Clinical Immunology 205 (2019) 57–64

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Epidermal growth factor receptor mimotope alleviates renal fibrosis in murine unilateral ureteral obstruction model

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ARTICLE INFO

Keywords:
Epidermal growth factor receptor
Macrophage
Mimotope
Renal fibrosis
CD9

ABSTRACT

Macrophages have been recognized as a vital factor that can promote renal fibrosis. Previously we reported that the EGFR mimotope could alleviate the macrophage infiltration in the Sjögren's syndrome-like animal model. In current study, we sought to observe whether the active immunization induced by the EGFR mimotope could ameliorate renal fibrosis in the murine Unilateral Ureteral Obstruction (UUO) model. A series of experiments showed the EGFR mimotope immunization could ameliorate renal fibrosis, reduce the expressions of fibronectin, α-SMA and collagen I and alleviate the infiltrations of F4/80+ macrophages in UUO model. Meanwhile, the EGFR mimotope immunization could inhibit the EGFR downstream signaling. Additionally, the frequency of and F4/80+CD9+/FAS+ macrophages significantly increased in spleen after the EGFR mimotope immunization. These evidence suggested that the EGFR mimotope could alleviate renal fibrosis by both inhibiting EGFR signaling and promoting macrophages apoptosis.

1. Introduction

Chronic kidney disease (CKD), which leads to aggressive and irreversible kidney destruction, is a devastating disease and therefore recognized a worldwide health problem that presents tremendous socioeconomic challenges [1,2].

Renal fibrosis, the common characteristic of CKD, is generally observed in almost every type of CKD during progression to the end stage of the disease.

Chronic fibrosis is exacerbated by the persistence of kidney inflammation, which involves both immune cells and activation of intrinsic renal cells. Thus pro-fibrotic cytokines and growth factors are released by these cells to attract more inflammatory cell infiltration served as major driver of persistent fibrosis [3,4]. Macrophages have been recognized as a vital factor that can promote renal fibrosis. Macrophages can activate and support myofibroblast survival by recruiting inflammatory cells and secreting growth factors such as TGF-β1 [5]. Meanwhile, macrophages could be induced to develop into collagen-producing a-SMA+ myofibroblast type cells in renal fibrosis environment [6,7]. Macrophages can also produce galectin-3, a beta-galactoside binding lectin, promote renal fibrosis through the profibrotic activation of renal fibroblasts independent of TGF-β expression or downstream signaling [8]. Macrophages are also a major source for matrix metalloproteinase (MMP), which can alter MMP-mediated extracellular matrix (ECM) homeostasis to promote fibrosis [9–11]. Therefore, targeting macrophage could be a potential effective therapeutic strategy.

Despite EGFR inhibitors and monoclonal antibodies (mAbs) are widely used in the clinical management, several common limitations of EGFR inhibitors and mAbs still need to be overcome: (i) retreatment is required to achieve disease control, but this exposes patients to the risk of the delayed-type hypersensitivity infusion reactions; (ii) a relatively high cost of production may not be acceptable to patients for a long-term treatment; (iii) inadequate patients compliance to the repeated infusions planned on a regular basis [12–14].

A mimotope is one kind of small peptides that structurally mimic a given antibody-binding site but are composed of different amino acids [15,16]. Active immunization using mimotopes has been developed as a strategy to overcome these practical limitations of EGFR inhibitors and monoclonal antibodies via initiation of the ongoing production of similar but more effective antibodies, which could recognize the mimicked natural epitope, and thereby prevent downstream signaling

Abbreviations: EGFR, Epidermal Growth Factor Receptor; CKD, Chronic kidney disease; MMP, matrix metalloproteinase; CFA/IFA, complete Freund adjuvant/incomplete Freund adjuvant; ECM, extracellular matrix; UUO, Unilateral Ureteral Obstruction; ADAM, metalloproteinase domain-containing protein; ALCAM, activated leukocyte cell adhesion molecule

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https://doi.org/10.1016/j.clim.2019.05.014
Received 6 February 2019; Received in revised form 24 April 2019; Accepted 28 May 2019
Available online 29 May 2019
1521-6616/

