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Effects of Netrin-1 and NHE1 Participation on the Migration of Macrophages Driven by CCL2

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ABSTRACT

This experiment was designed to investigate the relationship between NHE1 gene expression differences between Netrin-1 and NHE1. For this purpose, the blank control, CCL2, CCL2 + Netrin-1 groups were constructed, and cell migration ability was detected by scratch tests and Transwell experiments; Commercial over-expressed NHE1 adenovirus vector (over-expressed NHE1 group), shRNA adenoviral vector silencing NHE1 (silencing NHE1 group) and negative control without carrying virus (negative control group) were subjected to RT-PCR test 24h after infection and pH recovery rate after acid loading was measured. The percentage of wound healing area and the number of cell migration of macrophages in the blank control group, CCL2 group, CCL2+Netrin-1 group, over-expressed NHE1 group, silencing NHE1 group and negative control group were compared. Results showed that in terms of migration ability, the percentage of wound healing area and migration in CCL2 increased ($P < 0.05$), in CCL2 + Netrin-1 ($P < 0.05$) and increased NHE1 mRNA ($P < 0.05$), and not in NHE1 ($P < 0.05$).pH response rate after acid load (NHE1 activity) showed that NHE1 activity was enhanced compared with the blank group, while NHE1 activity in silent NHE1 group decreased ($P < 0.05$); from macrophage migration ability after overexpression/silencing, the percentage of macrophage wound healing area and cell migration increased/decreased compared with CCL2 group and Netrin-1 + CCL2 group ($P < 0.05$). Then Upregulation of NHE1 can promote CCL2-driven macrophage RAW264.7 cell migration, and the downregulation of NHE1 can inhibit its cell migration; Netrin-1 can inhibit CCL2-driven RAW264.7 cell migration regardless of NHE1 regulation.

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Introduction

Atherosclerosis (AS) is a chronic inflammatory disease of arteries that is difficult to subside. It is mainly characterized by the fact that monocytes/macrophages and other cells phagocytize oxidized low-density lipoprotein cholesterol (Ox-LDL) and other cells to form foam cells and stay in the arterial wall continuously (1). Macrophages/foam cells can move out of the plaque under certain conditions, promoting the reduction of plaque. The dynamic changes of monocytes/macrophages in and out of plaque determine the formation, development and instability of AS plaque (2). Netrin-1 is the most widely studied axon guiding factor in the Netrins family. In the cardiovascular field, Netrin-1 can promote macrophage retention plaque and accelerate atherosclerosis (3). Sodium/hydrogen exchanger 1(NHE1/SLC9A1) is a ubiquitous and complete membrane protein expressed in mammalian cells, and NHE1 is involved in cell migration (4-6). Cell migration plays a fundamental role in many physiological and pathophysiological processes such as embryogenesis, immune defense, wound healing or metastasis. Understanding how NHE1 and Netrin-1 participate in macrophage migration and their related mechanisms will help to determine the mechanism of inhibition of macrophage movement at the cellular level. In view of this, in this study, the mouse macrophage line RAW264.7 was used as the experimental cell line, which was induced by chemokine CCL2. In this experiment, under the up/down-regulation of NHE1, the changes in Netrin-1's cell migration ability and related mechanisms were clarified.

Materials and Methods

Cells and reagents

Mouse macrophage cell line RAW264.7 purchased from Cell Center, Basic Institute of Chinese Academy of Medical Sciences; Fetal bovine serum purchased from BI Company, Israel; DMEM high-sugar medium purchased from Gibco Company, USA; Penicillin/streptomycin mixture, purchased from BI Company, Israel; Trypsin purchased from Gibco Company, USA; PBS buffer purchased from BI company, Israel; mouse IgG, primary anti-NHE1 antibody, and rabbit IgG, primary Anti-Giantin antibody (Glogi Marker), provided by Abcam Company, USA; primary anti-ROCKI mouse IgG provided by Affinity Company, China. Primary anti-GAPDH mouse IgG produced by GeneTex Company, USA; recombinant mouse Netrin-1 and recombinant mouse CCL2 provided by R&D Company, USA; Alexa Fluor 488 labeled goat anti-

