HDAC2 ALLEVIATES IL-17A-MEDIATED AIRWAY REMODELING IN COPD BY INHIBITING AIRWAY INFLAMMATION AND FIBROBLAST ACTIVITY

Yahui Cao, Jianfeng Peng, Fan Yang, Xiaoyi Hu, Chaowen He*
Department of Respiratory and Critical Care Medicine, Shenzhen Longhua District Central Hospital, Shenzhen 518110, Guangdong Province, China

ABSTRACT

Objective: *To investigate the effect of histone deacetylase 2 (HDAC2) and interleukin-17A (IL-17A) on airway remodeling in chronic obstructive pulmonary emphysema (COPD).*

Methods: *Ten male SPF wild type (WT) mice were randomly divided into a WT air group and a WT CS group. Ten $HDAC2^{+/-}$ mice were randomly divided into an $HDAC2^{+/-}$ air group and an $HDAC2^{+/-}$ CS group. Ten $IL\text{-}17A^{-/-}$ mice were randomly divided into an $IL\text{-}17A^{-/-}$ air group and an $IL\text{-}17A^{-/-}$ CS group. The COPD model was induced by cigarette smoke (CS) exposure in the CS group, $HDAC2^{+/-}$ CS group and $IL\text{-}17A^{-/-}$ CS group, at 100 cigarettes a day, 5 days a week for 3 months. After 3 months, the mice were killed and their right lungs were taken for experiment. The expression of the HDAC2 protein in lung tissue of the WT air group and the WT CS group, and the expression of the IL-17A protein in lung tissue of the WT air group, WT CS group, $HDAC2^{+/-}$ air group and $HDAC2^{+/-}$ CS group were observed. The levels of chemokine ligand 1 (CXCL1), CXCL2 and IL-6, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissue of the CS group, $HDAC2^{+/-}$ CS group, $IL\text{-}17A^{-/-}$ air group and $IL\text{-}17A^{-/-}$ CS group were recorded. Normal human airway epithelial cells were randomly divided into the WT air group, WT CS group and $HDAC2^{+/-}$ CS group. The cells in the WT air group were not treated. The cells in the WT CS group and $HDAC2^{+/-}$ CS group were induced by CS to establish a COPD cell model. The expression levels of HDAC2 in the WT air group and WT CS group, and the IL-17A expression level in the WT air group, WT CS group and $HDAC2^{+/-}$ CS group, were compared.*

Results: *The expression of HDAC2 protein in lung tissue of the WT CS group was significantly lower than that of the WT air group ($P<0.05$). The levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in the WT CS and $HDAC2^{+/-}$ CS groups were higher than those in the WT air group ($P<0.05$), and the levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissue of the $HDAC2^{+/-}$ CS group were higher than those of the WT group CS group was significantly higher ($P<0.05$). There was no significant difference in CXCL1, CXCL2, IL-6, TGF-β-1 levels, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia between the WT air group and $HDAC2^{+/-}$ air group ($P>0.05$). The expression levels of IL-17A protein in lung tissue of the WT CS group and the $HDAC2^{+/-}$ CS group were significantly higher than that of the WT air group ($P<0.05$), and the expression level of IL-17A protein in lung tissue of the $HDAC2^{+/-}$ CS group was significantly higher than that of the WT CS group ($P<0.05$). There was no significant difference in IL-17A protein expression between the WT air group and the $HDAC2^{+/-}$ air group ($P>0.05$). The expression of HDAC2 in airway epithelium of the WT CS group was significantly lower than that of the WT air group ($P<0.05$). The expression level of IL-17A in airway epithelium of the WT CS group and the $HDAC2^{+/-}$ CS group was significantly higher than that of the WT air group ($P<0.05$), and the expression level of IL-17A in the $HDAC2^{+/-}$ CS group was significantly higher than that in the WT CS group ($P<0.05$). The levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in the $IL\text{-}17A^{-/-}$ CS group were lower than those in the WT CS group ($P<0.05$).*

Conclusion: *HDAC2 can alleviate airway remodeling in COPD induced by IL-17A by blocking airway inflammation and fibroblast activity.*

