

# Anti-inflammatory effect of HGF responses to traumatic oral ulcers using an HGF-Tg mouse model

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## Research article

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# Abstract

**Background:** Hepatocyte growth factor (HGF) has been implicated in inhibiting diverse types of inflammation and promoting wound healing. In this study, we demonstrate the anti-inflammatory effect of HGF on traumatic ulcer of oral mucosa using HGF overexpression transgenic C57BL/6 (HGF-Tg) mice. In total, 29 C57BL/6 (WT) and 29 HGF-Tg mice were used in this study. Traumatic ulcer of left mucosa was performed by abrasion using a n 15 surgery knife. Ulcer tissues and serum samples were collected on 5 th day, histological score, expression of inflammatory cells and serum cytokines expression were measured and analyzed.

**Results:** There existed statistical significant difference in connective Ly6G positive cells and NF- $\kappa$ B positive expression, HGF-Tg mice showed lower number of positive cells (  $p = 0.048$ ,  $p = 0.020$  ). Eotaxin ( t -test,  $p = 0.002$ ), MIP-1 $\gamma$  ( t -test,  $p = 0.011$ ), BLC ( t -test,  $p = 0.03$ ), Eotaxin-2 ( t -test,  $p = 0.027$ ), RANTES ( t -test,  $p = 0.038$ ), Lix ( t -test,  $p = 0.04$ ) and IL-3 ( t -test,  $p = 0.047$ ) had statistical significance higher expression in HGF-Tg mice. No significant difference of blood T cells (  $p = 0.998$ ), Neutrophils (  $p = 0.331$ ), Macrophages (  $p = 0.470$ ) and CD4+/CD8+ ratio (  $p = 0.451$ ) between HGF-Tg and WT group.

**Conclusions:** We observed that HGF-Tg animals presented less Ly6G-positive neutrophil infiltration, higher levels of circulating cytokines and less NF-  $\kappa$  B expression in connective tissue, with a positive effect on the healing of oral traumatic ulcers. Key words: HGF, oral ulcer, cytokine, inflammation

## 1. Background

Ulceration of the oral mucosa are relatively frequent and feature discontinuity of the epithelium [1,2]. They may be induced by many underlying etiologic factors including trauma, autoimmune/immune-mediated disorder, infection, causing tissue broken and ulceration [3]. Clinically, these ulcers are usually can be painful and may affect chewing, swallowing speaking and dramatically affect the patient's life quality. Healing happens in spontaneous manner that may vary from 1 to 2 weeks, however, medication intervention is needed in case of chronic or severe ulcer. Antiseptic mouthwashes, steroids, alternate immunosuppressant medications and protective films/gels were recommended clinically to reduce pain and promote healing of oral ulcer [2,4]. Recent years, regenerative medicine concept is developed for an alternative to promote tissue repair with the understanding of the biological mechanism of wound healing. Application of growth factors has been investigated to promote ulcer healing [5]. Hepatocyte growth factor (HGF) is one of the growth factors that is considered as a new potential clinical drug in tissue healing [6].

HGF expression was found to be upregulated following a variety of injuries; it was postulated that cells of the immune system contribute to regulating the expression of HGF and c-Met following injury and inflammation [7,8,9]. HGF promotes the development of immune cells and contributes to immune responses and tissue repair [10,11]. Moreover, HGF was reported to inhibit the secretion of inflammatory cytokines and to promote secretion of anti-inflammatory cytokines [12,13]. HGF was more recently reported to modulate the inflammatory environment and enhance wound healing and regeneration in animal models of inflammation-associated diseases in skin inflammation models [14], multiple sclerosis models [15] and models [16].

HGF can mediate skin wound healing by accelerating regeneration of epithelial cells [17] and by maintaining normal immune function [14]. HGF also regulates desirable wound healing by mediating oral mucosal

fibroblast function [18]. Accordingly, we hypothesized that using HGF will promote oral ulcer healing and remodeling. Because the short biological half life of internal HGF protein, intravenous medicine delivery can not ensure the effective function of HGF in the traumatic tissues, we established HGF-overexpressing transgenic (HGF-Tg) mice whose HGF expression level is higher than in wild type (WT) mice [19]. Our previous study found that HGF exerted anti-inflammatory effects by reducing both epithelial apoptosis and connective tissue *TNF $\alpha$*  expression in oral traumatic ulcers [19]. In this study, we tested the effects of HGF on inflammatory cells and *cytokine* expression in healing from traumatic injury of oral mucosa using HGF-Tg mice.

## 2. Methods

### 2.1 Establishment of an HGF-overexpressing mouse model

All animal experiments were performed according to the Ethical Principles for Animal Experimentation of Guangdong province and were approved by the Ethics Committee for Animal Research (no. 2016-067) of Guangzhou Medical University. Wild-type C57BL/6 (WT) obtained from the Guangdong Experimental Animal Center (Guangzhou Guangdong) and HGF-overexpressing transgenic C57BL/6 (HGF-Tg) mice were used in this study[19]. HGF is high expressed in oral mucosa of HGF-Tg mice(Fig 1). In total, 29 WT and 29 HGF-Tg mice aged 8–10 weeks ( $22.8 \text{ g} \pm 5.2 \text{ g}$ , 8–10 weeks) were used in this experiment. Mice were kept under 12-hour/12-hour light/dark cycles, with free access to food and water, in the Central Animal Laboratory of Guangzhou Medical University.

