Interleukin 21 and Its Receptor Play a Role in Proliferation, Migration and Invasion of Breast Cancer Cells

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Abstract. Interleukin 21 (IL21) is a cytokine produced predominantly by cluster of differentiation 4 (CD4+) T-cells and natural killer T-cells. There exists evidence that IL21 is implicated in various immunological processes through its specific receptor (IL21R). However, the participation of IL21 in the pathogenesis of solid tumors is not fully conclusive. In the present study, we demonstrated that there was differential expression of IL21R in breast cancer cells using reverse transcription-polymerase chain reaction (RT-PCR), western blotting and sequence analysis. The expression of IL21R was stronger in MDA-231 cells, weaker in MCF7 but negative in ZR-75.1 cells. The invasion and migratory capacity of IL21R+ MDA-231 cells was enhanced by IL21 in a dose-dependent manner. After IL21R was knocked-down by siRNA gene silencing, the response of MDA-231 to treatment with IL21 was attenuated. We found that siRNA silencing of IL21R also spontaneously suppressed cell proliferation. However, IL21 had no additional effect on the proliferation of MDA-231 cells. We also found that IL21R was involved in signaling pathways of matrix metalloproteinases (MMPs), that are crucial for spreading and migration of metastatic MDA231 cells. In conclusion, we unveiled the roles of IL21R in breast cancer cells, which enhances our knowledge on immunological regulation of cancer cells through the axis of IL21 and its receptor.

Human interleukin 21 (IL21) is a cytokine encoded by IL21 gene, which is mapped to chromosome 4q26-q27. The mature polypeptide of IL21 consists of a 131-amino-acid four-helix-bundle cytokine domain with sequence and structural homology to IL15 and IL2, but has also other multiple biological properties (1). IL21 is preliminarily expressed in activated cluster of differentiation 4 (CD4+) T-cells and natural killer (NK) T-cells. IL21 receptor (IL21R) is a class I cytokine receptor that shares a similar structure to the other members of the receptor family such as IL2R, IL4Rα and IL15R. IL21R requires dimerization with the indispensable common gamma chain (γc) subunit to bind IL21 (2). IL21R has been found to be expressed by T-, B- and NK T-cells.

The binding of IL21 and its receptor leads to the intracellular activation of the phosphorylated activation of multiple downstream signaling inducers including janus kinase 1 (JAK1), JAK3, signal transducer and activator of transcription 1(STAT1), and STAT3, which regulate actions on multiple lymphoid and myeloid cell populations, as well as on epithelial cells. The immune regulation involved in IL21 and its receptor is diverse depending on cancer type. For instance, IL21 may have an antitumor effect in patients with melanoma and renal cell carcinoma by inducing immune activity of T- and NK cells (3, 4). In contrast, T helper 17 (Th17) cell-associated IL21 plays a prominent role in tumor growth of colitis-associated colorectal cancer as a result of impaired tumor immunosurveillance (5). Abundant expression of IL21R in follicular lymphoma cells is also associated with tumor progression (6). Therefore, as an immune modulator, IL21R is a double-edged sword with therapeutic potential.

Clinical trials of IL21R-targeted antitumor therapies have been conducted by the administration of either IL21 or blockades such as specific monoclonal antibodies and antagonists, depending on the malignancy (7). Phase I and II clinical trials of IL21 in metastatic renal cell carcinoma, metastatic melanoma and relapsed/refractory indolent non-Hodgkin’s lymphoma show the acceptable safety profile and encouraging single-agent activity of IL21 (8). A phase I trial of IL21 in combination with an epidermal growth factor receptor inhibitor (cetuximab) in patients with metastatic colorectal cancer resulted in activation of immune response biomarkers such as serum CD25 (9).

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We hypothesized that breast cancer cells may also express IL21R, which participates in the modulation of tumor cell invasion.