Keywords: *COPD, HDAC2, IL-17A, airway remodeling.*

DOI: 10.19193/0393-6384_2022_1_47

Received *March 15, 2020;* ***Accepted*** *October 20, 2020*

Introduction

Chronic Emphysema (COPD), as a chronic airway inflammatory disease, has a high fatality rate[1]. According to the World Health Organization, there are about 230 million cases of COPD worldwide. At present, COPD has become the fourth leading cause of death for urban residents and the first leading cause of death for rural residents in China[2]. COPD poses a serious threat to patients' quality of life and life safety, so revealing the pathogenesis of the disease and new treatment methods have become the mainstream of the current clinical field. Many clinical scholars have proposed that airway immune response may be involved in the development of COPD and plays an important role[3]. Airway

remodeling is an extremely important pathological feature of COPD, and clinical studies have shown that the accumulation of extracellular matrix around the trachea drives the proliferation, migration, and repair of structural cells, which play a key role in the occurrence and development of airway remodeling[4]. A number of previous reports have explored the characteristics of COPD and other aspects, and although some achievements have been made, the mechanism of occurrence and development in the process of airway remodeling in COPD has not been fully clarified[5]. Histone deacetylase 2 (HDAC2), a member of the Class I HDAC family, plays its role by regulating the expression level of inflammatory genes[6]. Related reports of COPD have shown that the expression level of HDAC2 in peripheral lung and alveolar macrophages in patients with this disease is significantly reduced[7]. It further causes chronic airway inflammation, which is closely related to the disease severity in COPD patients[8]. Current studies have confirmed that interleukin-17A (IL-17A) is closely related to the pathogenesis of COPD, and its expression level is significantly increased in peripheral blood and lung tissues of patients with this disease[9]. However, studies on IL-17A and COPD mostly focus on airway inflammation, and whether IL-17A is involved in COPD airway remodeling has not been found. Therefore, this study aims to explore the influence and mechanism of HDAC2 and IL-17A on the occurrence and development of airway remodeling in COPD.

Materials and methods

Experimental materials

Ten male SPF wild type (WT) mice were purchased from Changzhou Cavens Experimental Animal Co., Ltd. (Batch number: SCXK (Jiangsu) 2018-0005). Another 10 mice each with $HDAC2^{+/-}$ (knockout of HDAC2 gene, background of C57BL/6), and $IL\text{-}17A^{-/-}$ (knockout of IL-17A gene, background of C57BL/6) were purchased from Wuhan Hualianke Biotechnology Co., Ltd. (Batch number: SCXK (Hubei) 2018-0001). Human normal airway epithelial cells were purchased from Saibaekang (Shanghai) Biotechnology Co., Ltd.

Main reagents

Reagents

Marlboro cigarettes were purchased from China Tobacco Hunan Industrial Co., Ltd. A BCA protein concentration quantitative kit was purchased from Beijing Kangjia Hongyuan Biotechnology Co., Ltd. An ELISA kit was purchased from Jiangxi Blue Pure Biological Reagent Co., Ltd. Mouse anti-HDAC2 and IL-17A monoclonal antibodies were purchased from Beijing Luyuan Bird Biological Technology Co., Ltd. The immunohistochemical kit was purchased from Changzhou Beiyuanxin Biotechnology Co., Ltd.

Methods

• Ten male SPF WT mice were randomly divided into a WT air group and a WT CS group; 10 $HDAC2^{+/-}$ mice were randomly divided into an $HDAC2^{+/-}$ air group and an $HDAC2^{+/-}$CS group. Ten $IL\text{-}17A^{-/-}$ mice were randomly divided into an $IL\text{-}17A^{-/-}$ air group and an $IL\text{-}17A^{-/-}$ CS group, in which the WT air group and $HDAC2^{+/-}$ air group mice were not treated with any treatment. The COPD model was established in the WT CS group, $HDAC2^{+/-}$ CS group and $IL\text{-}17A^{-/-}$ CS group after exposure to cigarette smoke (CS), at 100 cigarettes a day, 5 days a week for 3 months. Three months later, the mice in each group were sacrificed and their right lung was taken and stored in an ultra-low temperature refrigerator.

• Protein was extracted from the right lung tissue of mice in each group, and the protein expression level of HDAC2 in the lung tissue of the WT air group and the WT CS group, and the protein expression level of IL-17A in the lung tissue of the WT air group, WT CS group, $HDAC2^{+/-}$ air group and $HDAC2^{+/-}$ CS group were detected by Western Lot.