### 2.2 Oral mucosa traumatic ulcer protocol

Oral mucosa traumatic ulcers were generated using the method described by Cavalcante in 2011 [4]. Briefly, the mice were anesthetized with 0.1–0.2 ml of pentobarbital sodium (0.1%) via intraperitoneal injection, and the oral mucosa was sterilized using 0.12% chlorhexidine gluconate. The ulceration was performed on the left oral mucosa using a number 15 scalpel blade; a 5-mm marker was used for standardization. The protocol was performed by the same investigator (Guanying Chen) for standardization. We choose 5th day as the sacrifice day because the mice lose weight during the 5 first days [4].

### 2.3 Blood and tissue sample collection

The mice were weighed on day zero and on the day of sacrifice (the 5<sup>th</sup> day),  $\text{Weight loss} = (\text{final weight} - \text{initial weight})$ . The mice were sacrificed by ether anesthesia and neck dislocation. Blood samples were collected and centrifuged for 10 min at  $200 \times g$ , after which the separated serum was collected and stored at  $-80 \text{ }^\circ\text{C}$ . The blood cells were collected for flow cytometry analysis. The mucosa containing the ulcers were collected and fixed in 10% formalin. After being embedded in paraffin, the tissue was sectioned into  $2\text{-}\mu\text{m}$  slices that were then mounted on slides and stained with hematoxylin and eosin (HE) for inflammation score evaluation (from 0 to 4 according to published evaluation criteria) [2]. Weighing, blood collection and sample collection were performed separately on the 5<sup>th</sup> day.

## 2.4 Serum cytokine analysis

Serum cytokines were tested in duplicate alongside a standard with the Mouse Cytokine Array Q5 (RayBio, QAM-CYT-5) according to the manufacturer's instructions. The fluorescent signal intensity was measured using an InnoScan 300 Microarray Scanner (Parc d'Activités Activestrel, 31390 Carbonne, France) at a wavelength of 532 nm and a resolution of 10  $\mu$ m. All results were analyzed using Q-Analyzer software for QAM-cyt-5. Healthy wild-type mice (n=3), WT mice with ulcers (n=5) and HGF-Tg mice with ulcers (n=8) were included in this analysis.

## 2.5 Flow cytometry analysis

Immune cells from WT (n=29) and HGF-Tg mice (n=29) blood were isolated after red blood cell lysis. Cells were washed and resuspended in PBS ( $1 \times 10^6$ /ml), and cells were stained for 30 min at 4 °C with the following antibodies: CD45-APC-Cy7 (BD, Lot: 7096638) CD4-PE (BD, Lot: 7138675) CD8-FITC (Invitrogen, Lot: 4329219) CD11b-PerCy5.5 (BD, Lot: 7066558) Ly6G-FITC (BD, Lot: 7052879) and F4/80-PE-Cy7 (Invitrogen, Lot: 4323732). The cells were sorted by flow cytometry (BD FACSAria III), and the data were analyzed using FlowJo software (BD FACSDiva software).

## 2.6 Immunohistochemical staining for NF- $\kappa$ B, CD45, and Ly6G

Slides bearing 4- $\mu$ m tissue slices were deparaffinized and rehydrated and then processed according to a standard immunohistochemical staining manual. Briefly, endogenous peroxidase was blocked with 3% H<sub>2</sub>O<sub>2</sub>, and then the samples were incubated for 1 hour at room temperature with primary antibodies against CD45 (Lot: 7096638, BD, USA, dilution 1:100), NF- $\kappa$ B (bs-0465R, Bioss, USA, dilution 1:50) and Ly6G (bs-2576R, Bioss, USA, dilution 1:100), after which they were incubated with HRP secondary antibodies and substrate. The percentages of NF- $\kappa$ B-positive cells in epithelium were scored as follows: 0: 0% positive cells; 1: 1–33% positive cells; 2: 34–66% positive cells; and 3: 67–100% positive cells [2]. The score of Ly6G-positive cells among inflammation was calculated according to Li with a minor change [20]: the average positive cell ratios were photographed and counted in 3–5 fields (400X) and then scored as follows: 0: 0% positive cells; 1: 1–33% positive cells; 2: 34–66% positive cells; and 3: 67–100% positive cells. CD45 was qualified by the average positive cell number that was counted in 3–5 fields (400X).

## 2.7 Statistical analysis

SPSS 19.0 (IBM, USA) was used to calculate statistical significance between groups. The unpaired t-test and ANOVA were used to compare the differences between groups. Quantitative data are expressed as the mean  $\pm$  standard error (mean $\pm$ SE). GraphPad Prism 6 software (La Jolla, CA, USA) was used to plot the comparison of means, and  $p \leq 0.05$  was considered a statistically significant difference.