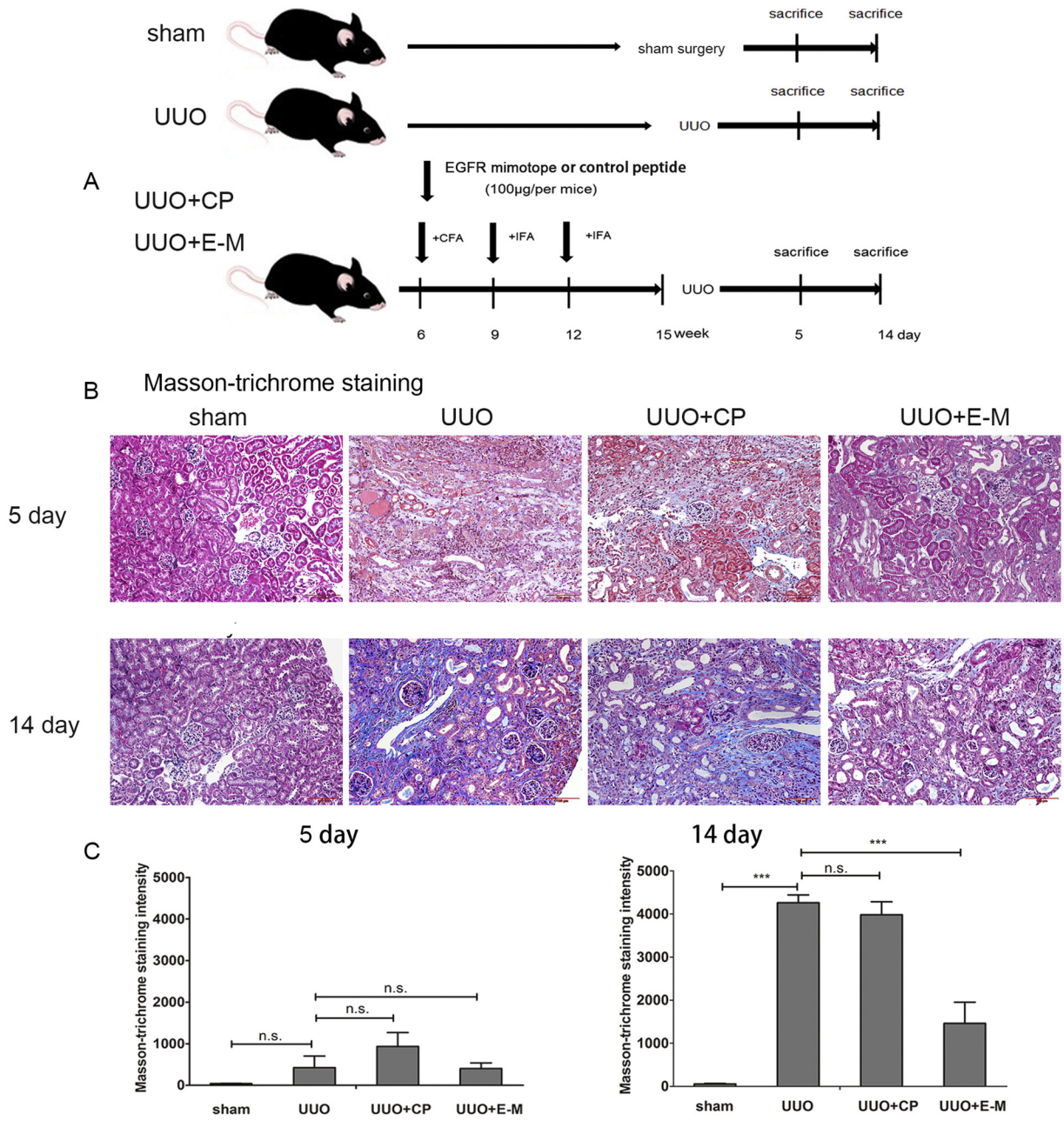

Fig. 1. Mimotope immunization ameliorated renal fibrosis in UUO model at day 14 after UUO. (A) Procedures of immunization and UUO. (B) Kidneys were harvested at days 5 and 14 after UUO for masson-trichrome staining. The results showed that a significant increase collagen accumulation could be observed in the kidneys at day 14, but not at day 5. EGFR mimotope immunization could significantly reduce the collagen deposition induced by UUO surgery. Although collagen accumulation was not observed in the kidneys at day 5 after UUO, we found severe nephron structure destructions in the kidneys from UUO and UUO + CP groups and the EGFR mimotope immunization could prevent the destructions obviously. (Mean ± SEM; ***, $p < .001$; **, $p < .005$; n.s.: non-significant; Representative image, n = 6 mice per group).

[17].

In previous study, we reported that the EGFR mimotope could alleviate the macrophage infiltration in exocrine glands in an autoimmune disease animal model [18]. In light of the important role of macrophage in renal fibrosis, we sought to observe whether the EGFR mimotope could ameliorate renal fibrosis in the murine Unilateral Ureteral Obstruction (UUO) model by inhibit EGFR signaling and promote macrophage apoptosis.

2. Materials and methods

2.1. Animals

Female 6-week-old C57BL/6J mice were purchased from shanghai laboratory animal center (Shanghai, China). The animals were maintained in a pathogen-free facility of Laboratory Animal Science of Hangzhou Normal University. Ethical approval for the use of animals in this research study was obtained from the Ethics Committee of Hangzhou Normal University. All procedures involving animals were performed according to the Research Animal Administration Guidelines of China and the Guidelines for the Care and Use of Laboratory Animals