• The levels of CXCL1, CXCL2, IL-6 and chemokine(C-X-C motif) ligand 1 in lung tissue of the WT air group, WT CS group, $HDAC2^{+/-}$ air group, $HDAC2^{+/-}$CS group, $IL\text{-}17A^{-/-}$ air group, $IL\text{-}17A^{-/-}$ air group, and $IL\text{-}17A^{-/-}$ CS group were detected by ELISA. Goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia were detected by Schiff reaction with periodate, Masson trichrome staining and immunohistochemistry.

• Human normal airway epithelial cells were cultured and randomly divided into a WT air group, a WT CS group and a $HDAC2^{+/-}$ CS group. The cells of the WT air group were not treated, and the cells of the WT CS group and the $HDAC2^{+/-}$ CS group were induced by CS to establish a COPD cell model. The expression level of HDAC2 in the WT air group and the WT CS group, and the expression level of IL-17A in the WT air group, WT CS group and $HDAC2^{+/-}$ CS group were detected by immunohistochemistry.

Statistical methods

All data in this study were analyzed and processed by SPSS23.0. The measurement data of CXCL1, CXCL2 and IL-6 levels in each group were expressed by ($\bar{x}\pm s$). The comparison between two groups was performed by a t-test, and the comparison between multiple groups was performed by one-way analysis of variance.

Results

Comparison of HDAC2 protein expression levels in lung tissues of mice in each group

The protein expression level of HDAC2 in lung tissue of mice in the WT CS group was significantly lower than that in the WT air group (P<0.05), ss shown in Figure 1.

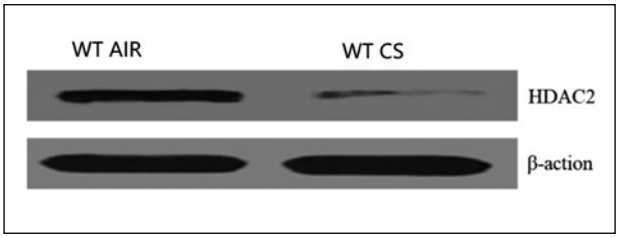

Figure 1: Comparison of HDAC2 protein expression levels in lung tissues of mice in each group.

Comparison of the levels of CXCL1, CXCL2 and IL-6 in lung tissues and related indicators of airway remodeling in each group

The levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissues of mice in the WT CS and HDAC2$^{+/-}$ CS groups were increased compared with those in the WT air group (P<0.05). In addition, the levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissues of mice in the HDAC2$^{+/-}$ CS group were significantly increased compared with those in the WT CS group (P<0.05). The levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissues of mice in the WT air group were not significantly different from those in the HDAC2$^{+/-}$ air group (P>0.05). See Table 1 and Table 2.

Comparison of IL-17A protein expression levels in lung tissues of mice in each group

The protein expression level of IL-17A in lung tissue of mice in the WT CS group and the HDAC2$^{+/-}$ CS group was significantly increased compared with that in the WT air group (P<0.05). The expression level of IL-17A protein in lung tissue of mice in the HDAC2$^{+/-}$ CS group was significantly higher than that in the WT CS group (P<0.05).

The expression level of IL-17A protein in lung tissue of mice in the WT air group was not significantly different from that in the HDAC2$^{+/-}$ air group (P>0.05), as shown in Figure 2.

Group	CXCL1 (pg/mg)	CXCL2 (pg/mg)	IL-6 (pg/mg)	TGF-β (ng/mg)
WT air group	13.25±1.89	1.65±0.56	66.13±1.49	399.26±12.45
WT CS group	17.16±2.06*	3.71±1.05*	80.13±2.15*	689.45±22.45*
HDAC2$^{+/-}$ air group	12.99±1.98	1.70±0.42	63.00±1.55	389.16±15.45
HDAC2$^{+/-}$ CS group	24.11±2.04*#	6.89±1.69*#	93.15±2.19*#	888.45±20.15*#

Table 1: Comparison of CXCL1, CXCL2 and IL-6 levels in each group ($\bar{x}\pm s$).
*Note: Compared with WT air group, *P<0.05; Compared with WT CS group, #P<0.05.*