## 3. Results

## 3.1 Weight loss and inflammation score analysis

All mice were alive on the sacrifice day, HGF-Tg mice ( $0.67 \pm 1.38$  g) had significantly less weight loss compared with WT mice ( $-0.07 \pm 1.94$  g) (*t*-test,  $p=0.038$ ); Microscopic analysis of the cheek trauma of HGF-Tg mice on the 5<sup>th</sup> day revealed the presence of less ulceration associated with moderate fibrosis and chronic inflammatory processes. In WT ulcer mice, we found a persistence of ulcers and mixed inflammatory infiltrates (neutrophils and mononuclear cells). HGF-Tg group (mean $\pm$ SE:  $2.55 \pm 0.67$ ) exhibited less ulceration and inflammation than did WT group (mean $\pm$ SE:  $3.32 \pm 0.65$ ) (*t*-test,  $p=0.000$ , Fig 2A).

## 3.2 RayBiotech (AAM-INF-G1-8) antibody array experiment

In this assay, compared with WT group, we detected 38 greater and 2 lesser (MIP-1 alpha, Fractalkine) amounts of production of serum cytokines in HGF-Tg group (Table 1). In terms of higher serum expression, we found 1–13.5-fold higher expression in HGF-Tg group. Eotaxin (*t*-test,  $p=0.002$ ), MIP-1 gamma (*t*-test,  $p=0.011$ ), BLC (*t*-test,  $p=0.03$ ), Eotaxin-2 (*t*-test,  $p=0.027$ ), RANTES (*t*-test,  $p=0.038$ ), Lix (*t*-test,  $p=0.04$ ) and IL-3 (*t*-test,  $p=0.047$ ) all showed statistical significance. *When comparing HGF-Tg group with the control group*, eotaxin (*t*-test,  $p=0.001$ ), MIP-1 gamma (*t*-test,  $p=0.007$ ), BLC (*t*-test,  $p=0.003$ ), MCSF (*t*-test,  $p=0.032$ ), RANTES (*t*-test,  $p=0.044$ ), Lix (*t*-test,  $p=0.037$ ) and IL-3 showed significantly higher expression levels than did control group. IL-6 (*t*-test,  $p=0.050$ ) was significantly lower and TCA-3 (*t*-test,  $p=0.034$ ) was significantly higher in WT group than in control group (Fig 3A and 3B).

## 3.3 Flow cytometry analysis

To characterize the impact of oral mucosa ulcer on circulating inflammatory cells, we performed flow cytometry analysis 5 days after oral traumatic ulcer induction. T cells were gated by CD45+/ CD8+ or CD4+; neutrophils were gated by CD45+/ CD11b+/Ly6G+; and macrophages were gated by CD45+/ Ly6G-/ CD11b+/ F4/80+. We found that there were no significant differences in terms of blood T cells ( $p=0.998$ ), neutrophils ( $p=0.331$ ) and macrophages ( $p=0.470$ ) between HGF-Tg and WT mice. Flow cytometry revealed that there were no significant differences in the CD4+/CD8+ ratio between the groups ( $p=0.451$ ) (Fig. 4).

## 3.4 Ly6G, CD45, and NF- $\kappa$ B immunostaining

*We next investigated Ly6G, CD45 and NF- $\kappa$ B expression using immunohistochemical staining. There was more Ly6G-positive neutrophil infiltration in WT mice, and it was significantly lower in HGF-Tg group (*t*-test,  $p=0.048$ , Fig. 2B ). In connective tissue, NF- $\kappa$ B expression was significantly lower in HGF-Tg mice (*t*-test,  $p=0.020$ , Fig. 2C). However, in epithelium, NF- $\kappa$ B expression in HGF-Tg mice was slightly lower than in WT mice, and the difference showed no statistical significance (*t*-test,  $p=0.570$ , Fig. 2C). The number of CD45-positive cells showed no differences between the HGF-Tg ulcer and WT ulcer (*t*-test,  $p=0.940$ , Fig. 2D).*

## 4. Discussion

The healing process of wound is a complex, programmed events of genetic and biological series. However, non-healing and chronic ulcer are often more challenging to clinical prognosis and public health [21]. Recent years, some reports illustrated the potential clinical usage of growth factors in wound healing and tissue repair. HGF is one of the potential growth factors which participate in the whole process of wound healing [6,22]. While the function of HGF in cancer development and epithelial cell activities has been well discussed, the role of HGF signaling in inflammation and wound healing is gaining increasing focus. High HGF expression has been shown to mediate anti-inflammatory action and regeneration in a variety of inflammatory and traumatic models [13,23,24,25]. The aim of this study was to evaluate anti-inflammatory activity of HGF on an oral mucosa traumatic ulcer model in rats, an appropriate model for clinical oral ulcer[4].