Materials and Methods

Cell culture. All human breast cell lines used for this study were obtained from the ATCC (Rockville, MD, USA). The human breast cancer cell lines MDA-231, MDA-368, MDA-453, MCF-7, ZR-75-1 and BT-20 were routinely maintained in Dulbecco’s modified Eagle’s medium/Ham’s F12 (Sigma-Aldrich, Irving, TX, USA) supplemented with 10% fetal calf serum (FCS), and 1X penicillin and streptomycin. Other cell lines including HL-60, THP-1 and Raji were maintained according to manufacturer’s instructions and applied as positive controls for IL21R expression. The HMECs (Life Technologies, Paisley, UK) were maintained using Medium 131 supplemented with the addition of Microvascular Growth Supplement (MVGS) and in flasks treated with Attachment Factor Protein (Life Technologies). All cells were incubated at 37°C, with 5% CO2 and 95% humidity.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from cells using TRI-Reagent® RNA isolation agent (Sigma-Aldrich). The concentration of RNA was determined using a spectrophotometer (WPA UV 1101; Biotec Photometer, UK). Reverse transcription was performed using a SuperScript® RT-PCR kit (Life Technologies), followed by PCR using a GoTaq® Green Master Mix (Promega, UK). Full primer sequences are outlined in Table I. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was used as the house-keeping gene control. PCR was conducted under the following reaction conditions: initial denaturing at 94°C for 5 min, followed by 40 cycles of 95°C for 30 s, 55°C for 40 s and 72°C for 30 s, and final extension at 72°C for 10 min. PCR products were loaded onto 2% agarose gel with SYBR® Safe DNA gel stain (Life Technologies, Paisley, UK). After electrophoresis, gels were photographed under UV light.

DNA Sequencing analysis. PCR products were loaded on 2% agarose gel and stained with SYBR® Safe DNA gel stain as mentioned above. After electrophoresis, the single DNA bands with the predicted size were collected using a clean scalpel with the assistance of a portable UV light. DNA samples from agarose gel were purified with a GenElute™ Gel Extraction kit (Sigma-Aldrich) and subjected to Sanger DNA sequencing analysis.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. Cancer cells were lysed with a SDS lysis buffer at 4°C on a rotator wheel before being spun at 13,000 x g to remove insolubles. Following lysis, total protein levels were quantified using the Bio-Rad DC Protein Assay kit (Bio-Rad Laboratories, Hertfordshire, UK), standardized to 2 mg/ml and half diluted with 2X Laemmli sample buffer (Sigma-Aldrich). Samples were boiled for 5 min before loading onto a 10% acrylamide gel and being subjected to electrophoretic separation. Once sufficient separation occurred as indicated by a BlueEye Prestained Protein Ladder (Geneflow Limited, Staffordshire, UK), the proteins were blotted onto a Poly(vinylidene fluoride) (PVDF) membrane (Amersham Biosciences, Bucks, UK), blocked in 10% milk and subjected to specific antibody probing. GAPDH was used as an internal control. Proteins were probed using a primary antibody at a concentration of 1:250 and a specific peroxidase-conjugated secondary antibody at a concentration of 1:1000. Monoclonal mouse anti-human antibodies against IL21R (sc-137120) and GAPDH (sc-47724) were purchased from Santa-Cruz Biotechnologies (CA, USA). Peroxidase-conjugated anti-mouse IgG secondary antibody was purchased from Sigma-Aldrich. Protein bands were visualized using a Supersignal™ West Dura system (Thermo Fisher Scientific, Runcorn, UK) and documented using a Syngene G:BOX gel documentation system (Syngene, Cambridge, UK).

Immunocytochemical staining for IL21R. Cultured breast cancer cells were fixed with 4% paraformaldehyde (PFA) and permeabilized with 0.1% Triton in PBS for 5 min. Following blocking with normal horse serum, mouse monoclonal anti-IL21R primary antibody was added at a concentration of 1:100 and incubated for 60 min. After extensive washing, slides were firstly incubated with a biotinylated anti-mouse secondary antibody for 30 min, and then with ABC reagent for another 30 min (Vector Laboratories, Peterborough, UK). Colour development was performed using 3 ′ 3 diaminobenzidine (DAB) substrate (Sigma-Aldrich). Representative images from the slides were subsequently captured under a microscope (Leica DM1000; Leica Microsystems, Wetzlar, Germany).

