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ORIGINAL ARTICLE

# Chemokine receptor CCR3 is important for migration of mast cells in neurofibroma

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**KEYWORDS**

Chemokine
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**ABSTRACT**

**Background** Neurofibroma consists of abundant extracellular matrix and many types of cells, including Schwann cells (SCs), mast cells (MCs), fibroblasts and endothelial cells. As SCs have been found to be the cell of origin for neurofibroma, how MCs may migrate into the tumor has not been fully clarified. Given that chemokine receptor CCR3 is found predominantly expressed by differentiated MCs, we postulated that CCR3 may play a role in the homing of MCs to neurofibroma. The goal of this study is to investigate the possible involvement of chemokine receptor CCR3 in the migration of MCs to the neurofibroma.

**Methods** Expressional and functional assays for CCR3 and its ligands were performed on MCs and SCs.

**Results** By real-time quantitative polymerase chain reaction and enzyme-linked immunosorbent assay, we found one of the CCR3 ligand, CCL7 was highly expressed by murine SC cell line SW10, and also moderately expressed by MCs. In serial chemotaxis assays, MCs were found specifically responsive to CCL7 and also condition medium from SW10 cells, indicating SCs may attract MCs by CCR3-mediated cell migration.

**Conclusion** The interaction of CCR3 and CCL7 may play important roles for MC migration toward SC in the neurofibroma.



## Introduction

Neurofibromatosis 1 (NF1), also known as von Recklinghausen's disease, is the most common neurocutaneous disorder mainly featured by localized or extensive cutaneous, intraneural, or even visceral neurofibromas which often cause life-long suffering of severe disfiguring and loss of function in patients.[1] The *NF1* gene, a tumor suppressor gene, has been identified as the main pathogenic gene for NF1 (in loss of function),[2] and its gene product, the neurofibromin protein, has been characterized as an antagonist of Ras protein,[3,4] a well-known oncogene product signaling cell proliferation and apoptosis resistance.[5]

Characterized by cellular heterogeneity, neurofibroma is composed of many types of cells including Schwann cells (SCs), mast cells (MCs), fibroblasts, endothelial cells (ECs), and perineural cells.[1] Several lines of evidences have indicated that SCs appear to be the major progenitor cells for neurofibroma formation; both *NF1* gene copies have been shown inactivated exclusively in the SCs, whereas a wild-type gene is still retained in the other neurofibroma cells, including fibroblasts.[6] Moreover, in vitro studies have revealed several unique tumorigenic features of the SCs in neurofibroma, including delayed senescence, lack of density-limited growth, and the tendency to form proliferative cell aggregates.[7]

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Functionally, it has also been shown that the neurofibroma SCs promote angiogenesis and invade basement membranes, while normal SCs and fibroblasts from neurofibroma do not.[8]

On the other hand, our knowledge about how MCs become one of the constituent cells of the neurofibroma and whether they may also contribute to neurofibroma tumor progression remains limited. Normally, MCs are originally derived from bone marrow progenitors, circulate in the peripheral blood as undifferentiated but committed progenitors, and then migrate into peripheral tissues (e.g. lung, gastrointestinal tract, or skin) where they mature into either mucosal-type MCs (MMCs) or connective tissue-type MCs (CTMCs).[9] MCs have long been considered as a key inflammatory effector cells in human allergic diseases like asthma; nevertheless, recent studies have also demonstrated many types of inflammatory cells, including MCs, play important roles in the initiation, progression, and angiogenesis of tumors.[10–12]

Chemokine receptor belongs to the family of seven-transmembrane, G protein-coupled receptor. Long well known for its ability to mediate directional migration of leukocyte, chemokine receptor has been shown to be essential for MC trafficking to airways and intestine in several constitutive or pathological conditions.[13,14] For example, several recent reports have demonstrated that chemokine receptor CCR3 plays multiple essential roles in MCs, including trafficking of MC progenitors and selective modification of MC phenotype and effector function.[15–20] Importantly, Romagnani et al[20] have demonstrated that CCR3 is primarily expressed by CTMCs distributed in skin and intestine submucosa, but not MMCs in intestine mucosa and lung, and CCR3 is the only chemokine receptor maintained in CTMCs after differentiation to mature cells.[20] We thus hypothesized that CCR3 may also play a role in the MC migration/homing to the neurofibroma. In present study, we have investigated the expressional status of CCR3 and its ligands in MCs and SCs respectively, followed by in vitro assays to test whether the migration and cell survival of MCs may be mediated in a CCR3-dependent fashion. Our data have provided further evidences that the chemokine receptor may also play a pivotal role in the process of MC migration to neurofibroma.