Recent studies have described the indispensable contribution of HGF to wounding healing by inhibiting immune cell infiltration and inflammation in tissues [8,26]. We found that HGF-Tg mice presented less weight loss, less epithelial ulceration and less infiltration of Ly6G+ neutrophils as shown by histological and immunohistochemical observation on day 5. Neutrophils are the major infiltrating cells in wounds, especially in the early phase [27], our finding indicates that the effect of HGF in the healing of oral wounds involves decreasing the number of neutrophils in connective tissue of wound sites rather than the number of circulating immune cells; the mechanism of this phenomenon merits deep study. One study showed that HGF-specific c-Met knockout mice showed significantly severe neutrophil infiltration in non-alcoholic fatty liver disease (NAFLD) [28]. HGF treatment can decrease the Lymphocytes of the spleen in mice model [16]. Overexpression of HGF was tested as a way to enhance connective inflammation repair rather than re-epithelialization of wounds [22,29]. In terms of the effect of HGF on circulating immune cells and CD45+ T cells in connective tissue of oral ulcers, HGF failed to induce a significant decrease in circulating immune cell numbers or tissue T cells compared with WT mice.

Inflammation cytokines mediate complex functions in traumatic ulcers, depending on their proportional concentrations [15,30,31]. HGF maybe a new drug target to treat metabolic disease by inhibiting the inflammation cytokines in adipose tissue in HGF-Tg mice [26]. Anti-inflammatory roles of HGF have been described, including inhibiting pro- and inflammatory cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , TGF- $\beta$  and iNOS. increasing anti-inflammatory cytokines such as RANTES, IL-4, IL-10 and blocking *NF- $\kappa$ B*. All these roles could promote the wound healing of oral ulcer [16,32]. We measured the effect of HGF on 40 inflammatory cytokines up to 5 days after the model was constructed. mice. Almost all cytokines had a higher fold-change of fluorescent signal intensity in HGF-Tg group compared with WT mice, except for fractalkine and MIP-1 alpha. The levels of LIX, IL3, eotaxin, eotaxin-2, MIP-1, BLC, RANTES and MCSF were significantly higher in HGF-Tg than in WT after injury. Eotaxin, LIX, Eotaxin-2, MIP-1, BLC, and RANTES are chemokines that are early signals from traumatic tissues to immune cells [30]. This suggests that HGF promotes a stronger signal for the recruitment of inflammatory cells into traumatic tissue and corresponding responses from immune cells [30]. The levels of colony-stimulating factors LIX and IL3 were also significantly higher in HGF-Tg, possibly suggesting better tissue recovery after injury [30]. We also observed that almost all the pro- and anti-inflammatory cytokines increased in HGF-Tg mice, although the differences showed no statistical significance. So HGF can contribute to anti-inflammatory effect and wound repair via adjusting cytokine production [10].

The transcription factor nuclear factor kappa-B (*NF- $\kappa$ B*) is important for inflammatory responses, mediating the expression of cellular signals such as cytokines and growth factors [33]. Several studies have shown that HGF

exerts anti-inflammatory action by disrupting *NF-κB* expression. HGF potently inhibits renal inflammation by preventing *p65/NF-κB* binding at its acting element [34], or by shutting down *NF-κB* activation through increasing *A20* expression [35], without side effects on normal kidney function [36]. By reducing the activity of *NF-κB*, increased HGF expression in platelet-rich plasma provides anti-inflammatory function in an articular chondrocyte inflammation model [8]. In this study, there was a significant decrease in connective *NF-κB* expression in HGF-Tg ulcers on the 5<sup>th</sup> day. However, this effect was not observed in ulcer epithelium; both groups expressed higher epithelial *NF-κB*. We observed that HGF may decrease connective inflammation of the oral mucosa by reducing connective *NF-κB*. The mechanism merits further study.

## Conclusions

In conclusion, we observed that HGF-Tg ulcermice had less Ly6G-positive neutrophil infiltration, higher levels of circulating cytokines and less *NF-κB* expression in connective tissue, with a positive effect on the healing of oral traumatic ulcers.

## Abbreviations

HGF:Hepatocyte growth factor

## Declarations

**Ethics approval and consent to participate:** All animal experiments were performed according to the Ethical Principles for Animal Experimentation of Guangdong province and were approved by the Ethics Committee for Animal Research (no. 2016-067) of Guangzhou Medical University.

**Consent for publication:** Not applicable.

**Availability of data and materials:** The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Competing interest:** The authors declare they they have no competing interests.

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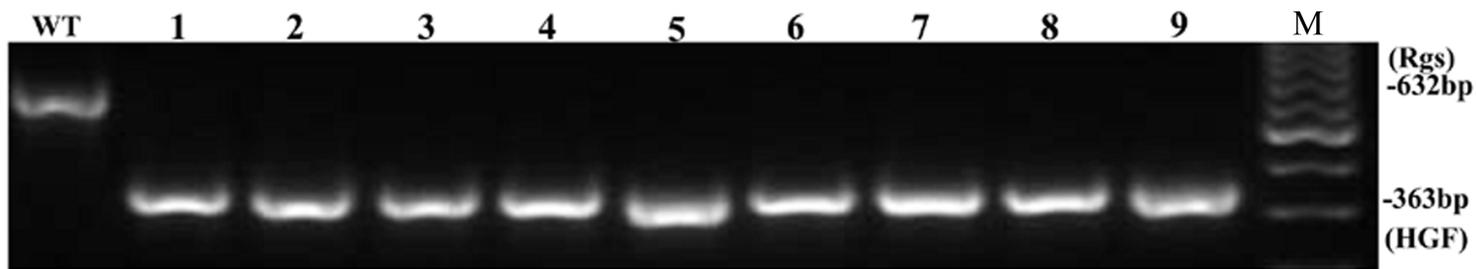
## Table

