IL-32 promotes breast cancer cell growth and invasiveness

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Abstract

Interleukin (IL)-32 is a newly identified cytokine in humans and primates. It has been established that IL-32 may antagonize cancer growth. However, to the best of our knowledge, the direct effect of IL-32 on breast cancer cell growth has not yet been investigated. In addition, rodents lack the expression of IL-32; hence, the effects of IL-32 on breast cancer xenografts in nude mice have not been studied. This study aimed to examine the potential regulatory effects of IL-32 on breast cancer cell lines and a tumor xenograft model, as well as the effects of IL-32 on apoptosis. The effects of IL-32 on cell proliferation and apoptosis were investigated by MTT assay and TUNEL staining, respectively. The results revealed that IL-32 increased the proliferation rate of cancer cells and decreased the rate of apoptosis. In addition, IL-32 was found to enhance the growth of tumor xenografts in vivo. In summary, IL-32 may represent a useful therapeutic target for human breast cancer.

Introduction

Interleukin (IL)-32, or NKA, is a novel cytokine which was originally isolated from activated T cells. Treatment of lymphocytes with IL-32 may induce the expression of different cytokines, including tumor necrosis factor (TNF)-α or IL-8, which have been found to be involved in multiple inflammatory processes and cancer progression. Notably, a gene homolog of IL-32 was not found in rodents, hence previous study of IL-32 may antagonize cancer growth. However, to the best of our knowledge, the direct effect of IL-32 on breast cancer cell growth has not yet been investigated. In addition, rodents lack the expression of IL-32; hence, the effects of IL-32 on breast cancer xenografts in nude mice have not been studied. This study aimed to examine the potential regulatory effects of IL-32 on breast cancer cell lines and a tumor xenograft model, as well as the effects of IL-32 on apoptosis. The effects of IL-32 on cell proliferation and apoptosis were investigated by MTT assay and TUNEL staining, respectively. The results revealed that IL-32 increased the proliferation rate of cancer cells and decreased the rate of apoptosis. In addition, IL-32 was found to enhance the growth of tumor xenografts in vivo. In summary, IL-32 may represent a useful therapeutic target for human breast cancer.

Materials and methods

Agents and cell culture

IL-32 was obtained from He nuo Biotech (Changsha, China). The MCF-7 cell line was purchased from Gongji Biotech (Shanghai, China), and cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies, Carlsbad, CA, USA) with 100

mg/ml penicillin (Sigma Corporation, Shanghai, China) and 10% fetal bovine serum (Sigma Corporation) at 37°C in 5% CO2. For 12 h glucose withdrawal, the glucose was removed from the medium for 12 h, with all other components maintained. At the end of the withdrawal period, the glucose-containing medium was used again.

3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

In order to examine the cell viability, an MTT assay was performed. A total of 6,000 cells were seeded into 96-well plates and cultured for 24 or 48 h in the presence of IL-32 at 10, 100 or 500 ng/ml. Following incubation for 24 or 48 h, 150 μl 5 mg/ml MTT solution was added for 2 h.

32 at 10, 100 or 500 ng/ml.

The supernatant was removed following centrifugation at 5,000 × g for 2 min at 4°C.
to transplantation in nude mice. The present study used an i.p. injection of IL-32, as mentioned in the previous study, colon cancer cells were directly transfected with IL-32γ, prior to cancer growth by silencing the NF-κB and STAT3 signaling pathways (12). It should be noted that in a previous study, the IL-32γ variant has been shown to inhibit inflammatory processes (13). IL-32 induces the expansion of hematopoietic progenitor cells (14). Increased cell proliferation, which is consistent with a previous finding showing that IL-32 increases the growth of tumor xenografts in vivo (15). IL-32 may act as an intracellular controller, and these results are consistent with previous studies showing the involvement of IL-32 in response to certain inflammatory processes (13–15). IL-32 may act as an intracellular controller, determining the cell survival and death (13). Indeed, the current study found that in vitro culture IL-32 seems to enhance survival of breast cancer cells in a glucose-withdrawn environment. Additionally, IL-32 increased the total number of total, as revealed by apoptosis in the control group (17). In the in situ deah detection kit (Roche Diagnostics) was used for staining and cell counting, according to the manufacturer’s instructions. Briefly, the cells were incubated with DNA-labeling solution for 60 min at 37°C, then washed three times with rinse buffer prior to incubation with the antibody-staining solution for 30 min at room temperature in the dark. Tumor size was measured every 5 days for 30 days and the volume was calculated as follows: Volume = 0.5 multiplied by length multiplied by width multiplied by width. On day 30, the mice were sacrificed by CO2 inhalation and the tumors were harvested and fixed in 10% formalin. The tumors were then cut into 10 μm sections in a Cryostat (Leica CM 3050S; Leica Microsystems, Wetzlar, Germany). A TUNEL in situ-cell deah dection kit (Roche Diagnostics) was used for staining and cell counting, according to the manufacturer’s instructions. Briefly, the cells were incubated with DNA-labeling solution for 60 min at 37°C, then washed three times with rinse buffer prior to incubation with the antibody-staining solution for 30 min at room temperature in the dark. Statistics Data are presented as the mean ± standard deviation and were analyzed using SPSS software, version 11.0 (SPSS, Inc., Chicago, IL, USA). A t-test and analysis of variance were used to compare differences between the groups. 