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mouse IgG(H+L), Cy3 labeled goat anti-rabbit IgG(H+L) ab97036 and SDS-PAGE protein loading buffer (5×) produced by Biyuntian Company, China; efficient RIPA tissue/cell lysate, Rainbow 180 broad-spectrum protein marker, SDS, glycine, Tris, anti-fluorescence attenuation sealing tablets (including DAPI) and TBST buffer (10×) produced by Solarbio Company, China; normal sheep serum for sealing (working solution) produced by Zhongshan Company, China; R6812 HP Total RNA Kit produced by omega Company, USA; SYBR-Green/ROX qPCR Maste Mix provided by Wuhan Servicebio Company; nigericin produced by Sigma Company, USA; SNARF-1 /AM produced by Invitrogen Company, USA.

Main instruments

All equipment is provided by the Key Molecular Biology Laboratory of Guizhou Medical University and the Clinical Medical Research Center of the Affiliated Hospital of Guizhou Medical University. The CO2 incubator was produced by Japan's SANYO Company, and the biosafety cabinet was purchased from China's Haier Company; the clean bench was produced by China Jinjing Company; Nikon Eclipse CI was produced by Nikon Company, Japan; the constant temperature water bath pot was provided by Guangming Company, China; Backman 21R refrigerated centrifuge was an instrument produced by Backman Company, USA.

Preparation of reagents

Complete culture medium containing 10% fetal bovine serum (adding fetal bovine serum, simple culture medium, penicillin/streptomycin mixed solution in turn, with the ratio of 10:89:1), cell cryopreservation solution (DMSO: fetal bovine serum =1:9, stored at 4℃ in refrigerator), Netrin-1 working solution (100 μg/ml in sterile PBS containing at least 0.1 % human or bovine serum albumin and configured to a maximum effective concentration of 200 ng/ml in a total volume of 7 ml using PBS as diluent), CCL2 working solution (100 μg/ml in sterile PBS containing at least 0.1 % human or bovine serum albumin and configured to a maximum effective concentration of 10 ng/ml in a total volume of 7 ml using PBS as diluent), WB electrophoresis solution (tris: 14.4g, glycine: 3.03 g, SDS: 1 g, added into a 1000 ml measuring cup. Adding pure water to 1000 ml, and mixing with a stirrer or stirring bar), WB membrane transfer solution (tris: 14.4g, glycine: 3.03 g, 200 ml methanol, added into a 1000ml measuring cup. Making the volume of pure water constant to 1000ml, mixing with a stirrer or stirring bar, and storing at 4℃).

Preparation of RAW264.7 cells

Rewarm macrophage RAW264.7 cells to 37℃ in a water bath box, and quickly shake to melt. Suck out the cell suspension in the cryopreservation tube with a pipette and add it into a 15 ml centrifuge tube. Add 3 ml of pure culture medium, and centrifuge at 1000rpm for 5min. Discard the supernatant, and add 3 ml complete culture medium to prepare cell suspension. Add 5ml of cell suspension with the concentration of 6×10^5 cells /ml in T25 cell culture bottle. Put the cell bottle in a CO_2 incubator at 37℃ for culture. Observe under the inverted microscope until the cell density grows to 80 %-90%. When the cells agglomerate, are round and transparent, and begin to be passaged, perform conventional passaging and cryopreservation.

Cell transfection

Commercial overexpression NHE1 adenovirus vector expressing the sequence of the full coding region of NHE1 and silencing NHE1 adenovirus vector expressing shRNA against NHE1 RNA were customized, and empty adenovirus was used as negative control. The shRNA sequence targeting NHE1 mRNA (7) is shown in Table 1.

Virus infection

Before infection, spread RAW2647.7 cells on a 6-well plate and culture overnight at 37℃. The cell density should be about 40% to 60% on the second day. Take out the silenced and overexpressed NHE1 adenovirus vector from the refrigerator and melt it slowly on the ice. After complete melting, centrifuge the virus vector at 1000 rpm× 5 min to the bottom of the bottle, and start the virus infection experiment. Then, suck off the original culture medium of the cells, wash with serum-free culture medium, and add 100 μl of virus solution according to the multiplicity of infection of 50, and shake well. Seal the mouth, put it in a flat angle centrifuge, centrifuge at a low speed of 500×g for 1.5h, and then put it in an incubator at 37℃ for 4 h. Add complete culture medium, culture for 24 h, and then perform PCR and WB detection of NHE1 mRNA and protein to detect the overexpression and silencing efficiency of virus vector, and detect NHE1 activity by fluorescence ratio and pH recovery rate after acid loading.