## Materials and methods

### *Antibodies, reagents, and cell lines*

Recombinant murine IL-3, recombinant murine CCL7 (also known as monocyte chemotactic protein-3), and recombinant murine stem cell factor (SCF) were purchased from Peprotech (Rockyhill, NJ, USA). Abs used in FACS analysis were anti-mouse CCR3 (R&D Systems, Minneapolis, MN, USA), anti-mouse FcεRIα (eBioscience, San Diego, CA, USA), and anti-mouse CD117 (Kit; eBioscience). The isotype control Abs were hamster IgG (Biolegend, San Diego, CA), rat IgG 2A (R&D Systems), goat IgG (R&D Systems), mouse IgG1 (Rockland, Gilbertsville, PA, USA), and rat IgG 2A (eBioscience). Anti-mouse CCL7 polyclonal Ab was from Biovision (Mountain View, CA), and HRP-conjugated anti-goat IgG was from Rockland. Other reagents used were calcein AM (Molecular Probe/Invitrogen, Rockville, MD, USA), and pertussus toxin (Calbiochem, San Diego, CA). The murine cell lines, including SC line (SW10), cytotoxic T cell line (CTLL-2), and immature dendritic cell line (JAWSII), were originally from American Type Culture Collection (Manassas, VA, USA) and purchased from local provider National Health Research Institute Cell Bank (Hsin Chu, Taiwan). All cell lines were cultured in growth media according to instruction of provider.

### *Isolation of mouse MCs*

Mouse bone marrow-derived CTMCs (BM-CTMCs) were isolated using BALB/c mice (6–8-week-old female) as previously described with modification,[21] under the approval of institutional animal care and use committee. Bone marrow cells were suspended in RPMI 1640 medium with 10% fetal bovine serum and cultured with SCF (40 ng/mL), and IL-4 (300 U/mL). Cells were incubated at 37ºC in a 5% $CO_2$ humidified atmosphere. Four weeks later, the cells were analyzed by flow cytometry for the expression of FcεRIα and Kit to characterize the differentiation phenotypes of BM-CTMCs. MCs in culture up to 4–8 weeks were used for experiments.

### *Detection of CCR3 and CCL7 expression by quantitative PCR (qPCR) and flow cytometry*

CCR3 expression by BM-CTMCs and CC Chemokines (CCL3, 7, 8, 13) expression by SW10 SCs were detected by real-time qPCR. Total RNA was isolated from cell pellets using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. First-strand cDNA was generated from total RNA using commercial kit for reverse transcription (Superscript First-Strand Synthesis System; Invitrogen, Frederick, MD, USA). qPCR was performed using an ABI PRISM 7900 DNA Sequence Detection System and SYBR Green (Applied Biosystems, Foster City, CA) to quantify the PCR product as previously described.[22] Mouse glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as an internal control. The primer pairs for CCL3 (5′-GCTTCTCC TACAGCCGGAAGA-3′ and 5′-GCAAAGGCTGCTGGTTTC A-3′), CCL7 (5′-GGATCTCTGCCACGCTTCTG-3′ and 5′-G GCCCACACTTGGATGCT-3′), CCL8 (5′-AGCTACGAGAGA ATCAACAATATCCA-3′ and 5′-CAGAGAGACATACCCTGCT TGGT-3′), CCL13 (5′-GAGACTCCCTCTGTGAATGTAATTC C-3′ and 5′-GCTGCCCGGCACATCTT-3′), CCR3 (5′-GGCT CCTGCCTCCACTGTAC-3′ and 5′-TGAGGATCAACACAAC CATTATGTT-3′), and G3PDH (CGTGTTCCTACCCCCAATGT and TGTCATCATACTTGGCAGGTTTCT) were designed using Primer Express software (Applied Biosystems). Cycle threshold

numbers (Ct) were derived from the exponential phase of PCR amplification. Fold differences in expression of gene *x* in cell populations *y* and *z* were derived by $2^k$, where $k = (Ctx - Ct_{G3PDH})$ population $y - (Ctx - Ct_{G3PDH})$ population *z*. For detection of CCR3 expression by BM-CTMCs at protein level, BM-CTMCs were stained with fluorescein-conjugated anti-mouse CCR3 antibody and were analyzed by flow cytometry.

### *Detection of the expression of CCR3 and CCL7 in neurofibroma lesions*

Tissue immunostain using anti-CCR3 antibody (Abcam, Cambridge, MA) and anti-CCL7 antibody (GenWay, San Diego, CA) was performed using 4-μm or 6-μm sections from the frozen (for immunofluorescent studies) biopsied specimens from neurofibroma lesions in NF1 patients, with informed consent and the approval of institutional review committee. Immunofluoresent staining was performed with confocal microscopy as previously described.[23] Briefly, frozen sections from representative neurofibroma lesions were stained with either anti-CCR3 or anti-CCL7 antibodies, followed by stain with respective FITC-conjugated secondary antibodies. Anti-glial fibrillary acidic protein (GFAP) and anti-MC tryptase antibody, from Abcam and Dako (Glostrup, Denmark) respectively, were used to stain and specifically label SCs and MCs with Rhodamine-conjugated secondary antibodies. 4′,6-diamidino-2-phenylindole (DAPI) was used to label cell nuclei.