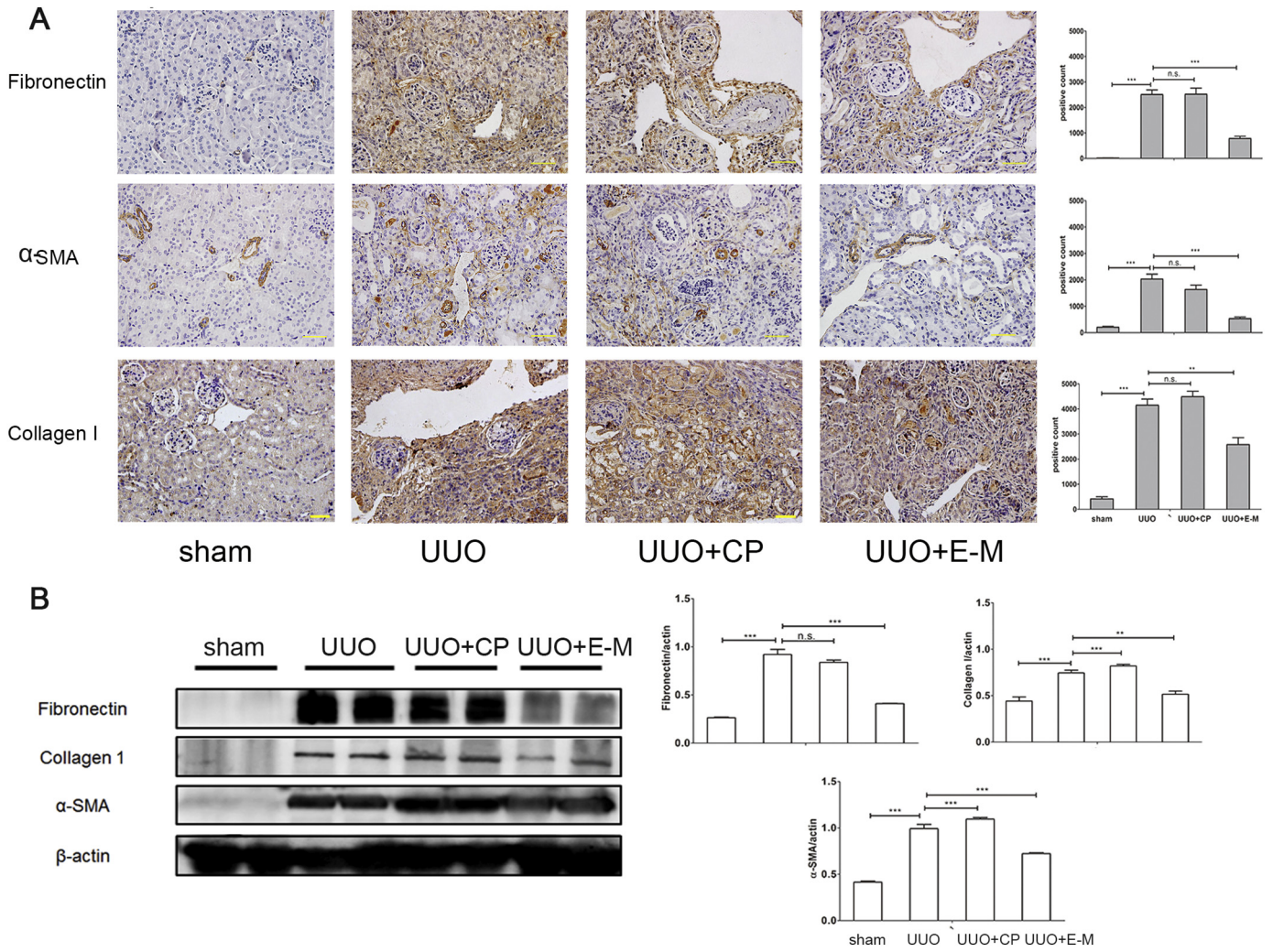

Fig. 2. Mimotope immunization reduced the expressions of fibronectin, α-SMA and collagen I at day 14 after UUO. (A) For immunohistochemistry staining, kidney sections were incubated with anti-fibronectin, -α-SMA, -collagen I, followed by incubation with anti-rabbit secondary IgG. The results showed that the EGFR mimotope immunization could significantly reduce the expressions of fibronectin, α-SMA and collagen I at day 14 after UUO. (B) Western blot assay showed the similar changes on the expressions of fibronectin, α-SMA and collagen I at day 14 after UUO. (Mean ± SEM; ***, $p < .001$; **, $p < .005$; n.s.: non-significant; Representative image, n = 6 mice per group).

in China.

2.2. Peptide immunization and unilateral ureteric obstruction (UUO)

The EGFR mimotope WHTEILKSYPHEGGGSGGGS [19] and control peptide (AIPLVVPFYSHSGGGSGGGS) (purity of the peptide was > 98%) were synthesized chemically via the solid-phase procedure by SBS Co. Ltd. (Beijing, China).

The C57BL/6J mice were divided into either the sham group (n = 12 mice), the UUO group (n = 12 mice), the UUO + control peptide group (UUO + CP, n = 12 mice), the UUO + EGFR mimotope group (n = 12 mice). UUO was performed by ligation of the left ureter as described previously [20]. Sham-operated control mice underwent an identical surgical procedure to the UUO mice except ligation of the ureter was not performed. The immunization and UUO procedures are shown in Fig. 1A. Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Renal fibrosis may not only be triggered by pro-inflammatory macrophages, but instead by profibrotic macrophages in the different stages of CKD [21,22]. Thus, we sacrificed mice at day 5 and 14 after UUO to observe renal fibrosis and macrophages.

2.3. Histology analysis

Kidneys were harvested at days 5 and 14 after UUO. Sections were thawed, dried, and then fixed with 4% paraformaldehyde PBS for 30 min. Paraffin-embedded sections were deparaffinized by immersion in xylene, followed by dehydration in ethanol. The tissue sections were prepared and stained with Masson-trichrome. For immunohistochemistry staining, sections were incubated with anti-fibronectin, -α-SMA, -collagen I, -F4/80 (fibronectin: sc-29011, santa cruz biotechnology; α-SMA: 19245, Cell Signaling Technology; collagen I: ab34710; F4/80: ab16911, Abcam). Sections were observed with a Leica microscope (DM3000, Leica). The images were obtained using a Leica Application Suite v3.6.

2.4. Flow cytometric analysis

In previous study, we reported that the EGFR mimotope could alleviate the macrophage infiltration in exocrine glands in an autoimmune disease animal model by upregulating the expression of FAS in macrophage. Additionally, CD9 was reported to be a negative regulator of macrophage activation [23,24]. Thus, we evaluated the expression of FAS and CD9 in macrophages at day 5 and 14 after UUO.