Western blot method

Take a proper number of cells, use Solarbio tissue protein extraction kit, 10 μl PMSF+1 ml RIPA, to prepare a mixture. Add 200 μl of the mixture to 20 mg of tissue, and lyse the tissue. Set the freezing centrifuge in advance at 4℃ and12000 rmp for 15 min, and take out the supernatant. Record the volume of the sucked-out supernatant, then add it to the loading buffer at the ratio of 4:1, heat the mixture in a water bath at 100℃ for 10min, cool it to room temperature, and store it in a refrigerator at -80℃. After glue preparation, electrophoresis and membrane transfer treatment, clamp the membrane transfer clamp and put into the membrane transfer tank. Put the membrane transfer tank into a foam box filled with crushed ice. It's best to make the ice water fully surround the electrophoresis tank. According to marker, determine the required membrane transfer time, take out PVDF membrane and put it in a membrane washing box, wash it with TBST for 3 times (100 rpm, 5 min each time). Add 5% skim milk, seal it for 2 h, and wash it with TBST for 3 times. Add the primary antibody overnight in a refrigerator at 4℃, recover the primary antibody and wash it with TBST for 3 times. Add

Table 1. NHE1 sequence.

	Positive-sense strand	Antisense strand
NHE1 shRNA	5'-CGAAGAGAUCCACACACAGtt -3'	5'-CUGUGUGUGGAUCUCUUCGtt-3'
Messy code short RN sequence	5'-UUCUCCGAACGUGUCACGUTT-3	5'-ACGUGACACGUUCGGAGATT-3'

secondary antibody and incubate at room temperature for 1 h. Wash with TBST for 3 times. Prepare 2 ml of exposure solution and add it into a 5 ml centrifuge tube (the ratio of exposure solution A to exposure solution B is 1: 1); put the contact surface between the membrane and the glue into an exposure instrument with the face up. Suck a small amount of exposure liquid and drop it on the membrane to make the exposure liquid fully contact with the membrane; expose and record WB experimental results.

Cell scratch test

Mark the date and group on the outer wall of the 6-well plate, and draw three straight lines through the holes (three parallel black lines at the bottom of the 6-well plate) with a marker and a ruler at the bottom of the culture plate. Discard the supernatant, add complete culture medium into one bottle to prepare 10 ml cell suspension, count the cells, take the cell suspension with a cell density of 2.0×10^5 cells /ml, and add 2 ml into a 6-well plate per well. Put the cell plate in a CO_2 incubator at 37℃ for culture. After the cells of the 6-well plate grow to 70%, start starving for 12 h. After 12 h, discard the old culture medium, draw three scratches along the black line perpendicular to the bottom of each well of the 6-well plate with a 10 μL gun head, and gently wash them with PBS for 2-3 times to try to wash off the cells in the middle of the scratches. Add 2ml of corresponding working solution into the holes of blank control group, CCL2 group, CCL2+Netrin-1 group, overexpressed NHE1+CCL2 group and silent NHE1+CCL2 group, record the electron microscope photos at 0h and 24h respectively, and analyze the percentage of wound healing area with Image J software.

$$\text{Percentage of wound healing area (\%)} = \frac{\text{0 hour wound area - 24 hour wound area}}{\text{0 hour wound area}} \times 100\% \quad [1]$$