### *Detection of CCL7 expression by enzyme-linked immunosorbent assay*

For CCL7 detection by ELISA, 96-well microtitre plates (Nunc-Immuno Module F8 MaxiSorp, NalgeNunc International, Roskilde, Denmark) were coated with 100-μL of (per well) SW10 culture medium. The wells were incubated overnight at room temperature. After washing, the wells were incubated with blocking solution (1% BSA/PBS) for 1 hour. 100-μL anti-mouse CCL7 polyclonal antibody (2 μg/mL) was then added to each well. After incubation for 2 hours, 100 μL of HRP-conjugated anti-goat IgG (1:35 000) was added. The plate was developed with TMB Plus, Liquid-1 Component (Amresco Inc., Solon, OH, USA) for 20 minutes. The reaction was then blocked with Stop Solution (1N $H_2SO_4$). The absorbance was measured by microplate reader at 450 nm.

### *Chemotaxis assay*

The chemotaxis assay was performed as previously described.[24] The migration of BM-CTMCs in response to CCL7 was analyzed using 96-well (8-μm pore size) ChemoTx Disposable Chemotaxis System (Neuro Probe; Gaithersburg, MD). MCs ($2.5 \times 10^4$) were assessed with exposure to recombinant mouse CCL7 at various concentrations or SW10 conditioned medium, with or without other reagents (to test CCL7-dependence) including pertussis toxin (PTX) (100 ng/mL), anti-mouse CCL7 neutralizing antibody (40 μg/mL), and goat IgG isotype control (40 μg/mL). BM-CTMCs were labeled with calcein AM, loaded atop of the filter, and the chemotaxis well was incubated at 37ºC for 3 hours. The number of fluorescent MCs migrating through filter was quantified by direct counting in three or more randomly selected microscopic fields (40× objective).[21]

### *CCR3 signaling pathways in MCs*

BM-CTMCs ($1 \times 10^6$/mL) were cultured in 24-well plates for 4 hours at 37º in serum-free medium and then treated with CCL7 (200 ng/mL), with or without neutralizing anti-CCL7. Cells were harvested at various time points (0, 1, 5, 10, 15, and 30 min) for total protein extraction. The Coomassie Protein Assay Kit (PIERCE, Rockford, IL) was used to measure the protein. Cell lysates containing 30-μg protein were separated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to PVDF membranes. Membranes were blotted with various antibodies, including antipan-Akt, anti-phospho-Akt, anti-pan-ERK1/ERK2, and antiphospho-ERK1/ERK2 (R&D systems) at 4ºC overnight. Secondary antibodies were then added, and the bands were developed by enhanced chemiluminescence.

### *Statistical analysis*

The data are presented as mean ± SD. Data were analyzed by Student's t tests (two-sided, parametric). Statistical significance was determined at $p < 0.05$. Experiments were performed at least three times. Representative data are presented.

## Results

### *CCR3 expression by BM-CTMCs*

Using qPCR and flow cytometry, we have measured the expression of chemokine receptor CCR3 by mouse BM-CTMCs. BM-CTMCs were isolated and cultured as previously reported,[21] with about 88% of cell population showing the expression of phenotype markers including Kit, FcεRIa (data not shown) and heparin-containing granules.[21,25] Relatively high expression of CCR3 by BM-CTMCs has been demonstrated using qPCR (Figure 1A), in comparison with other type of murine inflammatory cells including CTLL-2 (cytotoxic T cell line) and JAWSII (immature dendritic cell line), as these two cell types have also been reported to express CCR3.[26,27] CCR3 expression was also detected by flow cytometry (Figure 1B) and by immunostain (Figures 1C and D) using samples of neurofibroma lesions. Representative lesions were studied with both immunohistochemical (not shown)