In vitro Matrigel invasion and migration assays. An in vitro Matrigel invasion assay was used to assess the invasiveness of breast cancer cells with response to recombinant human IL21. Briefly, transwell inserts (8 μm pores) for 24-well plates were pre-coated with 50 μl/insert of 1 mg/ml Matrigel (BD Bioscience, UK), for 1 h at 37°C. 20,000 cells were seeded into each insert in medium with or without rhIL21. 650 μl culture medium containing 10% FCS was added to each well under the inserts. After incubation for 48 h, cells that penetrated the Matrigel-coated membrane and adhered to other side of the inserts were dissociated with cell dissociation solution (MerkMillipore, Watford, UK) containing Calcein AM (eBiosciences, Hatfield, UK) for 1 h at 37°C. The solution containing invaded cells was transferred to a 96-well black-well plate at a volume of 100 μl/well. Invaded cells labeled with Calcein AM were then quantified using a fluorescence plate reader (Promega, Southampton, UK). Migration assay was performed similarly to the invasion assay described above, but in the absence of Matrigel.

In vitro cell growth assay. Cells were seeded into 96-well plates at a density of 3,000 cells/well in medium with or without recombinant human IL21 (rhIL21) and cultured for 3 days. After incubation the cells were fixed in 4% formalin and stained with 0.5% crystal violet (w/v). The stained crystal violet was then extracted using 10% (v/v) acetic acid, and the absorbance was determined using a spectrophotometer (ELx800; Bio-Tek, Neufahrn, Germany) at a wavelength of 540 nm. The absorbance values are proportional to the number of cells.

Knock-down of IL21R expression using siRNA. MDA-231 cells (6x105) were seeded into a 6-well tissue culture plate and cultured for 24 h. SMARTpool small interfering RNA (siRNA; 100 nM) targeting human IL21R or non-target control (NTC) was transfected into cells using DharmaFECT Duo Transfection Reagent according to the manufacturer’s protocol (GE Healthcare, Little Chalfont, UK). A group of cells was treated with only DharmaFECT Duo (DD control) for cytotoxicity analysis of the transfection reagent. Cells
and MCF-7, MDA-361 and BT-20 cells. However, ZR-75.1 showed that there was gene expression of (Figure 1A). DNA sequencing analysis showed that the PCR, western blotting and sequencing analysis. RT-PCR data containing breast cancer cells at a density of 5×10^4 cells per well. The measurement of spreading, attachment and migration behavior of cancer cells. Briefly, cancer cells were seeded at a density of 4×10^4 cells/well and cultured for 24 h. Each transfection or treatment condition was set up with 6 repetitions. After transfection for 24 h, cell behavior was monitored in an ECIS system. Post-wound migration of cells was measured after electrical wounding at 2,600 μA for 20 s. The broad-spectrum matrix metalloproteinases (MMP) inhibitor Marimastat used in the ECIS assay was purchased from Tocris (Bristol, UK).

In vitro angiogenesis assay. A tubule-forming assay was performed to evaluate the effect of soluble factors produced by breast cancer cells on angiogenesis properties of endothelial cells. Briefly, Matrigel, a basement membrane matrix commonly used to study angiogenesis in vitro, was placed in a 24-well plate at 200 μl/well. The plate was then incubated at 37˚C for 1 h to allow Matrigel to solidify. HMVECs were then plated at a density of 1×10^5 cells per well in 450 μl EBM-2 media. Transwell inserts (0.4 μm pores) containing breast cancer cells at a density of 5×10^4 cells per well were subjected to transfection with IL21R or NTC siRNA regents for 24 h and then applied on top of the wells with HMVECs, respectively. Tubule formation of HMVECs on Matrigel was captured at 18 h with a Leica DMIL LED microscope (Leica Microsystems, Wetzlar, Germany) with a ×10 objective.

Statistical analysis. All data were analyzed using the SPSS version 20 for Windows (SPSS, Chicago, USA) and are presented as mean±SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA) for multiple groups or unpaired Student’s t-test for two groups. The significance of differences in the ECIS data was analyzed using repeated-measures ANOVA. p-Values less than 0.05 were considered statistically significant.