Obtaining NHE1 overexpression and silencing virus vector

Perform RT-PCR 24h after infection. The experimental process should be strictly aseptic. Sterile enzyme-free gun head and centrifuge tube are used. Use RNA extraction kit to treat cells: culture each group of cells in the 6-well plate. After sucking out the culture medium, add 400 μl Buffer GTC to lyse the cells, and vortex and mix the cells evenly. Transfer the lysate into a 1.5 mL centrifuge tube, and homogenize it in a homogenizer for 30 s. Insert the gDNA Removal Column into a 2 mL centrifuge tube, transfer the lysate to the column, and centrifuge it at 14,000 rpm for 3 min at room temperature. Transfer the filtrate to a new 1.5 mL centrifuge tube. Add absolute ethanol (concentration 2:1), vortex and mix for 30 s. Sleeve HiBind® RNA Mini Column into a new 2 mL centrifuge tube. Transfer the above mixture to the column, centrifuge at 10,000 rpm for 60 s at room temperature, and discard the filtrate. Add 300 μl RNA Wash Buffer I, centrifuge at 10,000 rpm for 60 s at room temperature, and discard the filtrate. Sleeve the centrifugal column into a 2 ml collecting tube, add RNA Wash Buffer I 400 μl, centrifuge at 13,000 rpm for 1min at room temperature, and discard the filtrate. Sleeve the centrifuge column in a 2 mL collection tube, add 500 μL RNA Wash Buffer II, centrifuge at 13,000 rpm for 1 min at room temperature, discard the filtrate, and repeat once; centrifuge the empty column at 12,000 rpm for 2 min. Sleeve the centrifuge column in a new 1.5 mL centrifuge tube. Add 30 μL DEPC-treated water to the center of the centrifuge column membrane. After standing at room temperature for 2 min and centrifuging at 10,000 rpm for 1 min, the filtrate obtained is RNA. Detect the concentration and purity of total RNA of the samples by nucleic acid quantitative analyzer, and identify the integrity of RNA samples. According to the RNA concentration, add 2 μg RNA into each tube, and calculate the sample volume: 2 μg/ concentration. Take 200 μL PCR tube, add 5×SweScript All-in-One SuperMix for qPCR 4.0 μL, gDNA Remover 1 μL and Total RNA/mRNA 2 μg, calculate the volume of each sample according to the volume = 2 μg/ concentration, and add Nuclease-Free Water to make up the total volume of 20 μL according to the total amount of the first three. Onboard processing: 5 min at 25℃, 30 min at 42℃, 5s at 85℃ and 30 min at 4℃. Label the cDNA obtained by reverse transcription and store it in the refrigerator at -80℃ for later use.

Transwell experiment

Add the cells that have grown to about 80-90% to a serum-free medium for culture for 12 h, then digest them with trypsin and collect them by centrifugation. According to different experimental groups, divide RAW264.7 cells into different treatment groups, and treat them according to the conditions of different treatment groups. They were divided into nine experimental groups: blank group (negative control group, CCL2 group, CCL2+Netrin-1 group), overexpression NHE1 group (negative control group, CCL2 group, CCL2+Netrin-1 group) and silence NHE1 group (negative control group, CCL2 group, CCL2+Netrin-1 group). Material preparation: reheat the Transwell chamber to room temperature, and put other spare parts into the biosafety cabinet to illuminate with an ultraviolet lamp for 30min; preparation of cell suspension: digest cells in the negative control group, cells in silenced NHE1 group and cells in overexpressed NHE1 group digested with trypsin, centrifuge them and resuspend them into cell suspension. Adjust the concentration of cell suspension to 2×10^5 cells /ml, and mark the corresponding experimental group on the tube wall; after preparing the working solution, take out the Transwell chamber, and add 0.1 ml/ well cell suspension to the upper chamber and 0.5 ml/ well cell working solution to the lower chamber according to different experimental groups. Put the cell suspension in a 5% CO_2 incubator at 37℃ for 24 h, take out the small chamber, suck out the culture medium, wipe the cells in the upper chamber with a sterile cotton swab, fix them with methanol for 30 min, and then dye them with 0.1% crystal violet for 15 min. Put it under an inverted microscope. Take random photos and make records.

Detection of the level of NHE1 protein by PCR method

Take GAPDH as an internal reference gene, and detect the mRNA level of NHE1 by SYBR Green Master. Repeat the measurements three times for all data. Use the 20μL of RT-PCR reaction system: 10μL of SYBR QPCR Master Mix, 1.2 μL of cDNA template, 8 μL of each sample, and 0.4 μL of upstream and downstream primers of each sample. After mixing, instantly centrifuge in a mini centrifuge for 15s to make the reaction system sink onto the bottom of the centrifuge tube. Reaction conditions: 95℃ for the 30s, 95℃ for 15 min, 60℃ for 30s, and 40 cycles of the last two steps. Gene primer sequences are shown in Table 2.