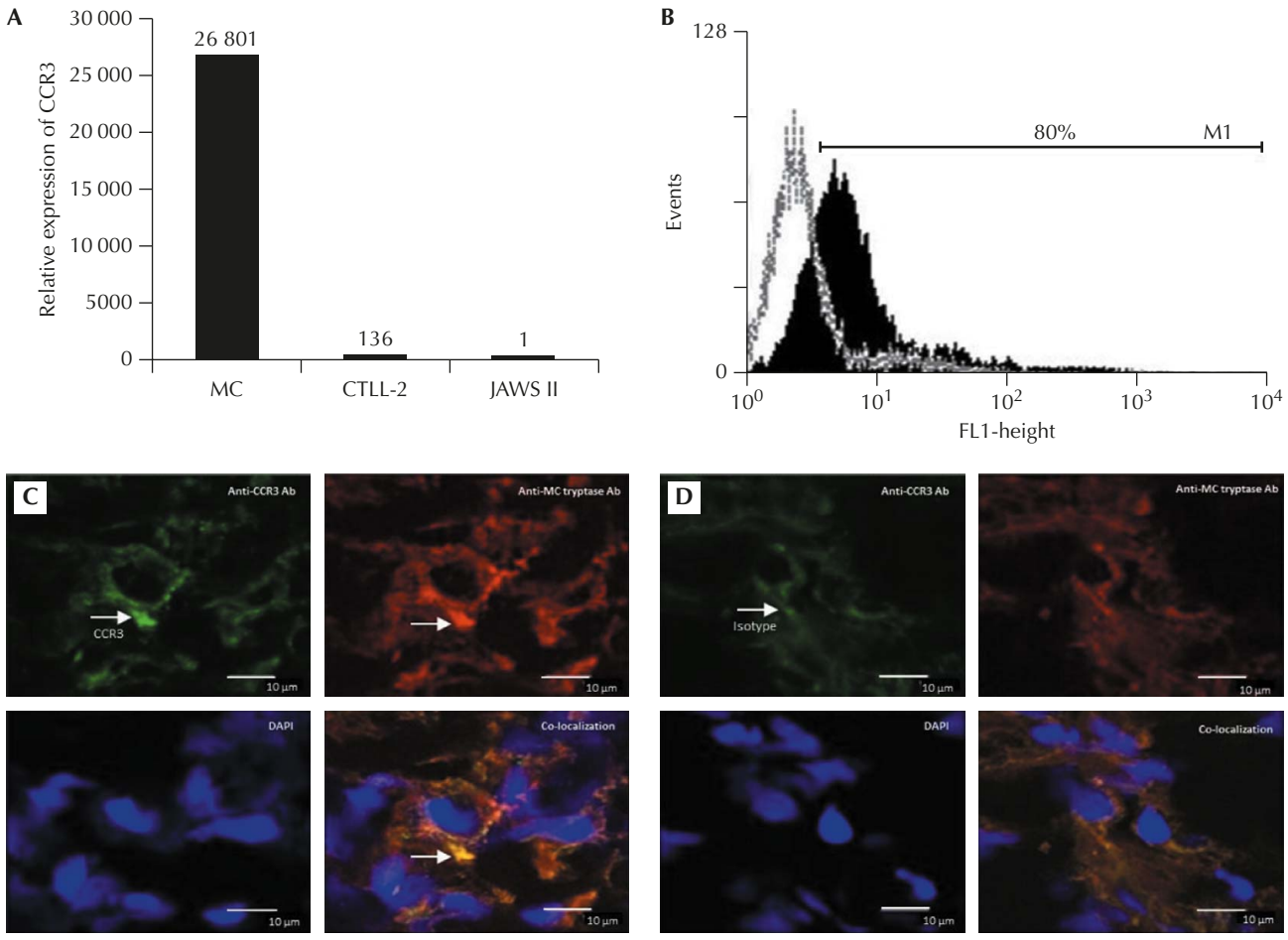


**Figure 1** Expression of CCR3 by mast cells (MC). (A) CCR3 expression by bone marrow–derived connective tissue–type MCs (BM-CTMC) was detected by real-time quantitative polymerase chain reaction, in comparison with other murine immunological/inflammatory types of cells (CTLL-2, cytotoxic T cell line; JAWSII, immature dendritic cell line). Relative expression level for each cell type is shown atop the black bars. CCR3 expression by MCs was also detected by flow cytometry (B) Dotted line indicates isotype IgG for anti-CCR3; solid area indicates anti-CCR3 antibody and immunostain. (C) CCR3 expression by MCs detected by immunofluoresent staining. Representative frozen sections from neurofibroma lesions were stained with FITC-conjugated anti-CCR3 antibodies (left upper panel, green). Rhodamine-conjugated anti-MC tryptase antibodies were used to stain and specifically label MCs (right upper panel, red). Colocalization (merged color) of CCR3, MCs, and cell nuclei (DAPI stain, blue; left lower panel) was shown in right lower panel. White arrow indicates a representative MC showing colocalization of CCR3 and MC tryptase. (D) Isotype control study for anti-CCR3 staining with similar setting as in (C), demonstrating insignificant baseline stain (left upper panel). White arrow indicates a representative MC. Immunofluorescent staining was detected by confocal microscopy.

and immunofluorescent (Figures 1C and D) methods, using isotype control (for anti-CCR3) and double staining (to label MCs), and the expression of CCR3 by MCs was thus confirmed.

### *CCL7 expression by SCs and MCs*

In order to determine whether SCs, the progenitor cells for neurofibroma, may attract MCs through the interaction of CCR3 and its ligand (s), the expressional status of all the four ligands for CCR3 (CCL3, CCL7, CCL8, and CCL13) in SW10 SC cells was examined by qPCR. As shown in Figure 2A, CCL7, but not other ligands for CCR3, was found predominantly expressed by SW10. CCL7 expression by SCs was further confirmed by ELISA, showing CCL7 secretion in conditioned medium derived from SW10 culture (Figure 2B).

To further determined the CCL7 expression in the neurofibroma lesions, representative lesions were studied with both immunohistochemical (not shown) and immunofluorescent (Figure 2C) methods as aforementioned, using isotype control (for anti-CCL7) and double staining (to label SCs and MCs) to confirm the expression of CCL7 by SCs. As shown in Figure 2C, immunofluorescent study by confocal microscopy demonstrated CCL7 expression around SCs and MCs (not shown).

