2 after indicated time intervals. VEGF-a treatment increased Saos-2 cell proliferation for all time intervals. According to statistical analysis, a maximum increase in cell proliferation was observed after 48 hours of incubation (1.7-fold).

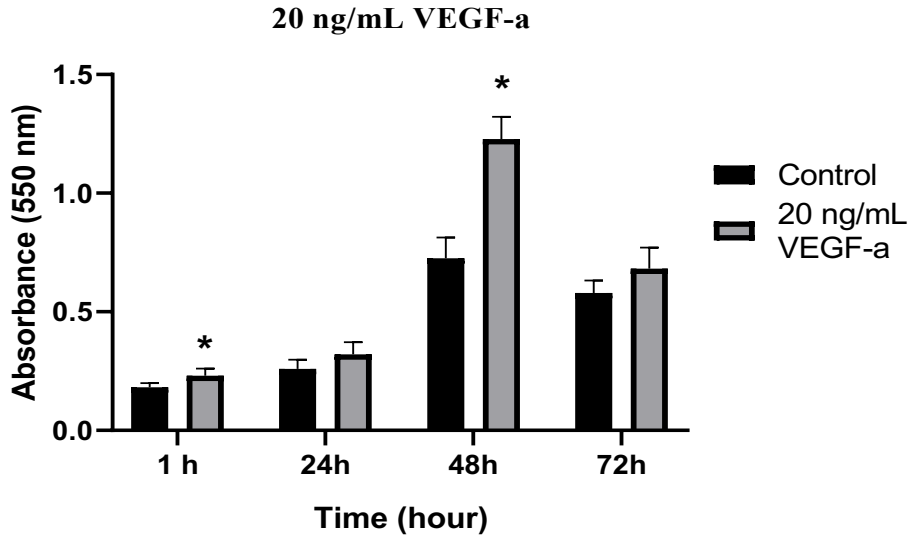

Figure 1: Effect of 20 ng/mL of VEGF-a treatment on Saos-2 cell proliferation. Untreated cells were used as the control for 1, 24, 48, and 72 h. Statistical analysis was carried out by ANOVA (one way) (Statistical significance was taken as * $p \leq 0.05$). The image shown is average results from independent experiments carried out at least three times

We also assessed the proliferative effect of the other angiogenesis-related growth factor FGF-1 on Saos-2 cells by MTT as described above. Treatment of the Saos-2 cell with 20 ng/mL of FGF-1 led to a very slight increase in cell proliferation. A maximum increase was observed after 72 hours of incubation (1.2 fold).

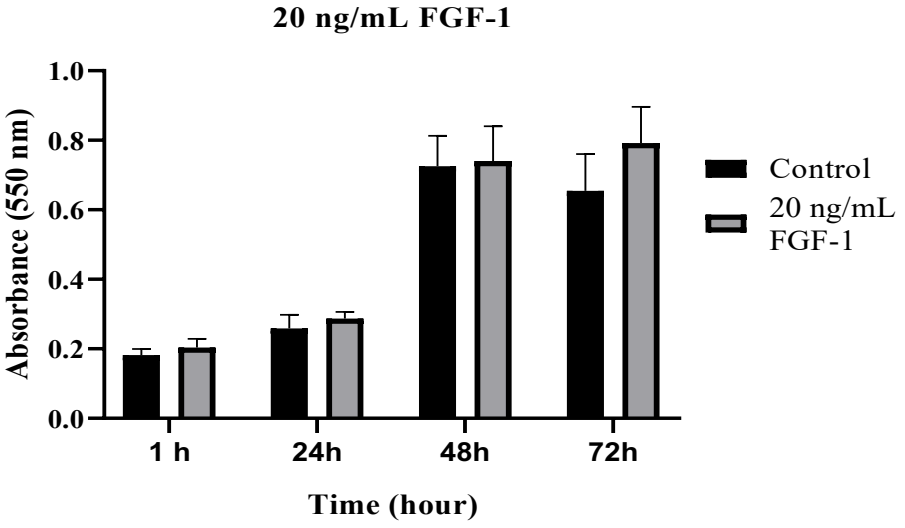

Figure 2: Effect of 20 ng/mL of FGF-1 treatment on Saos-2 cell proliferation. Untreated cells were used as control groups for the indicated time. The p-value was calculated by ANOVA (one way) analysis ($p > 0.05$). The image shown is the average results from independent experiments carried out at least three times