Table 2. The sequences of each primer.

Gene name	Forward primer	Reverse primer
NHE1	5'-CCCCATGCCAGTCAGAG-3'	5'-GGCCACATCAGA ATCGGT-3'
GAPDH	5'-GGTTGTCTCCTGCGACTTCA-3'	5'-TGGTCCAGGGTTTCTTACTCC-3'

The Ct values of all samples were read by fluorescence quantitative PCR instrument to obtain the Ct value of each gene in the samples. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Inspection of the recovery rate of pH value (NHE1 activity)

Carry out the inspection according to the NHE1 activity detection scheme in Literature (8). Plant the cells on the cover glass and infect them with different adenoviruses for 24 h. Take out the cover glass, wash it with PBS 3 times, add SNARF-1 /AM (final concentration 5 μmol /L), and incubate it at 37℃ for 30 min. After rinsing with PBS, put it on a self-made cover glass bracket, select a single transfected (green fluorescence) cell, and detect it under a laser confocal microscope. The excitation wavelength is 530 nm, and the emission wavelength is 580 nm and 630 nm, respectively. Calculate the fluorescence intensity ratio of the two emission wavelengths at the same excitation wavelength = 630 nm /580 nm. Detect the recovery rate of intracellular pH value after acid loading. Rise the cells with carbonate buffer containing 20 mmol /L NH4Cl for 3 min. After that, rinse them with sodium-free solution for 5 min and then with a sodium-containing solution. Adopt intracellular pH standard curve system. Incubate them with high potassium solutions of different pH, SNARF-1 /AM (final concentration of 5 μmol /L) and nigericin (final concentration of 5 μmol /L) for 30 min. Obtain the regression equation according to the ratio of the pH value to the corresponding fluorescence intensity, and make the intracellular pH standard curve.

Statistical analysis

Graphpad Prism 9 statistical software was used for statistical analysis, and the measurement data were expressed as mean standard deviation (x±s). After variance analysis, the LSD-t test was used for comparison of differences between groups, and Dunnett T3 test was used for variance discrepancy.

Results

Netrin-1 inhibits cell migration induced by CCL2 by

Results of migration assay

As seen from the migration ability, compared with the blank control group, the percentage of cell wound healing area and the number of cell migration in the CCL2 group increased (all P< 0.05), while that in the CCL2+Netrin-1 group decreased (all P < 0.05). (Figure 1)

Obtaining NHE1 overexpression and silencing virus vector

Results of RT-PCR and pH recovery rate of NHE1 overexpression

The expression of NHE1 mRNA and protein in the overexpressed NHE1 group increased (all *P*< 0.05) (Figs. 2A and 2b); the recovery rate of pH value (NHE1 activity) after acid loading showed that compared with the blank group, the NHE1 activity in the overexpression group was enhanced (Figs. 2C and 2D).

Results of RT-PCR and pH recovery rate of NHE1 silencing

The expression of NHE1 mRNA and protein decreased in the NHE1 silencing group (all *P*< 0.05) (Figs. 3A and 3B); the recovery rate of pH value (NHE1 activity) after acid loading showed that compared with the blank group, the NHE1 activity in the overexpression group decreased (Figs. 3C and 3D).

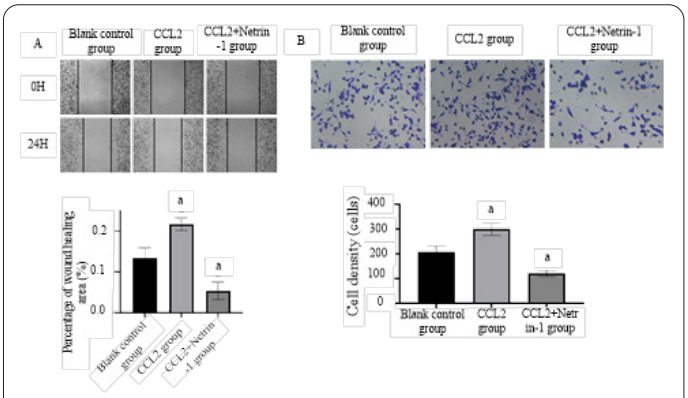

Figure 1. Effect of Netrin-1 on cell migration induced by CCL2. Note: A is the scratch test result (×100), and B is the Transwell result (×200); a: P< 0.05, *vs* blank control group, n = 3; b: P< 0.05, *vs* CCL2 group, *n* = 3.