Group	Goblet cell hyperplasia	Peribronchial collagen deposition (%)	Smooth muscle hyperplasia
WT air	0.51±0.12	12.05±1.02	1.25±0.14
WT CS	1.89±0.39*	30.16±5.15*	3.29±0.15*
HDAC2$^{+/-}$ air	0.49±0.15	12.19±1.15	1.21±0.09
HDAC2$^{+/-}$ CS	3.02±0.34*#	49.19±5.02*#	4.95±0.19*#

Table 2: Comparison of goblet cell hyperplasia, peribronchial collagen deposition, and smooth muscle hyperplasia in each group ($\bar{x}\pm s$).
*Note: Compared with WT air group, *P<0.05; Compared with WT CS group, #P<0.05.*

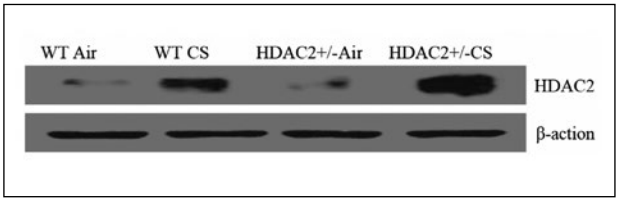

Figure 2: Comparison of IL-17A protein expression levels in lung tissues of mice in each group.

Comparison of HDAC2 and IL-17A expression levels in airway epithelial tissues of each group

The expression level of HDAC2 in airway epithelium of the WT CS group was significantly lower than that of the WT air group (P<0.05). The expression level of IL-17A in airway epithelium of the WT CS group and the HDAC2$^{+/-}$ CS group was significantly higher than that of the WT air group (P<0.05). The expression level of IL-17A in airway epithelium of the HDAC2$^{+/-}$ CS group was significantly higher than that of the WT CS group (P<0.05), as shown in Figures 3 and 4.

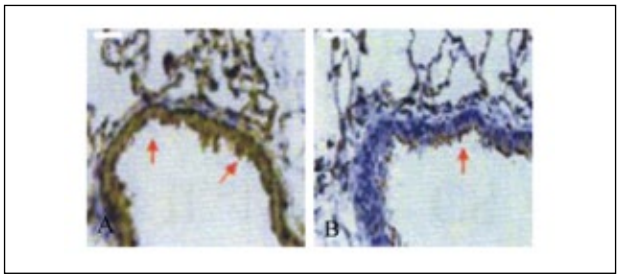

Figure 3: Comparison of HDAC2 expression levels in airway epithelial cells of each group.
Note: Figure 3A: WT air group; Figure 3B: WT CS group.

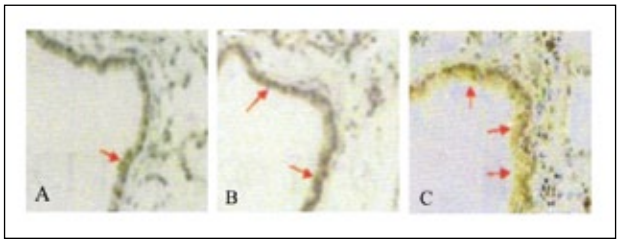

Figure 4: Comparison of IL-17A expression levels in airway epithelial cells of each group.
Note: Figure 4A: WT air group; Figure 4B: WT CS group; Figure 4C: HDAC2$^{+/-}$ CS group.

Comparison of inflammatory cell infiltration and airway remodeling in lung tissues of mice in each group

The levels of CXCL1, CXCL2, IL-6, TGF-β-1, goblet cell proliferation, peribronchial collagen deposition, and smooth muscle hyperplasia in lung tissues of mice in the IL-17A$^{-/-}$ CS group were significantly lower than those in the WT CS group (P<0.05) (see Table 3 and Table 4).