### *MCs are specifically responsive to CCL7 and conditioned medium from SCs*

We next determined whether CCR3 may be functionally active in BM-CTMCs. By standard chemotaxis assay, BM-CTMCs demonstrated typical chemotaxis responses to CCL7 at various concentrations (Figure 3A). The chemotaxis response was specific to CCL7, as it could be blocked by neutralizing anti-CCL7 Antibody and PTX (a nonspecific blocking agent for chemokine receptors), but not by isotype control IgG

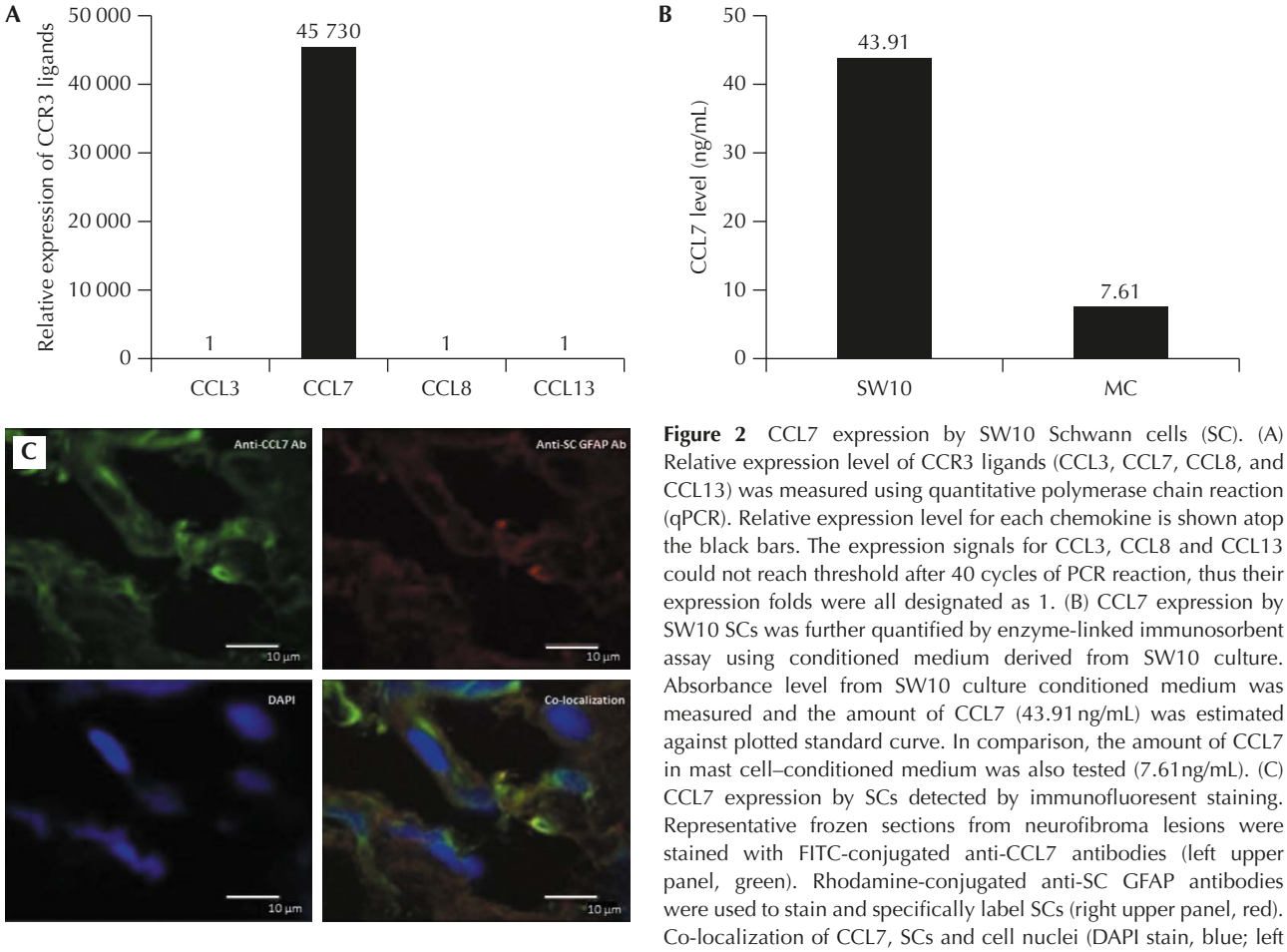


**Figure 2** CCL7 expression by SW10 Schwann cells (SC). (A) Relative expression level of CCR3 ligands (CCL3, CCL7, CCL8, and CCL13) was measured using quantitative polymerase chain reaction (qPCR). Relative expression level for each chemokine is shown atop the black bars. The expression signals for CCL3, CCL8 and CCL13 could not reach threshold after 40 cycles of PCR reaction, thus their expression folds were all designated as 1. (B) CCL7 expression by SW10 SCs was further quantified by enzyme-linked immunosorbent assay using conditioned medium derived from SW10 culture. Absorbance level from SW10 culture conditioned medium was measured and the amount of CCL7 (43.91 ng/mL) was estimated against plotted standard curve. In comparison, the amount of CCL7 in mast cell–conditioned medium was also tested (7.61ng/mL). (C) CCL7 expression by SCs detected by immunofluoresent staining. Representative frozen sections from neurofibroma lesions were stained with FITC-conjugated anti-CCL7 antibodies (left upper panel, green). Rhodamine-conjugated anti-SC GFAP antibodies were used to stain and specifically label SCs (right upper panel, red). Co-localization of CCL7, SCs and cell nuclei (DAPI stain, blue; left lower panel) were shown in right lower panel. Immunofluorescent staining was detected by confocal microscopy.