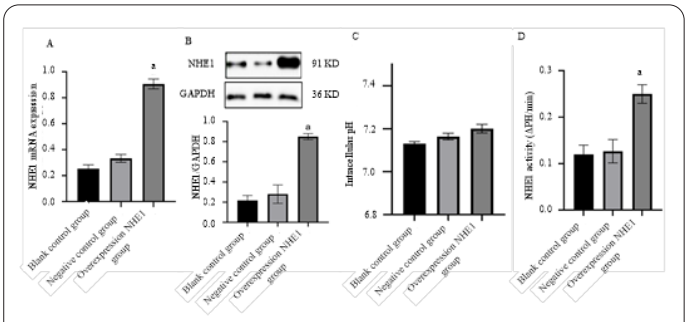

Figure 2. Netrin-1 inhibits intracellular NHE1 protein expression induced by CCL2. Note: a: *P*< 0.05 *vs* negative control group,*n*=3.

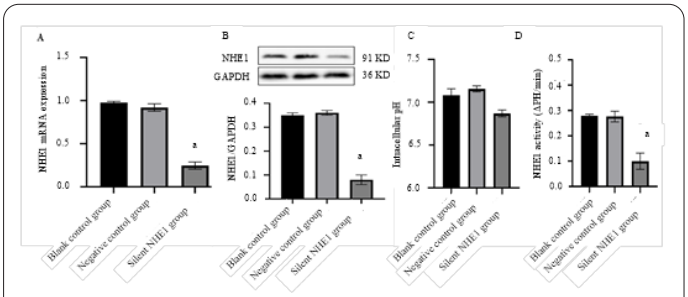

Figure 3. Transfection efficiency and activity of NHE1 silencing packaged by siRNA. Note: a: *P* < 0.05 vs negative control group, *n*=3.

Migration ability of macrophages after NHE1 overexpression/silencing

Results of migration assay

Compared with CCL2 group and Netrin-1+CCL2 group, the percentage of wound healing area and the number of cell migration of CCL2 macrophages treated by NHE1 overexpression/silencing increased/decreased ($P< 0.05$) (see Figure 4).

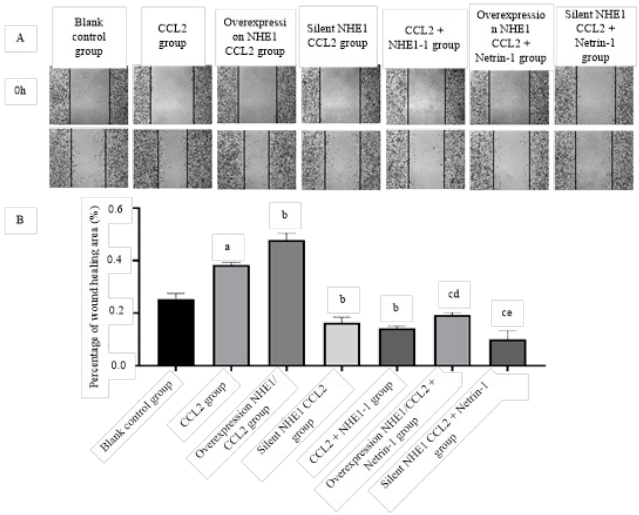

Figure 4. Observation of the effect of CCL2 on cell migration induced by Netrin-1 after Note: an NHE1overexpression/silencing. Note: A: scratch test; B: percentage of wound healing area, time: 24 h, 100× under a microscope; a: $P < 0.05$ *vs* negative control group, $n = 3$; b: $P < 0.05$ *vs* CCL2 group, $n = 3$; c: $P< 0.05$ *vs* CCL2+Netrin-1 group, $n = 3$; d: $P < 0.05$ *vs* overexpression/NHE1+CCL2 group, $n = 3$; e: $P < 0.05$ *vs* silencing NHE1/CCL2+Netrin-1 group, n=3.