Group	CXCL1 (pg/mg)	CXCL2 (pg/mg)	IL-6 (pg/mg)	TGF-β-1 (ng/mg)
WT air group	13.25±1.89	1.65±0.56	66.13±1.49	399.26±12.45
WT CS group	17.16±2.06	3.71±1.05	80.13±2.15	689.45±22.45*
IL-17A$^{-/-}$ air group	12.16±1.15	1.78±0.59	65.13±1.59	375.56±13.56
IL-17A$^{-/-}$ CS group	13.98±1.79#	2.53±0.76#	68.49±1.19#	423.35±11.02

Table 3: Comparison of CXCL1, CXCL2 and IL-6 levels in each group ($\bar{x}$±s).
*Note: Compared with WT air group, *P<0.05; and WT CS group, #P<0.05.*

Group	PAS	Masson (%)	α-SMA
WT air group	0.51±0.12	12.05±1.02	1.25±0.14
WT CS group	1.89±0.39	30.16±5.15	3.29±0.15
IL-17A$^{-/-}$ air group	0.61±0.09	12.16±0.99	1.15±0.10
IL-17A$^{-/-}$ CS group	0.86±0.25#	18.43±0.52#	2.23±0.18#

Table 4: Comparison of goblet cell hyperplasia, peribronchial collagen deposition and smooth muscle hyperplasia in each group ($\bar{x}$±s).
*Note: Compared with WT AIR group, *P<0.05; and WT CS group, #P<0.05.*

Discussion

COPD is a preventable and treatable malformation, of which smoking is the main predisposing factor, and airway remodeling is the main factor leading to incomplete reversible airflow limitation in COPD. However, its specific mechanism has not been fully revealed. Chronic airway inflammation is also a major cause of COPD, especially small airway inflammation, which is mainly mediated by the expression of inflammatory genes. HDAC2 can reverse the hyperacetylation of core histones and block the expression of inflammatory genes, which may be the key to regulating the inflammatory response[10]. HDAC2 regulates many processes such as cell proliferation, differentiation, and apoptosis[11]. It has been reported that HDAC2 levels are reduced in alveolar macrophages and bronchus in patients with asthma[11]. Clinical studies have reported that the activity of HDAC2 in peripheral blood monocytes and cells is significantly lower than that of the normal population, and the decreased expression level and activity of HDAC2 in patients with severe asthma are closely related to hormone resistance[13].

IL-17A plays an important role as a pro-inflammatory factor in the pathogenesis of COPD. Clinical reports have shown that HDAC2 inhibitors can enhance the acetylation of retinoid-related orphan nuclear receptor and its mediated expression of IL-17A. In addition, HDAC2 activates the nuclear factor-kappa B signaling pathway and regulates the release of inflammatory factors[14]. Previous reports have shown that IL-6, TNF-α, can activate Th17 cells and then induce differentiation of Th17 cells from naive CD4 T cells[15]. T cells differentiate into IL-17A, which is mainly differentiated by histone/protein deacetylase activity. At present, human and animal experiments have shown that CD4+T cell subtype Th17 cells can release IL-17A, IL-21 and other factors, among which IL-17A plays an important role in inflammatory response, cancer, and the onset and development of autoimmune diseases. In addition, IL-17A can also act on host defense processes such as cell and fungal infection. Studies relating to COPD have shown that the expression level of IL-17A in bronchial submucosa of patients with COPD is significantly up-regulated, and the occurrence of this phenomenon is correlated with airflow restriction. Other reports have shown that lung fibroblasts can produce and release epithelial mesenchymal transformation components such as

collagen, thereby causing COPD, asthma, and other chronic airway diseases. It has also been confirmed from studies relating to COPD that fibroblasts from patients with this disease are more sensitive to IL-17A, and their level of synthetic epithelial-mesenchymal transformation is significantly increased compared with non-COPD patients. TGF-β1 plays an important role in the initiation of airway remodeling and the maintenance of pulmonary collagen homeostasis. Clinical studies have shown that IL-17A can activate fibroblasts and accelerate cell proliferation and collagen synthesis. In addition, IL-17A also acts on inflammatory cytokines and pro-fibrogenic factors to activate fibroblasts. In this study, we selected HDAC2 gene knockout mice to establish a COPD model, and found that HDAC2 can significantly block airway inflammation, airway remodeling, and fibroblast activity induced by CS, while IL-17A can accelerate airway inflammation and airway remodeling, and enhance fibroblast activity induced by CS, by stimulating TGF-β-1. In addition, HDAC2 can improve the IL-7A–mediated airway remodeling in COPD by blocking airway inflammation and fibroblast activity.

In conclusion, HDAC2 can alleviate IL-17A–mediated airway remodeling in COPD, and its mechanism of action is achieved by blocking airway inflammation and fibroblast activity.