4. Discussion

The development of new blood capillaries is an important issue for tumor growth and metastasis [1]. Angiogenesis involves whole processes of the malignant osteosarcoma. VEGF, aFGF and bFGF are angiogenesis-related growth factors and released from tumor tissues and contribute to tumor progression. They have the ability to stimulate angiogenesis under hypoxic conditions. VEGF promotes cell migration by initiating a variety of signaling pathways. It is also a mediator of vessel permeability and enables endothelial progenitor cells to migrate from the bone marrow to neovascularization sites. It has been reported that increased VEGF production is important in the growth of solid tumors including osteosarcoma. Peng and colleagues were determined that silencing of the VEGF inhibited osteosarcoma growth and angiogenesis in Wistar rat model. Besides, they were established that the silencing of VEGF expression could promote apoptosis in Saos-2 cells [29, 39]. VEGF-a mainly conducts its pro-angiogenic activities through VEGFR-2 (VEGF receptor 2). Because of its critical role in tumor angiogenesis, the VEGF/VEGFR pathway has become a therapeutic target in cancer research [39, 40].

The human FGF family includes 23 members and they are expressed in many tissues. FGFs regulate a broad spectrum of physiological and pathological processes, including development, wound healing and neoplastic transformation. Further, FGFs play important roles in tumorigenesis increasing cell migration and invasion. For instance, FGF2, has angiogenic potential and promote tumor angiogenesis [42]. Shimizu and colleagues have determined that FGF-2 has function maintaining the aggressive profile of the osteosarcoma affecting cellular immaturity They have suggested that blocking of growth factor signaling pathways including FGF-2, might be useful in controlling the aggressiveness of osteosarcoma [1]. Han and colleagues reported a significant upregulation in FGF-5 expression levels in osteosarcoma cell models and patient tissues, compared to control cells and healthy tissues. Further, they presented evidence on FGF5 promoted osteosarcoma cell proliferation [34]. The FGFs promote tumor progression through specific (FGFR) FGF receptor signaling. The expression of *FGFR* genes has been detected in osteosarcoma, such as *FGFR1*, *FGFR2,* and *FGFR3*. However, the expression profile of FGFRs differs among the osteosarcoma cell models. FGFR1 expression is associated with poor response to chemotherapy in osteosarcoma. High expression levels of FGF2 and its receptor FGFR3 have been found in osteosarcoma and are significantly related with poor prognosis [4].

FGF1 has been determined as a proliferative factor for human preadipocytes and IEC-6, Caco-2, and HT-29 cell lines [43]. Although the proliferative effects of FGFs on different cell types have been studied, the effect of FGF-1 on osteosarcoma cell proliferation is unknown.

Because of the critical roles of the VEGF and FGF family on tumor growth and progression antiproliferative effect of these growth factors on the osteosarcoma was assessed. VEGF-a promoted Saos-2 cell proliferation gradually up 1.7 fold for 48 hours. This proliferative effect was started to decrease after 72 hours of incubation. FGF-1 had a slight effect on the proliferation of the osteosarcoma cells. In conclusion, in this work, we demonstrated that treatment of Saos-2 osteosarcoma cell with VEGF-a and FGF-1 in the absence of other growth factors activated cellular proliferation. These effects could contribute to the poor prognosis of osteosarcoma. Overexpression and/or silencing studies will provide more direct evidence for the contribution of these factors to osteosarcoma development.

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