Discussion

The inflammatory reaction in AS plaque increases oxygen consumption. As a result, the arterial plaque is relatively anoxic, and hypoxia-inducible factors accumulate. NHE1 and Netrin-1 are the target gene products of hypoxia-inducible factors. Chemokine CCL2 is the most famous CC chemokine, which can attract monocytes expressing CCL2 receptor CCR2 in the circulatory system, promote their entry into the surrounding damaged and inflammatory tissues, and transform them into tissue macrophages. Macrophages in plaque release a large amount of CCL2, which can recruit monocytes from circulating blood into plaque, where they devour oxidized LDL and become foam cells. Foam cells release inflammatory factors and promote plaque formation. CCL2 plays an important role in plaque formation (9). It binds to the CCR2 receptor of cells, which promotes the inflammatory reaction of cells, increases metabolic demand and oxygen consumption of cells, remodels metabolism during immune activation, and enhances glycolysis (10). To eliminate excessive H+, cells promote NHE1 synthesis. High inflammatory factors in arterial plaques compensate for elevated NHE1, exacerbating the state of plaque acidification and accelerating plaque development (11).

Netrin-1 is found in the ventral plate and neuroepithelial cells of the mammalian spinal cord, as well as in other parts of the nervous system, including the somatic mesoderm, pancreas, and myocardium. It-1 also plays a role in the development and formation of non-neural tissues, cardiovascular disease, renal disease, and other disorders (12) and is involved in the pathogenesis of acute and chronic inflammatory diseases (13). Although Netrin-1 was initially understood specifically to be involved in axonal guidance of the CNS, its expression also significantly increases in many cancers. (14). Netrin-1 receptors, UNC5B and DCC play a role in the nervous system, but immune cells are dominated by UNC5B, inhibiting cell migration (15).

In this study, it was found that inhibition of Netrin-1 could inhibit cell migration by regulating NHE1 in the NHE1 silencing or overexpression experimental group in the scratch test. Netrin-1 promotes cell migration of tumor cells (16), while it inhibits macrophage migration because tumor cells are mediated by Netrin-1 receptor or DCC and macrophages are mediated by UNC5B. In this experiment, the mouse phagocytic cell line RAW264.7 was used as the experimental cell line, which was induced by chemokine CCL2. The changes in cell migration ability and related mechanisms of Netrin-1 under the up-/down-regulation of NHE1 were observed. The results showed that CCL2 could promote cell migration and increase the expression and NHE1 activity protein, but this effect could be inhibited by Netrin-1. Furthermore, the adenovirus vector effectively knocking down NHE1 and overexpressing NHE1 was used to transfect cells and intervene NHE1 from two aspects, namely gene acquisition and gene deletion. It was found that up-regulation of NHE1 could promote cell migration, while down-regulation could inhibit cell migration. Up-regulation or down-regulation of NHE1 expression could enhance or weaken the ability of CCL2-induced cell migration, but Netrin-1 could reverse or inhibit the ability of CCL2-induced migration. At present, the joint study of NHE1 and Netrin-1 is only limited to the growth of neurite axis. NHE1 can promote the growth of neurite axis induced by Netrin-1, and its mechanism may be related to the up-regulation of rho GTP enzyme activity (17-18). Our current study showed that Netrin-1 inhibited cell migration, and the up-regulation of NHE1 did not reverse the inhibitory effect of Netrin-1. This suggests that the effect of Netrin-1 inducing cell migration may occur in the downstream of NHE1. Although it remains to be further studied whether NHE1 is the main bearer or bystander in this study, it is necessary to combine NHE1 with Netrin-1 to study arterial plaque. Given the high expression of NHE1 and Netrin-1 in tumors and nerve tissues, our research results may be of certain reference significance to the field of neurodevelopment or tumors.

To sum up, up-regulation of NHE1 could promote the migration of macrophage RAW264.7 driven by CCL2, while down-regulation of NHE1 could inhibit its migration; Netrin-1 inhibited the migration of macrophage RAW264.7 cells driven by CCL2, whether NHE1 was up-regulated or not.

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