**Results**

IL-32 increases cell proliferation

The results demonstrated that IL-32 treatment reduced the cell numbers compared with the untreated cells, as detected by light absorbance. After 24 or 48 h, the number of viable cells significantly increased following 100 or 500 ng/ml IL-32 treatment (P<0.01). In addition, this association appeared to be concentration-dependent (Table I).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>100 ng/ml IL-32</td>
<td>110 ± 6</td>
</tr>
<tr>
<td>500 ng/ml IL-32</td>
<td>120 ± 7</td>
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</tbody>
</table>

Table I. Effect of interleukin (IL)-32 on cell proliferation.

IL-32 decreases the levels of cancer cell apoptosis in vitro following glucose withdrawal

Given that IL-32 increased cell proliferation, the subsequent step was to investigate whether this treatment leads to a reduction in cell apoptosis after 12 h glucose withdrawal. Using TUNEL staining, the rate of apoptosis in the control group was revealed to be 56.7±8.9%, while those in the 10, 100 and 500 ng/ml IL-32-treated groups were 44.2±6.7% (P<0.01), 37.7±6.9% (P<0.01) and 34.3±5.1% (P<0.01) at the 24 h time point (12 h following the induction of glucose withdrawal).

IL-32 increases the growth of tumor xenografts

Following the results of the cell proliferation assay, it was investigated whether IL-32 contributed to tumor growth in vivo. It was revealed that IL-32 treatment at 0.2 mg/kg and 1 mg/kg increased the tumor graft growth compared with treatment with saline only (Fig. 1).

Discussion

To the best of our knowledge, the current study is the first to characterize the effects of IL-32 on cancer cells directly; specifically, on breast cancer cells. IL-32 showed cancer-promoting effects in vitro and in vivo. These results are consistent with previous studies showing the involvement of IL-32 in response to certain inflammatory processes (13–15). IL-32 may act as an intracellular controller, determining the cell survival and death (13). Indeed, the current study found that in culture IL-32 seems to enhance survival of breast cancer cells in a glucose-withdrawn environment. Additionally, IL-32 increased the total number of total, as revealed by increased cell proliferation, which is consistent with a previous finding showing that IL-32 induces the expansion of hematopoietic progenitor cells (16).

It should be noted that in a previous study, the IL-32γ variant has been shown to inhibit cancer growth by silencing the NF-κB and STAT3 signaling pathways (17). In the aforementioned study, colon cancer cells were directly transfected with IL-32γ, prior to transplantation in nude mice. The present study used an i.p. injection of IL-32, and 150 μl DMSO was added to each well for absorbance reading at a wavelength of 490 nm using a plate reader (Bio-Rad 680, Bio-Rad Laboratories, Shanghai, China). The assay was repeated six times.
therefore systemic IL-32 may trigger additional effects, for example the recruitment of other inflammatory signaling pathways. We hypothesized that since cancer tissues have an increased expression of IL-32, this indicated the potential involvement of this cytokine in cancer growth, for example in pancreatic cancer (18,19).

In conclusion, the results of the current study suggest that IL-32 exerts modulatory effects on the growth and survival of breast cancer cells. Therefore IL-32 may be a novel therapeutic target for breast cancer, and potentially other types of cancer as well. Future studies, which investigate the combination of IL-32 silencing and chemotherapy drugs for breast cancer treatment may be useful.

References


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