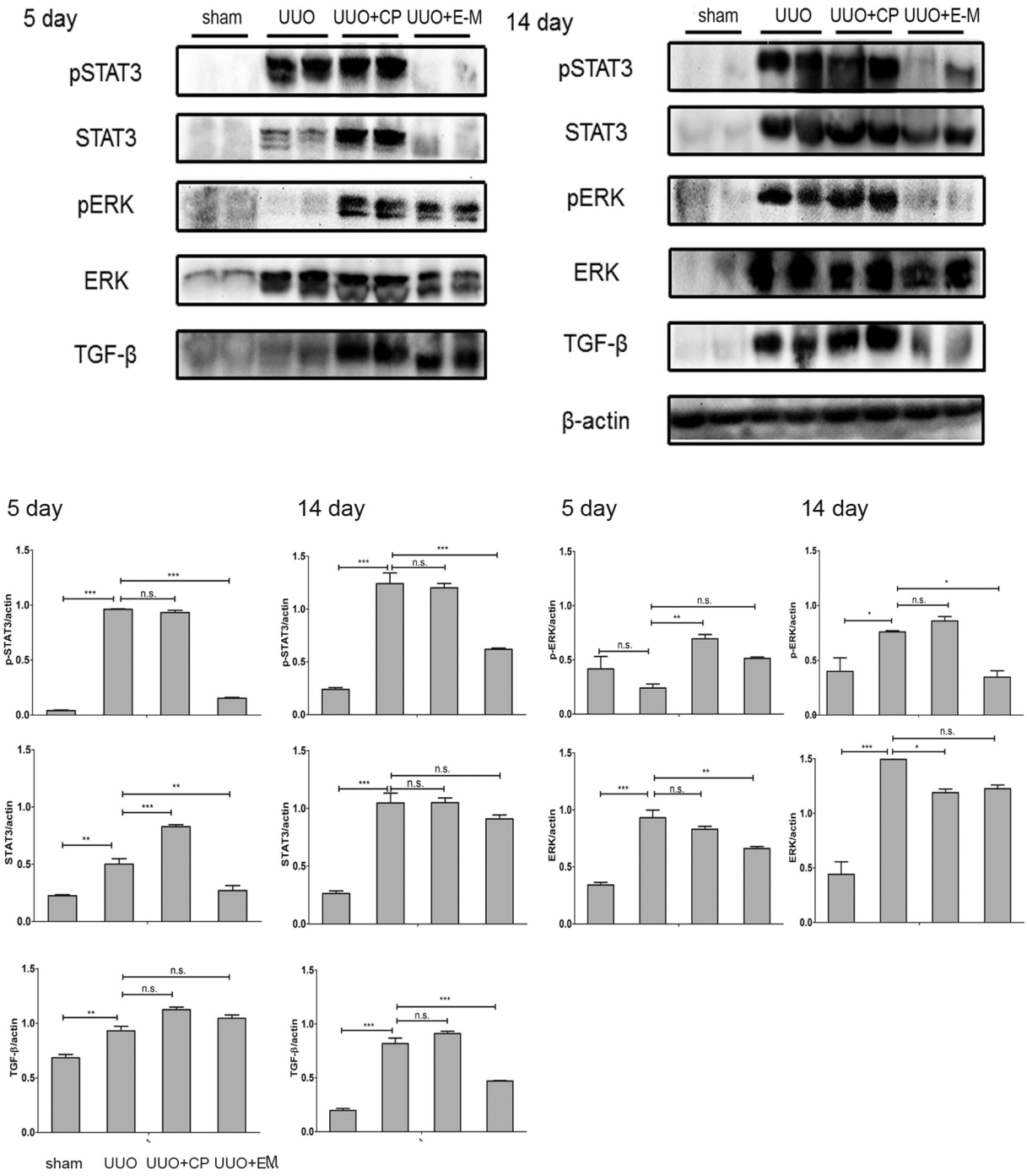

Fig. 3. Mimotope immunization inhibited EGFR downstream signaling. Kidneys were dissected and homogenized for signaling analysis by western blot assay. The membranes were incubated with anti- p-ERK, -ERK, -p-STAT3, -STAT3 and –TGF-β antibody. The results showed that the EGFR mimotope immunization could significantly inhibited EGFR downstream signaling induced by UUO. The experiment was performed in triplicate. Gray value ratio was calculated for statistical analyses by using Image J. (Mean ± SEM; ***, $p < .001$; **, $p < .005$; *, $p < .05$; n.s.: non-significant).

When we isolated the spleens, we found the spleens were significantly enlarged after UUO. Consequently, we measured the weight of the spleen. The spleen weight index was calculated using the formula [spleen weight (mg)/mouse weight (g) × 10]. After the red blood cells were removed from the splenocytes by treatment with 0.16 M NH_4Cl solution, the splenocytes were collected and incubated with APC-labeled anti-CD9, Alexa Flour 488-labeled anti-F4/80+, APC-labeled anti-FAS (CD9: 17-0091-82; F4/80+, MF48020; FAS: MA1-10313) for 30 min at room temperature. Then, the cells were analyzed by flow cytometry (BD, FACSCanto II).

2.5. Western blot analysis

Kidneys were dissected and homogenized in Tissue Extraction Reagent I (Invitrogen). After centrifugation at 15,000*g* for 15 min at 4 °C, equal amounts of protein lysate were loaded directly after immunoprecipitation onto 7%–15% SDS-PAGE, transferred onto Immobilon-P transfer membranes (merck millipore). For fibrosis analysis, the membranes were incubated with anti-fibronectin, -α-SMA, -collagen I (fibronectin: ab2413; α-SMA: ab32575; collagen I: ab34710, Abcam). For signaling analysis, The membranes were incubated with