Table1. Mean cytokine concentrations (pg/ml) with standard error of mean. \* indicate significantly higher concentrations in WT-RAU compared to control group ( $p < 0.05$ ); \*\* indicate significantly higher concentrations in HGF-RAU compared to control animals ( $p < 0.05$ ). \*\*\* indicate significantly higher concentrations in HGF-RAU compared to WT-RAU ( $p < 0.05$ ).

cytokine	Control mean±SD	WT-RAU mean±SD	HGF-RAU mean±SD	Fold change		
				WT- RAU vs control	HGF- RAU vs control	HGF- RAU vs WT- RAU
BLC	1201.2±207.6	1200.6±440.3	4467.4±3404.3	1	3.72**	3.72***
CD 30L	1453.8±287.3	2064.4±1729.1	3901.1±6808.1	1.42	2.68	1.89
Eotaxin	21527.1±381.3	25125.0±4256.8	51258.6±15606.5	1.17	2.38**	2.04***
Eotaxin-2	13929.9±3749.2	16765.8±3457	28012.3±11211.4	1.2	2.01	1.67***
Fas ligand	396.8±131.6	392.5±115.7	4512.3±5085.1	0.99	11.37	11.5
Fractalkine	730.7±186.9	538.6±194.8	537.5±285.5	0.74	0.74	0.99
GCSF	912±266.2	929.7±331.1	12558.1±22967.8	1.02	13.77	13.51
GM-CSF	2594.5±507.3	2810.6±801	10378±6320.5	1.08	2.44	2.25
IFN-gamma	3001.7±146.5	3492±1121.3	7697.9±9176.9	1.16	2.57	2.20
IL-1 alpha	876.7±378.3	680±304.2	1054.7±648.1	0.78	1.2	1.55
IL-1 beta	631.2±261.7	570±152.6	4114±4541.5	0.90	6.52	7.22
IL10	2779±824.9	3526.5±2292.4	5550.3±8044.8	1.27	1.99	1.57
IL- 12p40/p70	2450.5±755.2	3106.4±1673.7	7574.7±811.4	1.27	3.09	2.44
IL-12p70	2675.7±279.8	3038.2±680.5	5664.3±6470.1	1.14	2.12	1.86
IL-13	2218±435.8	2978.5±1516.6	9679.1±11064.7	1.34	4.36	3.25
IL-17	2270.1±228.9	2608±873.3	11182.7±6317.2	1.15	2.78	2.42
IL2	1517±160.5	1997.8±764.5	9589.5±17513.3	1.32	6.32	4.8
IL3	2604.4±636.2	2886.2±1639.9	13858±12876.3	1.11	5.32**	4.8***
IL4	2512.4±281.7	2775.6±377	6339.1±9660.8	1.11	2.52	2.28
IL6	804.9±251.2	436.6±178.4	492.2±282.2	0.54*	0.61	1.12
IL9	3117.1±462.2	4279.2±2336.8	5876.7±5637.1	1.37	1.89	1.37
I-TAC	352±150.4	330.6±183.3	329.4±180.2	0.94	0.94	1
KC	600.1±220	499.5±253.1	580.6±338.8	0.83	0.97	1.16
Leptin	298.2±92	289.9±136.3	505.8±269.4	0.97	1.69	1.74
LIX	13199.1±4719.1	15823.3±5557.3	25329.7±7926	1.19	1.92**	1.6***
Lymphotactin	1259.3±85.8	1617.7±614.6	2534.8±2865	1.28	2.01	1.56
MCP-1	2346.2±302	2487.9±498.6	1364.9±13504.8	1.06	4.42	4.17
MCSF	351.1±167.6	686.7±286	1020.1±433.3	1.96	2.91**	1.49
MIG	542.1±102.2	836±275.1	1380.3±627.6	1.54	2.55	1.65
MIP-1 alpha	664.5±372.3	548.3±112.9	483.8±346.5	0.83	0.73	0.88
MIP-1 gamma	16683.6±6245.7	172908±9376.2	236130.4±52249.1	1.04	1.42**	1.36***
RANTES	640.4±197.8	514±85.8	3890.8±3745.2	0.80	6.08**	7.57***
SDF-1	461.2±214.1	373.3±149	749.5±415.7	0.81	1.63	2.01
sTNF RI	3774.8±999.1	3070.3±826.6	3708.4±1348.8	0.81	0.98	1.21
sTNF RII	1609.2±22.6	2097.4±658.7	3452.2±3601.6	1.3	2.15	1.65
TCA-3	1521.8±529	2548.4±509.5	3820.9±1580.6	1.68*	2.51**	1.50
TECK	433.9±119.6	445.4±97.5	553.9±326.8	1.03	1.28	1.25
TIMP-1	2077.9±317	2196.5±327.4	12400.3±17114.6	1.06	5.97	5.65
TIMP-2	184371.8±19139.9	216848.1±60565.1	286402.6±113626.4	1.18	1.58	1.32