(Figure 3B). Moreover, BM-CTMCs also showed specific responses to the SW10-derived conditioned medium by chemotaxis assay (Figure 3C), as the chemotaxis migration could also be blocked by anti-CCL7 and PTX, but not by isotype control. These results indicate SW10 may attract BM-CTMCs through chemotaxis mediated by CCR3-CCL7 interaction, thus supporting that CCR3 may play an important role in MC migration and homing to neurofibroma.

### *Immunoblotting for tracing CCR3 signaling pathway in response to CCL7*

To further explore the involvement of CCR3/CCL7 in the migration/homing and the survival of MCs, we next investigated the possible signaling events of BM-CTMCs in response to CCL7 by immunoblotting. In response to CCL7 treatment, the time-dependent accumulation of phosphorylated Erk1/2 was found in BM-CTMCs (Figure 4, upper panel). Moreover, the phosphorylation of another important signaling effector molecule, Akt was also found increased above basal level with the presence of CCL7 (Figure 4, lower panel). These reactions were CCL7-specific, as they could be blocked by neutralizing anti-CCL7 (not shown).

## Discussion

Long well-known as a key player for allergic disease like asthma, MC has been found to be involved in the induction of both innate and adaptive immune response and actively mediating an expanding list of diverse diseases with autoimmune, inflammatory, or even neoplastic natures.[9–11] Although the increased number of MCs in neurofibroma is a long well-known phenomenon,[28] the mechanism to mediate the migration and homing of MC to neurofibroma has not been elucidated until recent years. In a recent study, by using nullizygous (*NF1*$^{-/-}$) SCs and heterozygous (*NF1*$^{+/-}$) MCs from transgene mice, Yang et al[29] have shown that neurofibromin-deficient SCs secret more Kit ligand (namely SCF), which may serve as a chemoattractant for MCs expressing Kit receptor and stimulate MC migration in vitro. This study has provided a theoretical base which not only provides an explanation, at least in part, to the phenomenon of mastocytosis in neurofibroma lesions, but also highlights the possible close interaction between SCs and MCs in the neurofibroma progression via paracrine-mediated mechanisms.

However, although SCF is well known as a cytokine capable of enhancing development and multiple functions