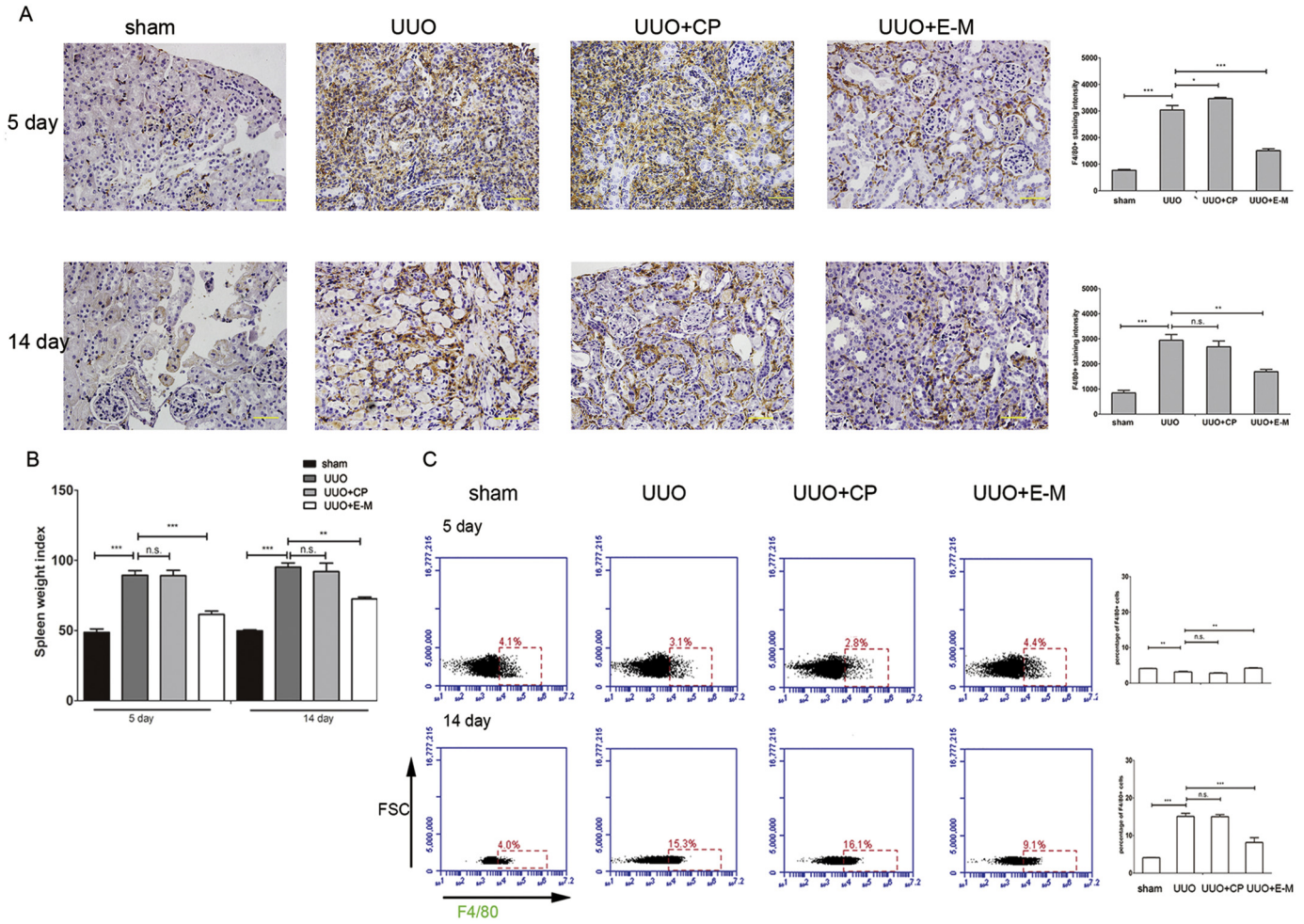

Fig. 4. Mimotope immunization ameliorated F4/80+ macrophage infiltration in the kidneys and reduced the frequency of F4/80+ macrophages from spleen. (A) F4/80+ macrophage infiltration in the kidneys was evaluated by immunohistochemistry staining. The results showed the EGFR Mimotope immunization could ameliorate F4/80+ macrophage infiltration in the kidneys induced by UUO. (B) The spleen weight index of mice from different groups. (C) The splenocytes were collected and incubated with Alexa Flour 488-labeled anti-F4/80 antibody. Then, the cells were then analyzed by flow cytometry. The results showed that the EGFR Mimotope immunization could reduce the frequency of F4/80+ macrophages from spleen. (Mean ± SEM; ***, $p < .001$; **, $p < .005$; *, $p < .05$; n.s.: non-significant. Representative image, N = 6 mice per group for immunohistochemistry staining; n = 3 mice per group for flow cytometry analysis)

anti- p-P44/42MAPK (ERK, Thr202/Tyr204), -ERK, -p-STAT3 (Tyr705), -STAT3, (p-ERK: 4307; ERK: 4695; p-STAT3: 9145; STAT3: 4904, Cell Signaling Technology) and –TGF-β antibody (ab311013, Santa Crus biotechnology) with a 1:1000 dilution overnight at 4 °C. The primary antibodies were detected with peroxidase-labeled goat anti-rabbit IgG or goat anti-mouse IgG and exposed on film by using enhanced chemiluminescence (merck millipore).

2.6. Statistical analyses

Image Pro Plus 6.0 (Media Cybernetics) was used for histology analysis. The positive count value was obtained from the analysis of five fields (×20 objective) by measuring the integrated optical density (IOD) for aniline blue- (Masson trichrome staining) or DAB- (Immunohistochemistry) stained region in every microscopic field. For western blot assay, the intensity of each band was quantified using ImageJ software (version 1.46r; NIH). For flow cytometric analysis, the frequency of cell was quantified using BD Accuri C6 software (BD, FACSCanto II). We performed the statistical analyses using GraphPad Prism 5.0 (GraphPad Software). The data are expressed as the mean ± SEM. Differences between groups were evaluated for statistical significance using the *t*-test or the one-way analysis of variance (ANOVA), and p values < .05 were considered to be statistically significant.

3. Results

3.1. Mimotope immunization ameliorated renal fibrosis in the UUO mouse model

We first evaluated the renal fibrosis of mice from each group with an analysis of Masson trichrome. As shown in Fig. 1B, a significant increase collagen accumulation could be observed in the kidneys at day 14, but not at day 5. EGFR mimotope immunization could significantly reduce the collagen deposition induced by UUO surgery. Although collagen accumulation was not observed in the kidneys of the mice from all groups at day 5 after UUO, we found severe nephron structure destructions in the kidneys and the destructions could be prevented by EGFR mimotope immunization obviously. Fibrosis can also be characterized through the detections of fibronectin, α-SMA and collagen I. Thus, we detected the expressions of fibronectin, α-SMA and collagen I in kidney tissues. As shown in Fig. 2, we found EGFR mimotope immunization could significantly reduce the expressions of fibronectin, α-SMA and collagen I at day 14 after UUO. Considering Masson trichrome staining at 5 day failed to show significant fibrosis, the results of the expressions of fibronectin, α-SMA and collagen I were showed in