Results

Expression of IL21R in breast cancer cell lines. Expression of IL21R in MDA-231, MDA-368, MDA-453, MCF-7, ZR-75.1 and BT-20 breast cancer cells was evaluated using RT-PCR, western blotting and sequencing analysis. RT-PCR data showed that there was gene expression of IL21R in MDA-231, MCF-7, MDA-361 and BT-20 cells. However, ZR-75.1 and MDA-453 cells were negative for IL21R gene expression (Figure 1A). DNA sequencing analysis showed that the obtained PCR product had a high level of sequence homology with IL21R in MDA-231 (100%) cells, while having a lower level of homology in MCF-7 cells (89%) (Figures 1B and C). Protein expression of IL21R was stronger in MDA-231 compared to MCF-7 cells as shown by western blotting (Figure 1D). We also observed both 97-kDa (glycosylated) and 58-kDa (non-glycosylated) protein bands of IL21R from MDA-231 cells. There was only a weak 97-kDa band of IL21R protein observed for MCF-7 cells. Data from immunostaining also confirmed IL21R staining was strong in MDA-231 cells, but weak in MCF-7 cells (Figure 2).

IL21 promotes proliferation, migration and invasion of MDA-231 cells but not of MCF-7 and ZR-75.1 cells. The effect of recombinant human IL21 on cellular behavior of the selected breast cancer cells was investigated. We demonstrated that IL21 promoted the invasion capacity of MDA-231 in a dose-dependent manner using Matrigel-coated transwell inserts with 8-μm pore size. The peak stimulation dose of IL21 was 25 ng/ml. IL21 had no effect on invasion of MCF-7 and ZR-75.1 cells (Figure 3A). In a similar pattern, IL21 stimulated the migration of MDA-231 cells in a dose-dependent manner using transwell inserts with 8-μm pore size in the absence of Matrigel. The peak stimulation dose of IL21 was also 25 ng/ml. IL21 did not increase migration of MCF-7 and ZR-75.1 cells, and a higher dose of IL21 appeared to have an inhibitory effect on these two cell lines (Figure 3B). In addition, IL21 enhanced the growth of MDA-231 cells after treatment with 1 (p<0.05), 5 (p<0.01) and 25 ng/ml (p<0.01) of IL21, respectively for 3 days (Figure 3C). IL21 did not enhance the growth of MCF-7 cells until treatment for 4 days (p<0.05, respectively). However, treatment for 4 days with 50 ng/ml of IL21 had an inhibitory effect on the growth of ZR-75.1 cells (p<0.05).

IL21R gene silencing abolished the response of MDA-231 cells to IL21. We established IL21R knock-down using different concentrations of siRNA SMARTpool and compared this to the same concentration of NTC. Raji cells (human Burkitt lymphoma cell line) were applied as a positive control for IL21R gene expression. After transfection for 48 h, IL21R siRNA knocked-down IL21R gene expression at both concentrations of 100 nM and 500 nM (Figure 4A). A concentration of 100 nM of IL21R siRNA

<table>
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<th>Gene</th>
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<th>Accession Size of PCR amplicon (bp)</th>
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<td>NM_002046 455</td>
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Table 1. Primers used for reverse transcription-polymerase chain reaction (RT-PCR) and sequencing.
appeared to be optimal and was selected for the subsequent cellular assays.

As shown in Figure 4B, after transfection with NTC siRNA for 48 h, migration capacity of MDA-231 cells dose-dependently increased in response to IL21 (p<0.01 vs. vehicle), that confirmed our previous data using wild-type cells. However, after treatment with IL21R siRNA, MDA-231 cells did not show any increase of migration in response to IL21, indicating that IL21 stimulated migration of MDA-231 cells through binding with its receptor.

For invasion (Figure 4C), after treatment with NTC siRNA for 48 h, the invasion capacity of MDA-231 cells increased in response to 50 ng/ml IL21. However, after treatment with IL21R siRNA, MDA-231 cells did not show any increase of invasion in response to IL21, indicating that IL21 stimulated migration of MDA-231 cells through binding with its receptor.

We also evaluated the effect of IL21R siRNA gene silencing on growth of MDA-231 cells (Figure 4D). It appeared that in NTC siRNA-treated cells, IL21 did not have any effect on cell growth. However, after IL21R siRNA silencing, treatment with 25 ng/ml of IL21 slightly increased the cellular growth (p<0.05).