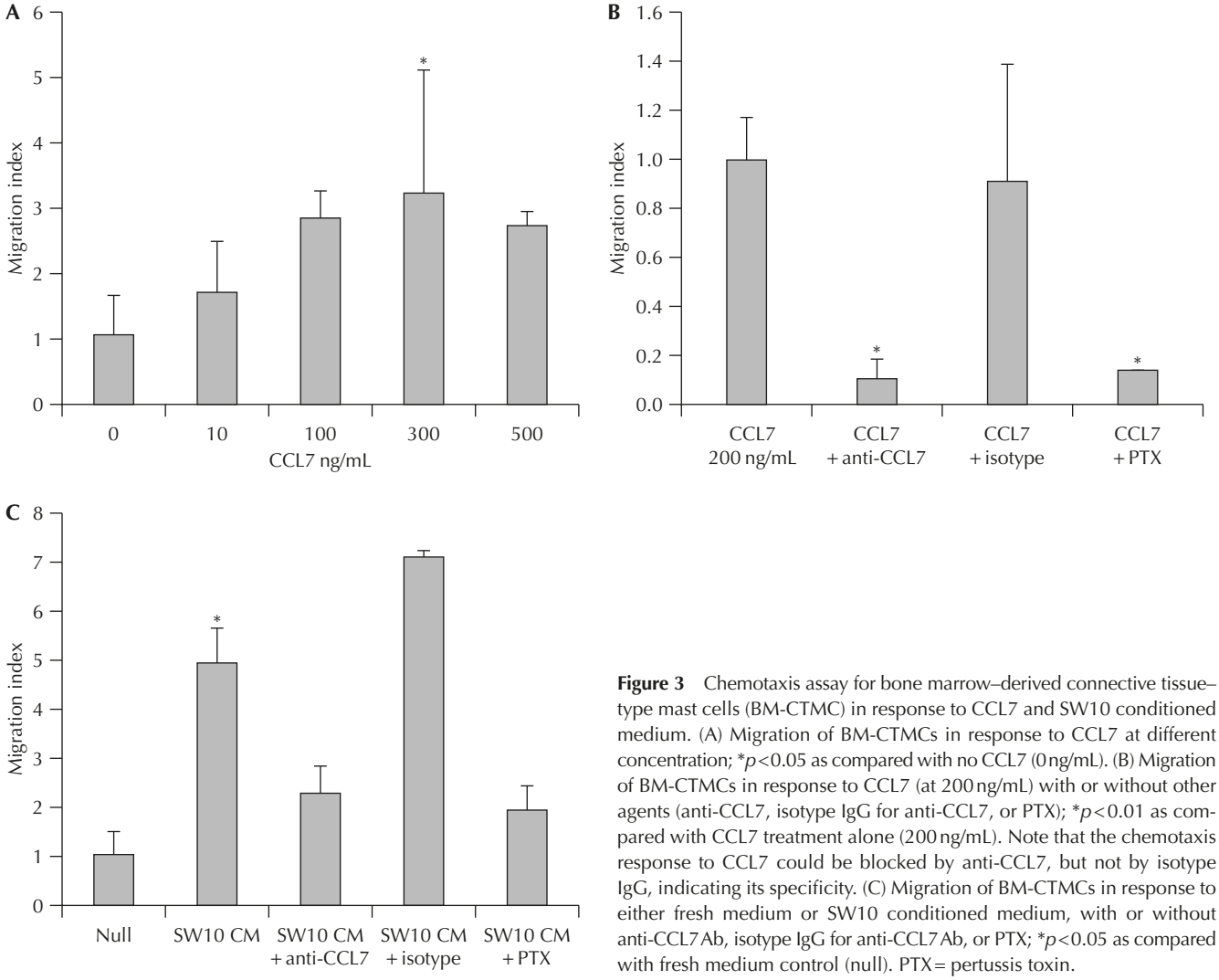


**Figure 3** Chemotaxis assay for bone marrow–derived connective tissue–type mast cells (BM-CTMC) in response to CCL7 and SW10 conditioned medium. (A) Migration of BM-CTMCs in response to CCL7 at different concentration; *$p<0.05$ as compared with no CCL7 (0 ng/mL). (B) Migration of BM-CTMCs in response to CCL7 (at 200 ng/mL) with or without other agents (anti-CCL7, isotype IgG for anti-CCL7, or PTX); *$p<0.01$ as compared with CCL7 treatment alone (200 ng/mL). Note that the chemotaxis response to CCL7 could be blocked by anti-CCL7, but not by isotype IgG, indicating its specificity. (C) Migration of BM-CTMCs in response to either fresh medium or SW10 conditioned medium, with or without anti-CCL7 Ab, isotype IgG for anti-CCL7 Ab, or PTX; *$p<0.05$ as compared with fresh medium control (null). PTX = pertussis toxin.

of MCs including migration,[30] given the marked cellular heterogeneity of neurofibroma and the complicated long-travelled migration pathway of MCs, it is quite unlikely that SCF is the only factor to mediate MC migration and homing to neurofibroma. It should also be noted that SCF may also have inhibitory effect on chemotaxis of MCs according to a recent report.[31] Other paracrine-mediated pathways, therefore, may well possibly also play important roles in the recruitment and homing of MCs to the lesions of neurofibroma from peripheral blood. As chemokine and chemokine receptors have been well established to be essential for the migration of inflammatory or malignant cells to the lesions of many inflammatory or cancerous diseases,[32] we therefore hypothesized that chemokine and chemokine receptors may also play a role in the MC migration to the neurofibroma. Indeed, there have already been quite a few studies demonstrating that chemokine and chemokine receptors are important for MC trafficking to airways and intestine in either constitutive or pathological conditions.[13,14] However, to date, very few reports[21] have specifically described whether chemokine system may also play a role in the homing/migration pathway of MC to neurofibroma.

We next asked which chemokine receptor may be important for MC migration to neurofibroma. As CCR3 has been shown to be the only chemokine receptor maintained in CTMC after differentiation to mature cells[20] and may play multiple pivotal roles in trafficking, effector function, and phenotype modification of MCs,[15–20] we hence further hypothesize that SCs, being the progenitor cells for tumor formation of neurofibroma, may attract MCs by CCR3-mediated mechanisms. Using mouse BM-CTMCs (a model of CTMC mimicking skin MCs)[21] and SC line SW10, herein we have shown that CCR3-expressing BM-CTMCs are able to migrate in response to SW10-derived conditioned medium in a CCL7-dependent fashion, thus supporting our postulation that CCR3 may also play a role in the migration and homing of MCs to the SC-initiated neurofibroma lesions.

We further found that survival of BM-CTMCs can be significantly hampered by neutralization of CCL7 (data not shown), and hence postulated that autocrine-mediated, rather than paracrine-mediated reaction may govern the survival need for the MCs residing in the neurofibroma lesions, as evidenced by our finding that MC itself also express comparable amount (in comparison with SW10 SC) of CCL7, which is in