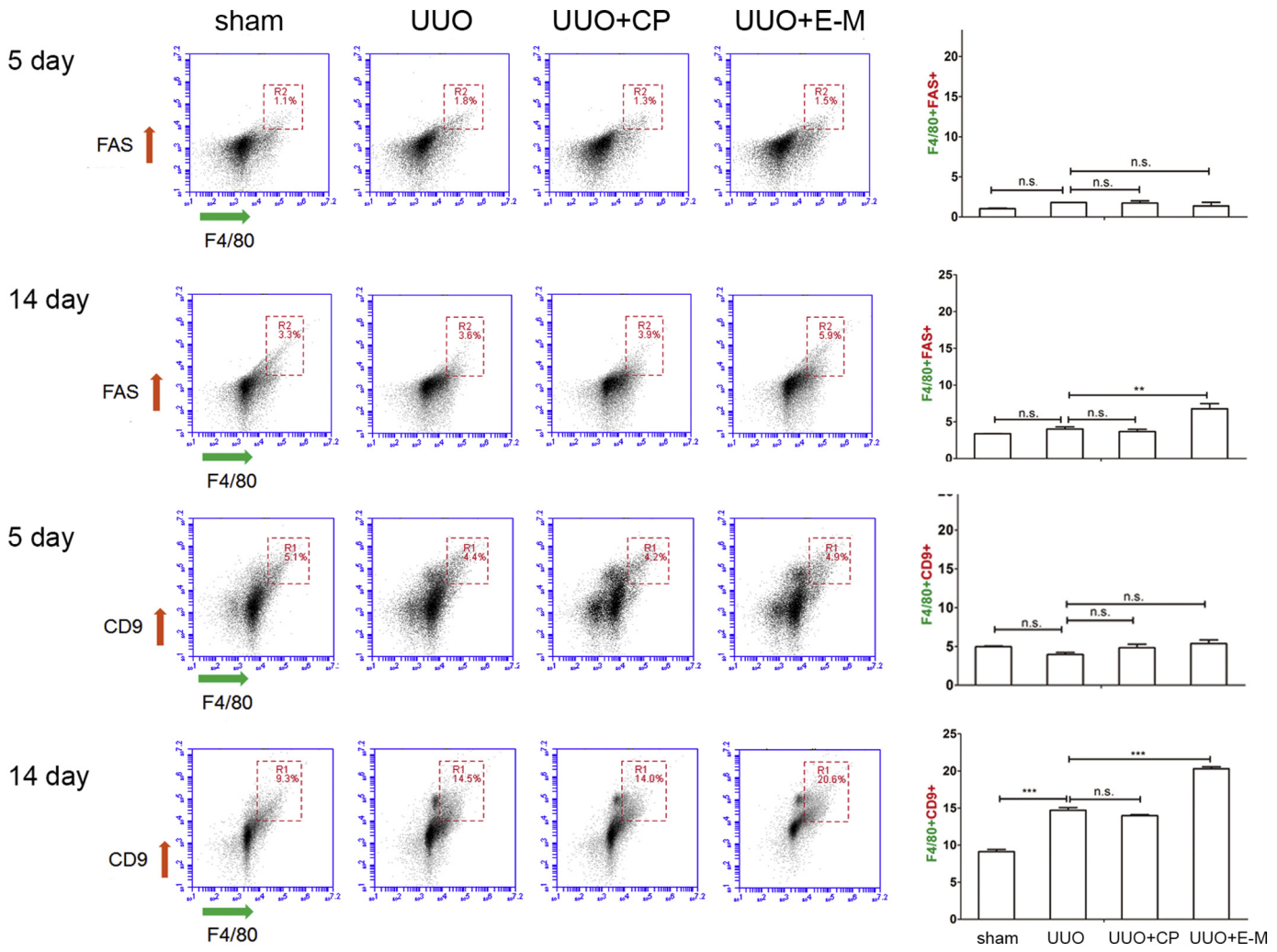

Fig. 5. Mimotope immunization increased the expression of CD9 and FAS in F4/80+ macrophages. The splenocytes were collected and incubated with APC-labeled anti-CD9, Alexa Flour 488-labeled anti-F4/80+ and APC-labeled anti-FAS. Then, the cells were then analyzed by flow cytometry. The results showed that the EGFR Mimotope immunization could increase the expressions of CD9 and FAS in F4/80+ macrophages. (Mean ± SEM; ***, $p < .001$; **, $p < .005$; *, $p < .05$; n.s.: non-significant; n = 3 mice per group).

Supplemental Fig. 1.

3.2. Mimotope immunization inhibited the EGFR downstream signaling induced by UUO

To observe what changes would be induced on the EGFR downstream signaling in the kidneys by peptide immunization, kidneys were dissected, homogenized and used in signaling pathway analysis. The results showed that additional mimotope immunization could significantly down-regulate the expressions of pSTAT3, STAT3, pERK, ERK and TGF-β induced by UUO at day 5 and 14. However, we noticed that the expression of pERK and TGF-β increased after CP immunization, which could be down regulated by EGFR mimotope at day 5 after UUO (Fig. 3).

3.3. Mimotope immunization could up-regulate CD9/FAS expressions in F4/80+ macrophages in spleen

To observe whether EGFR mimotope immunization could alleviate the macrophage infiltration in the kidneys after UUO, we performed the immunohistochemistry staining. The results showed that the macrophage infiltration decreased significantly in the kidneys with the EGFR mimotope immunization at day 5 and day 14 after UUO. The spleen weight index showed the similar changes to those of macrophage infiltration. The frequency of F4/80+ cells was significantly increased in the spleens after UUO, and these cells were statistically inhibited by EGFR mimotope immunization at day 14 after UUO, but not at day 5 (Fig. 4). The EGFR mimotope immunization could increase the expressions of both FAS and CD9 in macrophages at day 14 after UUO (Fig. 5).

4. Discussion

The major finding of our study was that immunization with an EGFR mimotope could alleviate renal fibrosis by inhibiting EGFR signaling. Meanwhile, the EGFR mimotope could up-regulated expression of CD9 and FAS in macrophages, which may promote macrophages apoptosis in UUO animal model.

The initial development of mimotope was to overcome the limitations of monoclonal antibodies, which were widely used in treatment of cancer. Active immunotherapy relies on the injection of mimotope at longer intervals and offers the advantage of generating an Ag-specific humoral- and/or cell-mediated complete-self-immune response. Furthermore, this response would be polyclonal, and induce more effective biological effects than those observed with the passive administration. However, few studies reported the mimotope immunization

could induce macrophages apoptosis. In previous study, we reported that the EGFR mimotope could alleviate the macrophage infiltration in an autoimmune disease animal model. Thus, we sought to confirm whether the EGFR mimotope has the similar functions on macrophage populations in a renal fibrosis animal model.