References

1) Godtfredsen NS, Jørgensen DV, Marsaa K, Ulrik CS, Andersen O, et al. Soluble urokinase plasminogen activator receptor predicts mortality in exacerbated COPD. Respir Res 2018; 19(1): 97.
2) Hohlfeld JM, Vogel-Claussen J, Biller H, Berliner D, Berschneider K, et al. Effect of lung deflation with indacaterol plus glycopyrronium on ventricular filling in patients with hyperinflation and COPD (CLAIM): a double-blind, randomised, crossover, placebo-controlled, single-centre trial. Lancet Respir Med 2018; 6(5): 368-378.
3) Alotaibi NM, Chen V, Hollander Z, Hague CJ, Murphy DT, et al. Phenotyping COPD exacerbations using imaging and blood-based biomarkers. Int J Chron Obstruct Pulmon Dis 2018; 13: 217-229.
4) Wang J, Lv H, Luo Z, Mou S, Liu J, et al. Plasma YKL-40 and NGAL are useful in distinguishing ACO from asthma and COPD. Respir Res 2018; 19(1): 47.
5) Orchard P, Agakova A, Pinnock H, Burton CD, Sarran C, et al. Improving Prediction of Risk of Hospital Admission in Chronic Obstructive Pulmonary Disease: Application of Machine Learning to Telemonitoring Data. J Med Internet Res 2018; 20(9): e263.
6) Göder A, Emmerich C, Nikolova T, Kiweler N, Schreiber M, et al. HDAC1 and HDAC2 integrate checkpoint kinase phosphorylation and cell fate through the phosphatase-2A subunit PR130. Nat Commun 2018; 9(1): 764.
7) Yoon S, Kook T, Min HK, Kwon DH, Cho YK, et al. PP2A negatively regulates the hypertrophic response by dephosphorylating HDAC2 S394 in the heart. Exp Mol Med 2018; 50(7): 1-14.
8) Qi Z, Wang C, Jiang J. Synthesis and Evaluation of C15 Triene Urushiol Derivatives as Potential Anticancer Agents and HDAC2 Inhibitor. Molecules 2018; 23(5): 1074.
9) Jee MH, Johansen JD, Buus TB, Petersen TH, Gadsbøll AØ, et al. Increased Production of IL-17A-Producing γδ T Cells in the Thymus of Filaggrin-Deficient Mice. Front Immunol 2018; 9: 988.
10) Sun XJ, Li ZH, Zhang Y, Zhong XN, He ZY, et al. Theophylline and dexamethasone in combination reduce inflammation and prevent the decrease in HDAC2 expression seen in monocytes exposed to cigarette smoke extract. Exp Ther Med 2020; 19(5): 3425-3431.
11) Elnaggar MM, Abdellrazeq GS, Dassanayake RP, Fry LM, Hulubei V, et al. Characterization of αβ and γδ T cell subsets expressing IL-17A in ruminants and swine. Dev Comp Immunol 2018; 85: 115-124.
12) Tang D, Zhao YC, Qian D, Liu H, Luo S, et al. Novel genetic variants in HDAC2 and PPARGC1A of the CREB-binding protein pathway predict survival of non-small-cell lung cancer. Mol Carcinog 2020; 59(1): 104-115.
13) Zhang T, Wei D, Lu T, Ma D, Yu K, et al. CAY10683 and imatinib have synergistic effects in overcoming imatinib resistance via HDAC2 inhibition in chronic myeloid leukemia. RSC Advances 2020.
14) Helegbe GK, Huy NT, Yanagi T, Shuaibu MN, Kikuchi M, et al. Elevated IL-17 levels in semi-immune anaemic mice infected with Plasmodium berghei ANKA. Malar J 2018; 17(1): 169.
15) Li N, Zhu Q, Li Z, Han Q, Zhang G, et al. IL17A gene polymorphisms, serum IL-17A and IgE levels, and hepatocellular carcinoma risk in patients with chronic hepatitis B virus infection. Mol Carcinog 2014; 53(6): 447-57.

Corresponding Author:
Chaowen He
187 Guanlan Avenue, Longhua District, Shenzhen City, Guangdong Province, China
Email: b7fjjp@163.com
(China)