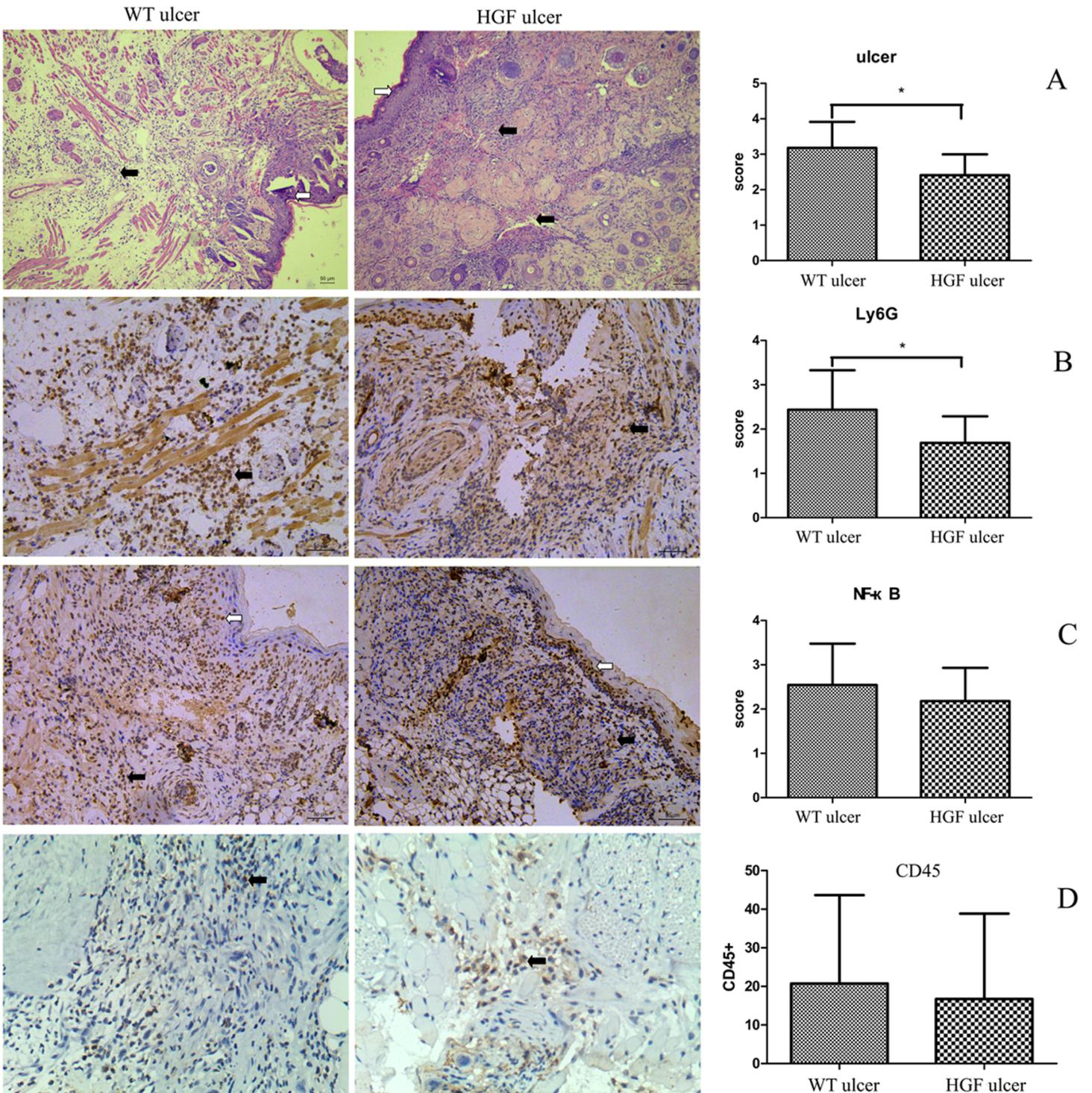
\* indicate significantly higher concentrations in WT-RAU compared to control group (p<0.05); \*\* indicate significantly higher concentrations in HGF-RAU compared to control animals (p<0.05). \*\*\* indicate significantly higher concentrations in HGF-RAU compared to WT-RAU (p<0.05). Results of **TNF $\alpha$**  and **IFN $\gamma$**  have been cited in previous study[19].