As we expected, the EGFR mimotope could significantly reduce the infiltration of macrophages in the kidney of mice in the different UUO injury stages. Notably, flow cytometric analysis showed that the frequency of macrophages in spleens increased significantly in mice at day 14 after UUO, but not at day 5 and the EGFR mimotope could increase the expression of FAS in these macrophages generated in spleens. Since the renal fibrosis was significantly enhanced at day 14, we assumed that the renal fibrosis may be triggered by the macrophages generated from spleen in a later stage in UUO model, but not the macrophages existed in the kidneys in the early stages of injury. The increased expression of FAS in these later generated macrophages induced by the EGFR immunization may help to alleviate renal fibrosis in UUO model.

CD9 is a 21–24 kDa member of the tetraspanin protein family and shows a wide cellular and tissue distribution. It was implicated in a range of cellular functions, including motility, proliferation, differentiation, fusion, and adhesion [25,26]. CD9 regulates activated leukocyte cell adhesion molecule (ALCAM) -mediated cell adhesion and T cell activation through its ligand CD6 [27]. Meanwhile, CD9 is the only tetraspanin that has been reported to associate directly with disintegrin metalloproteinase domain-containing protein (ADAM) 17 and inhibit ALCAM shedding induced by ADAM17 and to increase ALCAM levels on the cell surface and augmented ALCAM-mediated cell adhesion [28]. In addition, CD9 was reported to be a negative regulator of macrophage activation and lung inflammation because deletion of CD9 in mice enhances macrophage infiltration and TNF-α production [23]. In current study, we detected the expression of CD9 in macrophages from spleen of mice and the results showed the expression of CD9 was significantly higher in the EGFR mimotope immunized mice. The results suggested the EGFR mimotope immunization could increase cell adhesion or inhibit macrophage activation by up-regulating the expression of CD9 in macrophages. Importantly, ADAM17 has been reported to be involved in EGFR ligand processing [29]. Thus we assumed that EGFR mimotope may inhibit EGFR signaling in UUO model not only through neutralizing antibodies induced by immunization, but also by CD9 mediated-ADAM 17 regulations.

We noticed that the UUO + CP group shows worse collagen I accumulation and macrophage infiltration compared to UUO alone or UUO + EM at day 5 after UUO. We assumed that these outcomes may relate to the application of adjuvant. When the mice of UUO + CP and UUO + E-M groups received peptide immunization, the CFA were used. The CFA contains Drakeol 5NF, Arlacel A and Mycobacterium butyricum dry cells. Mycobacterium butyricum dry cells in adjuvant could attract macrophages and other cells to the injection site which enhances the immune response. In light of the important role of macrophages in renal fibrosis, we assumed that the worse outcomes showed by UUO + CP group may due to the CFA.

In conclusion, the EGFR mimotope could alleviate renal fibrosis in UUO model. The mechanism involved may offer a potential alternative pathway to fight against CKD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.05.014.

Acknowledgement

This work was supported by the Scientific Research Foundation of Shanghai Municipal Population and Family Planning Commission (no. 201740047), the National Natural Science Foundation of China (no. 81770716), the Natural Science Foundation of Shanghai (no. 16ZR1426900), the Traditional Chinese Medicine Science Innovative Project (no. ZYKC201601030), the Special funds for Clinical Medical Research of Chinese Medical Association (no. 17010040673) and Minhang Nature Science Research Program of Shanghai (no. 2018MHZ060).

Author contributions

Study conception and design: Lin Yang, Jianying Niu and Yong Gu. Acquisition of data: Haoran Yuan, Ying Yu, Nan Yu and Lilu Lin. Analysis and interpretation of data: Lin Yang and Jianying Niu. Manuscript preparation: Lin Yang.

Conflicts of interest

None of the authors has any potential financial conflict of interest related to this paper. No financial or other relationships could lead to a conflict of interests. The article implies that the work described has not been published previously.