Taken together, our results show that IL21 is highly involved in migration and invasion of MDA-231 cells through the interaction with its receptor, which may not be the consequence of growth alteration.

IL21R plays a role in the regulation of MMPs in MDA-231 cells. To understand whether IL21R participates in the regulation of MMPs in MDA-231 cells, we monitored attachment/spreading and migration dynamics of cells in response to Marimastat, a broad-spectrum MMP inhibitor, using

Figure 1. Expression of interleukin 21 receptor (IL21R) in human breast cancer cells. A: Reverse transcription-polymerase chain reaction (RT-PCR) assay shows strong expression of IL21R gene in MDA-MB-231 cells. There is also weak expression of IL21R in MCF-7, BT-20 and MDA-361 cells. ZR-75.1 and MDA-451 cells do not express IL21R. Immune cells including HL-60, THP-1 and Raji were used as positive controls. B: The sequencing data of the PCR product using IL21R primers in MDA-231 cells. C: The sequence of the IL21R amplicon in MDA-231 cells can be fully aligned to the IL21R mRNA using standard nucleotide BLAST from the National Center for Biotechnology Information (NCBI). D: Western blotting data show that MDA-231 cells expressed both 97 kDa (glycosylated) and 58 kDa (non-glycosylated) isoforms of IL21R protein, while MCF-7 cells only expressed the 97 kDa isoform of IL21R.
the ECIS system. As shown in Figure 5A, in NTC siRNA-treated MDA-231 cells, Marimastat appeared to inhibit the attachment and spreading of cells compared to DMSO over the course of 18 h (repeated-measures ANOVA, \( p = 0.021 \)), and the most significant treatment effect was observed after treatment for approximately 12 h (\( p = 0.03 \)). Marimastat inhibited the attachment and spreading of cancer cells compared to DMSO (\( p < 0.0001 \)) after MDA-231 cells were transfected with IL21R siRNA (Figure 5B). In addition, IL21R silencing augmented the sensitivity of MDA-231 cells to Marimastat compared to NTC (\( p = 0.034 \)). For MCF-7 cells (Figures 5C and D), Marimastat did not have a significant effect on the attachment of cells, whether they were pre-transfected with NTC (\( p > 0.05 \) vs. DMSO) or IL21R siRNA (\( p > 0.05 \) vs. DMSO).

After electrical wounding by the ECIS system, MDA-231 cells transfected with NTC siRNA did not show any change in migration (32,000 Hz readings) in response to Marimastat (\( p > 0.05 \) vs. DMSO) (Figure 6A). However, as shown in Figure 6B, MDA-231 cells transfected with IL21R siRNA showed response to Marimastat with inhibitory effect on migratory capacity (\( p = 0.029 \) vs. DMSO), which indicated that IL21R gene silencing sensitized the response of MDA-231 cells to Marimastat. In contrast, Marimastat did not have any significant effect on post-wound migration of MCF-7 cells, whether cells were transfected with IL21R siRNA or NTC (\( p > 0.05 \) vs. DMSO, respectively) (Figures 6C and 6D). As MMP proteins play a key role in invasion of tumor cells, these data further indicate that IL21R may participate in the invasion of MDA-231 cells through regulation of MMP signaling pathways.

IL21R appeared to be involved in the modulation of tumor-associated angiogenesis. We used a transwell co-culture system to evaluate the effect of secreted factors from tumor cells seeded to 0.4-μm inserts on tubule formation of HMVECs on Matrigel. As shown in Figure 7A and B, knockdown of IL21R using siRNA appeared to enhance the stimulating effect of MDA-231 cells on angiogenesis of HMVECs compared to NTC. In contrast, MCF-7 cells did not stimulate the tubule formation of HMVECs, and IL21R siRNA treatment did not alter this result (Figures 7C and 7D).
Discussion