## Figures



**Figure 1**

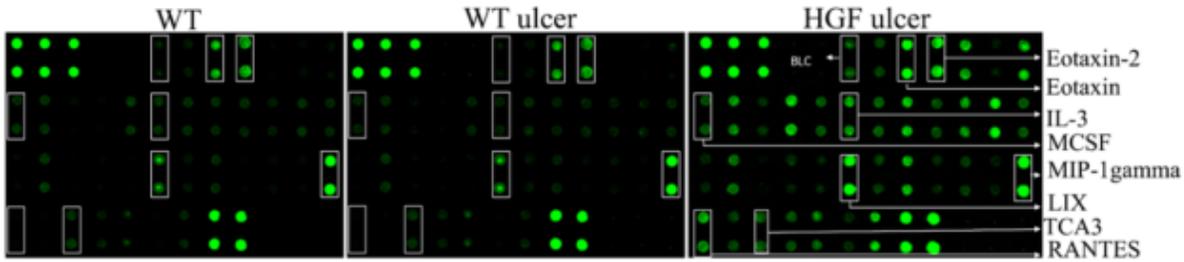
Representative HGF expression from HGF-Tg mice oral mucosa. WT: C57BL/6 mice; 1-9: C57BL/6 (HGF-Tg) mice.



**Figure 2**

Oral traumatic ulcers in the HGF-Tg and WT groups. (A) mice (HE, scale bar=50  $\mu$ m) ( $p < 0.05$ ). White arrow: epithelium; black arrow: connective tissue. Oral traumatic ulcers with Ly6G-positive neutrophil infiltration in both the HGF-Tg group and the WT group. (B) (immunohistochemistry, scale bar=50  $\mu$ m) ( $p < 0.05$ ). Oral traumatic ulcers with NF- $\kappa$ B in both epithelium ( $p > 0.05$ ) and connective tissue ( $p < 0.5$ ) in the HGF-Tg group and WT group. (C) (immunohistochemistry, scale bar=50  $\mu$ m). Oral traumatic ulcers with CD45 infiltration in both the HGF-Tg group and the WT group (D) (immunohistochemistry, scale bar=50  $\mu$ m) ( $p > 0.05$ ). White arrow: positive cells of epithelium; black arrow: positive cells of connective tissue.