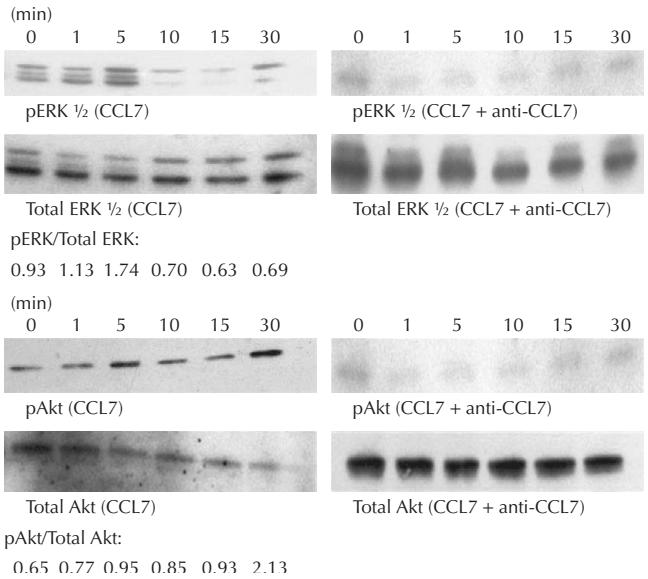


**Figure 4** Cell signaling assays for bone marrow–derived connective tissue–type mast cells (BM-CTMC). CCL7 treatment induces ERK and Akt phosphorylation in BM-CTMCs. In response to CCL7 treatment, the time-dependent accumulation of phosphorylated Erk 1/2 (pErk 1/2) was found in BM-CTMCs by immunoblotting (upper left panel). Moreover, the phosphorylation of another important signaling effector molecule, Akt, was also found increased (pAkt) above basal level with the presence of CCL7 (lower left panel). Neutralizing anti-CCL7 Abs were able to block both signaling event as shown in upper right panel (ERK) and lower right panel (Akt).

line with previous report that MCs may also express CCL7.[33] Certainly, it is quite possible that other paracrines or autocrines from MCs or other types of cells, in the complicated milieu of neurofibroma lesion, may also play a role in enhancing survival of MCs in the tumor.

Our finding that BM-CTMCs showed activation of ERK and Akt kinase pathway after CCL7 treatment is in accordance with previous reports.[34,35] As Mocsai et al[34] have demonstrated that stimulation of GPCR may induce the phosphorylation of ERK and Akt in BMMCs, Woo et al[35] have shown that migration of leukemia MCs to another CCR3 ligand, CCL11 (also known as eotaxin), is dependent on ERK pathway. Our data of CCL7-induced signaling thus further support the role for CCR3 to mediate the migration of MCs to neurofibroma lesions with expression of CCR3 ligands.

As *NF-1* has been identified as the pathogenic gene for neurofibroma and has been shown to be important to mediate MC migration (in deficient expression),[1,2] we have also investigated the level of *NF-1* expression by SW10 cells which serve as the model SC cells in our study. By qPCR, there is indeed less expression of *NF-1* by SW10, in comparison with mouse fibroblast (NIH/3T3) and mouse endothelial (MS-1) cells (data not shown). As previous reports have emphasized the indispensable role of $NF1^{-/-}$ SCs as the tumorigenic cells for neurofibroma, the fact that SW10 cells express less *NF1* than other types of cells that are also present in the neurofibroma lesions (fibroblast and endothelial cells) is in accordance with current theory and hence support the use

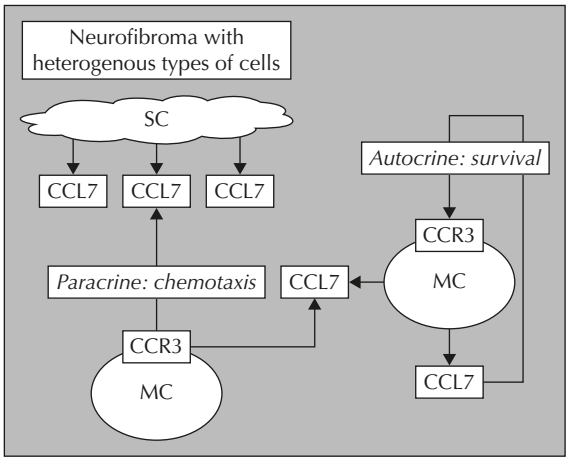


**Figure 5** Proposed model for the involvements of CCR3 and CCL7 in the mastocytosis of neurofibroma. CCR3 may play a role in the chemotactic migration/homing of mast cells (MC) to CCL7-secreting Schwann cells (SC) in neurofibroma (paracrine), while CCL7 may be important to sustain the survival of MC in neurofibroma lesions (autocrine) and may also help to attract more MC to the already formed tumor.

of SW10 cells as the representative SCs in this study. It will be interesting to see if further knockdown of *NF1* gene will enhance tumorigenic natures of SW10.[7]

In present study, we have provided evidences that chemokine receptor CCR3 and its ligand CCL7 may play a role in the migration of MCs toward progenitor cells (SCs) for neurofibroma (paracrine-fashion) and may sustain MC's survival in an autocrine way (Figure 5). Given that chemokine network has emerged as a promising therapeutic target for inflammatory disease, cancer, and viral infection, and there is still no effective therapy to date to contain the tumorigenesis of neurofibroma for NF1 patients, CCR3 or CCL7 may well serve as a potential target for the development of novel therapeutics for neurofibromatosis. Indeed, as Das et al[36] have demonstrated that small molecule antagonist against CCR3 may selectively inhibit eosinophil influx into the lung in mouse model of allergic inflammation, it will be important to investigate whether the migration of CCR3-expressing MCs and the subsequent tumor progression of neurofibroma may also be blocked by the similar approach.

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