Previous studies have demonstrated the profound and diverse function of IL21 in immune cells including CD4+ T-cells (3) and NK cells (10). Given the accumulated evidence that IL21 augments cellular cytotoxicity initiated by CD4+ T-cells and NK T-cells, various trials have been conducted in order to elucidate the efficacy, pharma-dynamics, safety and tolerability of immunostimulatory drugs which target IL21 or IL21R (9, 11). Those trials were conducted on patients with malignancies including leukemia, melanoma, metastatic renal cell carcinoma, lung cancer, colorectal cancer and ovarian cancer (7). Some phase I trials have demonstrated anti-tumor activity and acceptable safety of such agents in patients with cancer. However, it is worthy to note that quite often, IL21R is combined with inhibitors of other crucial signaling checkpoints in patients with metastatic solid tumors in order to optimize efficacy and minimize adverse effects. For example, the combination of IL21R and anti–programmed death-1 antibody has been studied to treat patients with advanced or metastatic solid tumors (ClinicalTrials.gov Identifier: NCT01629758). A phase I/II

Figure 3. Effect of recombinant human interleukin 21 (IL21) on invasion, migration and growth of breast cancer cells. Cells were treated with IL21 for 48 h before migration and invasion assays, respectively. A: Transwell invasion assay shows that IL21 promotes invasion ability of MDA-231 cells in a dose-dependent manner. There was no treatment effect of IL21 on MCF-7 and ZR-75.1 cells. B: Transwell migration assay showed that IL21 enhances migration capacity of MDA-231 cells in a dose-dependent manner. MDA-231 exhibited the highest migration as a response to 25 ng/ml of IL21. IL21 appeared to inhibit the migration of MCF-7 cells randomly at two doses (5 and 30 ng/ml). IL21 did not have any effect on migration of ZR-75.1 cells. C: Growth assays showed that IL21 was able to enhance growth of MDA-231 and MCF-7 cells with dose dependence. However, MDA-231 cells responded to IL21 treatment earlier, from day 3, while MCF-7 cells responded later, from day 4. IL21 did not increase growth of ZR-75.1 cells, and the highest dose (50 ng/ml) appeared to have cytotoxic cellular effect. *p<0.05, ** p<0.01 compared to vehicle solution or as indicated.
study on the combination of IL21 and vascular endothelial growth factor (VEGFR) tyrosine kinase inhibitor (sorafenib) was conducted for the treatment of metastatic renal cell carcinoma (12). However, those trials were not able to consider the specific effect of IL21 on tumor cells which express IL21R. In addition, the combination of IL21 and ipilimumab [targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4)] has been evaluated for the treatment of patients with metastatic melanoma, which resulted in favorable clinical outcome at Phase II (13). However, although IL21/IL21R-targeted drugs have attracted enormous interest for clinical benefits, little is known on whether those drugs have any direct effect on behaviors of tumor cells, apart from their activation of the immune cells.

IL21R is preliminarily expressed on hematopoietic cells but there are also studies showing the expression of IL21R on non-immune cells such as Hodgkin lymphoma cells, fibroblasts, keratinocytes and endothelial cells (14, 15). Our data herein suggest that IL21R is also expressed in breast cancer cells at different levels. For instance, the expression of IL21R is strong in MDA-231 cells, but weak in MCF-7 cells. We further confirmed this finding by western blotting and DNA sequencing analysis. In MDA-231 cells, the PCR products with IL21R-specific primers have 100% identity with the original sequence. The expression of IL21R-negative MCF-7 cells was confirmed by PCR as well. The expression levels of IL21R in different breast cancer cell lines were analyzed by flow cytometry, and the results showed that IL21R was expressed in more than 90% of breast cancer cells, with significantly higher expression in MDA-231 cells than in MCF-7 cells.

Figure 4. Knock-down of interleukin 21 receptor (IL21R) using siRNA eliminated the response of MDA-231 cells to IL21. A: Gene expression assay shows the optimal dose of IL21R siRNA to knock-down IL21R was 100 nM. B: Transwell migration assay (n=3) shows that after transfection with non-target control (NTC) siRNA, IL21 promoted the migration of MDA-231 cells with dose dependence, that was similar to the response of wild-type cells. However, after transfection with IL21R siRNA, MDA-231 cells lost their response to IL21 treatment. C: Transwell invasion assay (n=3) indicates that after transfection with NTC siRNA, IL21 at a concentration of 50 ng/ml promoted the invasion of MDA-231 cells. In contrast, functional response of MDA-231 cells to IL21 was not observed after cells were transfected with IL21R siRNA. D: Crystal violet growth assay (n=6) shows that in MDA-231 cells with NTC siRNA transfection, IL21 did not change the growth rate. However, in cells transfected with IL21R siRNA, only 25 ng/ml of IL21 slightly increased the growth ratio of MDA-231 cells. *p<0.05, **p<0.01 compared to respective vehicle solution or as indicated.
homogeneity with the sequence of IL21R. Therefore although IL21R is primarily expressed in immune cells, it can also be expressed by advanced solid tumor cells such as breast cancer cells.