A



B

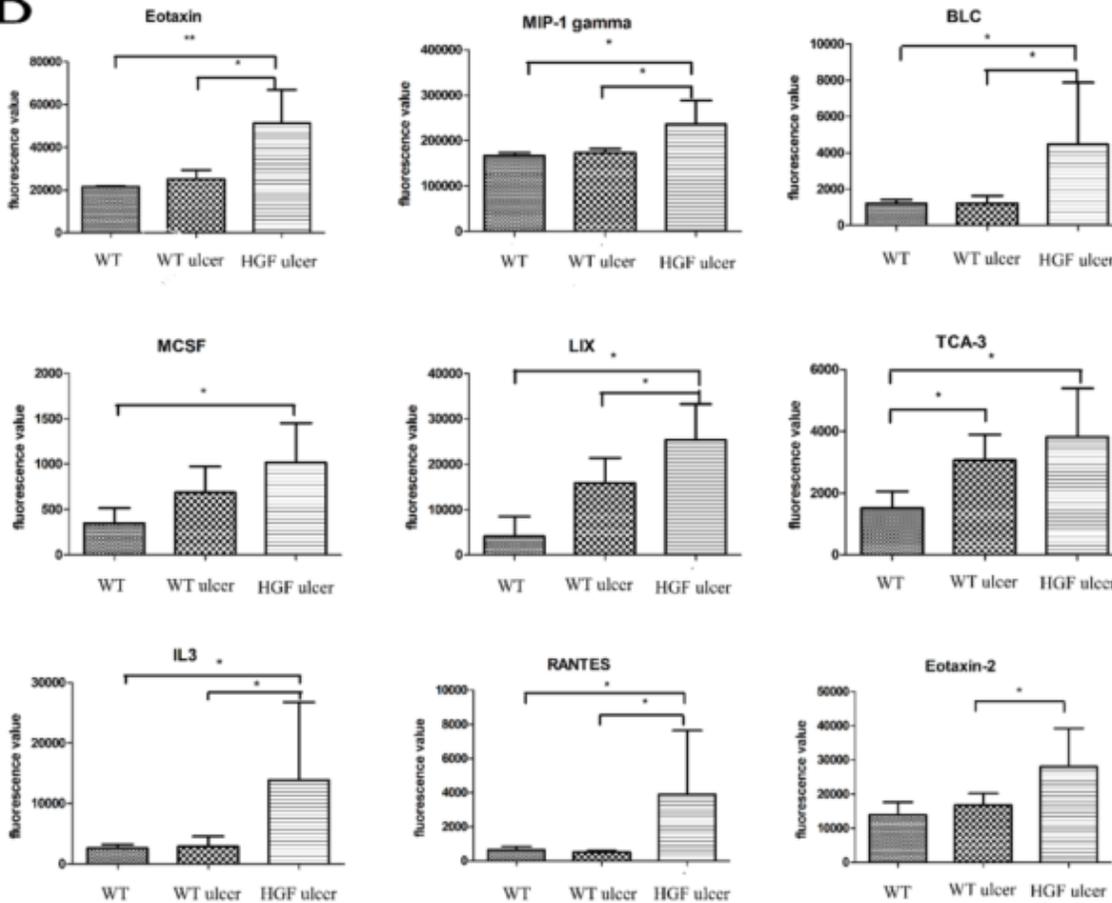
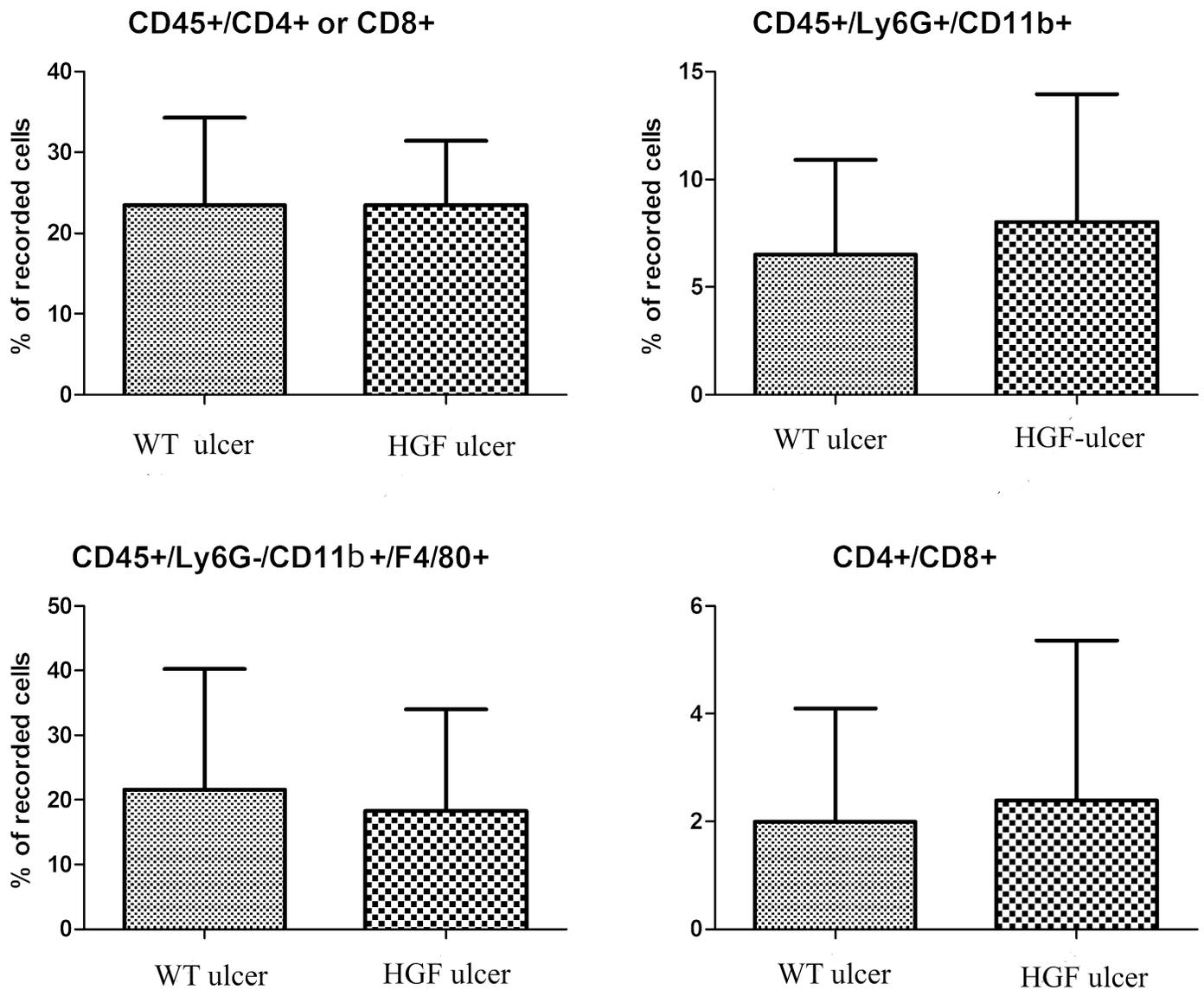


Figure 3

Antibody arrays against 39 cytokines comparisons. Representative images of antibody arrays against 39 cytokines (fig 3A). Significant increased concentrations of serum eotaxin, MIP-1 gamma, BLC, eotaxin-2, RANTES, Lix and IL3 and were observed in the HGF-Tg ulcer mice compared with control mice ( $p < 0.05$ ). When comparing HGF-Tg with normal mice, eotaxin, MIP-1 gamma, BLC, MCSF, RANTES, Lix and IL-3 had significantly higher expression levels than those in normal mice ( $p < 0.05$ ). IL-6 levels were significantly lower and TCA-3 levels were significantly higher in WT ulcer mice than in normal mice ( $p < 0.05$ ) (fig 3B).



**Figure 4**

The blood lymphocyte immunophenotypes were labeled with fluorescence antibodies and detected using fluorescence activated cell sorting (FACS). There were no significant differences in terms of T cells, neutrophils, macrophages and the CD8+/CD4+ ratio between the HGF-Tg and WT groups ( $p > 0.05$ ).

## Supplementary Files

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