References

[1] A.C. Webster, E.V. Nagler, R.L. Morton, P. Masson, Chronic kidney disease, Lancet 25 (2017) 1238–1252, https://doi.org/10.1016/S0140-6736(16)32064-5.
[2] A. Pani, J. Bragg-Gresham, M. Masala, D. Piras, A. Atzeni, M.G. Pilia, et al., Prevalence of CKD and its relationship to eGFR-related genetic loci and clinical risk factors in the SardiNIA study cohort, J. Am. Soc. Nephrol. 25 (2014) 1533–1544, https://doi.org/10.1681/ASN.2013060591.
[3] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, Inflammatory processes in renal fibrosis, Nat. Rev. Nephrol. 10 (2014) 493–503, https://doi.org/10.1038/nrneph.2014.114.
[4] M. Zeisberg, E.G. Neilson, Mechanisms of tubulointerstitial fibrosis, J. Am. Soc. Nephrol. 21 (2010) 1819–1834, https://doi.org/10.1681/ASN.2010080793.
[5] W.A. Border, N.A. Noble, Transforming growth factor beta in tissue fibrosis, N. Engl. J. Med. 331 (1994) 1286–1292.
[6] D. Pilling, R.H. Gomer, Differentiation of circulating monocytes into fibroblast-like cells, Methods Mol. Biol. 904 (2012) 191–206, https://doi.org/10.1007/978-1-61779-943-3_16.
[7] D.J. Nikolic-Paterson, S. Wang, H.Y. Lan, Macrophages promote renal fibrosis through direct and indirect mechanisms, Kidney Int. Suppl. 4 (2014) (2011) 34–38.
[8] M. Nishida, Y. Okumura, S. Ozawa, I. Shiraishi, T. Itoi, K. Hamaoka, MMP-2 inhibition reduces renal macrophage infiltration with increased fibrosis in UUO, Biochem. Biophys. Res. Commun. 354 (2007) 133–139.
[9] N.C. Henderson, A.C. Mackinnon, S.L. Farnworth, T. Kipari, C. Haslett, J.P. Iredale, et al., Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis, Am. J. Pathol. 172 (2008) 288–298, https://doi.org/10.2353/ajpath.2008.070726.
[10] A.P. Abraham, F.Y. Ma, W.R. Mulley, E. Ozols, D.J. Nikolic-Paterson, Macrophage infiltration and renal damage are independent of matrix metalloproteinase 12 in the obstructed kidney, Nephrology. 17 (2012) 322–329, https://doi.org/10.1111/j.1440-1797.2012.01567.x.
[11] T.K. Tan, G. Zheng, T.T. Hsu, S.R. Lee, J. Zhang, Y. Zhao, et al., Matrix metalloproteinase-9 of tubular and macrophage origin contributes to the pathogenesis of renal fibrosis via macrophage recruitment through osteopontin cleavage, Lab. Investig. 93 (2013) 434–449, https://doi.org/10.1038/labinvest.2013.3.
[12] Q. Ghafoor, S. Baijal, P. Taniere, B. O'Sullivan, M. Evans, G. Middleton, Epidermal growth factor receptor (EGFR) kinase inhibitors and non-small cell lung cancer (NSCLC) - advances in molecular diagnostic techniques to facilitate targeted therapy, Pathol. Oncol. Res. 24 (2018) 723–731, https://doi.org/10.1007/s12253-017-0377-1.
[13] H. Liang, X. Liu, M. Wang, Immunotherapy combined with epidermal growth factor receptor-tyrosine kinase inhibitors in non-small-cell lung cancer treatment, Onco Targets Ther. 25 (2018) 6189–6196, https://doi.org/10.2147/OTT.S178497.
[14] H.H. Loong, S.S. Kwan, T.S. Mok, Y.M. Lau, Therapeutic strategies in EGFR mutant non-small cell lung cancer, Curr. Treat. Options in Oncol. 29 (2018) 58, https://doi.org/10.1007/s11864-018-0570-9.
[15] F. Felici, A. Luzzago, A. Folgori, R. Cortese, Mimicking of discontinuous epitopes by phage-displayed peptides, II. Selection of clones recognized by a protective monoclonal antibody against the *Bordetella pertussis* toxin from phage peptide libraries, Gene. 128 (1993) 21–27.
[16] A. Luzzago, F. Felici, A. Tramontano, A. Pessi, R. Cortese, Mimicking of discontinuous epitopes by phage-displayed peptides, I. Epitopemapping of human H ferritin using a phage library of constrained peptides, Gene. 128 (1993) 51–57.
[17] A.B. Riemer, E. Jensen-Jarolim, Mimotope vaccines: epitope mimics induce anticancer antibodies, Immunol. Lett. 113 (2007) 1–5.
[18] L. Yang, Y. Wang, R. Xing, L. Bai, C. Li, Z. Li, X. Liu, Mimotope mimicking epidermal growth factor receptor alleviates mononuclear cell infiltration in exocrine glands induced by muscarinic acetylcholine 3 receptor, Clin. Immunol. 163 (2016) 111–119, https://doi.org/10.1016/j.clim.2016.01.006.
[19] L. Yang, H. Jiang, B. Shi, H. Wang, J. Li, H. Wang, M. Yao, Z. Li, Identification and characterization of Ch806 mimotopes, Cancer Immunol. Immunother. 59 (2010) 1481–1487, https://doi.org/10.1007/s00262-010-0872-7.
[20] J. Hughes, R.J. Johnson, Role of Fas (CD95) in tubulointerstitial disease induced by

unilateral ureteric obstruction, Am. J. Phys. 277 (1999) F26–F32, https://doi.org/10.1152/ajprenal.1999.277.1.F26.
[21] Y. Wang, D.C.H. Harris, Macrophages in renal disease, J. Am. Soc. Nephrol. 22 (2011) 21–27, https://doi.org/10.1681/ASN.2010030269.
[22] H.-J. Anders, M. Ryu, Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis, Kidney Int. 80 (2011) 915–925, https://doi.org/10.1038/ki.2011.217.
[23] M. Suzuki, I. Tachibana, Y. Takeda, P. He, S. Minami, T. Iwasaki, et al., Tetraspanin. CD9 negatively regulates lipopolysaccharide-induced macrophage activation and lung inflammation, J. Immunol. 182 (2009) 6485–6493, https://doi.org/10.4049/jimmunol.0802797.
[24] K. Kaji, S. Takeshita, K. Miyake, T. Takai, A. Kudo, Functional association of CD9 with the Fc gamma receptors in macrophages, J. Immunol. 166 (2001) 3256–3265.
[25] R. Reyes, B. Cardeñes, Y. Machado-Pineda, C. Cabañas, Tetraspanin CD9: a key regulator of cell adhesion in the immune system, Front. Immunol. 9 (2018) 863, , https://doi.org/10.3389/fimmu.2018.00863.
[26] C. Brosseau, L. Colas, A. Magnan, S. Brouard, CD9 tetraspanin: a new pathway for the regulation of inflammation? Front. Immunol. 9 (2018) 2316, , https://doi.org/10.3389/fimmu.2018.02316.
[27] A. Gilsanz, L. Sánchez-Martín, M.D. Gutiérrez-López, S. Ovalle, Y. Machado-Pineda, R. Reyes, et al., ALCAM/CD166 adhesive function is regulated by the tetraspanin CD9, Cell. Mol. Life Sci. 70 (2013) 475–493, https://doi.org/10.1007/s00018-012-1132-0.
[28] M.D. Gutiérrez-López, A. Gilsanz, M. Yáñez-Mó, S. Ovalle, E.M. Lafuente, C. Domínguez, et al., The sheddase activity of ADAM17/TACE is regulated by the tetraspanin CD9, Cell. Mol. Life Sci. 68 (2011) 3275–3292, https://doi.org/10.1007/s00018-011-0639-0.
[29] W.B. Melenhorst, G.M. Mulder, Q. Xi, J.G. Hoenderop, K. Kimura, S. Eguchi, et al., Epidermal growth factor receptor signaling in the kidney: key roles in physiology and disease, Hypertension 52 (2008) 987–993, https://doi.org/10.1161/HYPERTENSIONAHA.108.113860.