To evaluate whether IL21R expressed by MDA-231 cells has any biological activity, we investigated the treatment effect of IL21 on properties of MDA-231 cells. Our data indicate that IL21 promotes proliferation, migration and invasion of MDA-231 cells but not of MCF-7 and ZR-75.1 cells. This suggests that IL21 may enhance the invasive properties of tumor cells which have strong expression of IL21R. Previous studies have shown that in IL21R+ Hodgkin lymphoma cells, IL21 protects cells from apoptosis via activation of STAT3 signaling pathways, and attracts regulatory T-cells via up-regulation of macrophage inflammatory protein-3α (16). Future studies may be necessary to dissect the signaling mechanism underlying IL21R-modulated functions in breast cancer cells.

After IL21R was knocked-down by siRNA gene silencing, MDA-231 cells lost their response to IL21 of migration and invasion. This suggests that the observed effects of IL21 on MDA-231 cells are the consequences of the interaction between IL21 and its receptor. However, this study cannot thoroughly exclude the possibility that IL21 may have binding...
ability with other receptors, particularly in the IL2 family. This is mainly because IL21 has a high level of sequence and structural homology to IL15 and IL2, and IL21R has sequence and domain similarity to IL2R, IL9R and IL4Rα (17-19). IL21R may also function synergistically in an interchangeable complex with those domain sharers in solid tumors, although more evidence needs to be obtained (20, 21).

We have demonstrated that MDA-231 cells treated with IL21R siRNA are first more susceptible to the inhibitory effect of Marimastat, affecting spreading, attachment and post-wound migration capacity of the cells. Marimastat is a broad-spectrum MMP inhibitor, therefore, IL21R might be associated with MMP signaling in breast cancer cells. It is known that MMP family members are associated with tumor stage and participate in tumor progression, invasion and metastasis in advanced breast cancer (22-24). It might be possible to sensitize cells to MMP-targeted antitumor therapy by knocking-down the expression of IL21R. It also appears that IL21R siRNA silencing is able to enhance MDA-231 stimulated in vitro angiogenesis. Previous studies have shown that there is co-localization of IL21R and VEGF in the keratinocytes of patients with skin sclerosis, and VEGF production is modulated by IL21 through IL21R (25, 26). Additional studies need to investigate whether IL21 is associated with tumor angiogenesis in a VEGF-dependent manner.

Figure 6. Knock-down of interleukin 21 receptor (IL21R) using siRNA sensitizes cells to the effect of MMP inhibitor Marimastat on post-wound migration of MDA-231 cells, as indicated by the electric cell-substrate impedance sensing (ECIS) assay. Cells were loaded into the ECIS plates and transfected with NTC and IL21R siRNA, respectively for 48 h. Post-wound migration of cells was measured after electrical wounding at 2,600 μA for 20 s in the ECIS system. The graphs show the normalized reading of electric resistance (32,000 Hz) from individual cellular populations which indicates migratory properties of cells. Data are shown as means±SD (n=6). A: MDA-231 cells transfected with NTC siRNA. B: MDA-231 cells transfected with IL21R siRNA. C: MCF-7 cells transfected with NTC siRNA. D: MCF-7 cells transfected with IL21R siRNA.
To the best of our knowledge, this is the first study to demonstrate the expression and function of IL21R in breast cancer cells. It will be interesting to unveil which sub-types of patients with breast cancer carry IL21R+ breast cancer cells by cohort screening, and whether IL21R-targeted therapy may have an impact on personalized breast cancer treatment.

Conflicts of Interest

The Authors declare no conflict of interest.

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