

## Original Article

# Decreased expression of stem cell factor mRNA and protein in the gallbladders of guinea pigs fed on high cholesterol diet

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Received February 1, 2015; Accepted April 4, 2015; Epub April 15, 2015; Published April 30, 2015

**Abstract:** Objective: Sufficient evidence suggests that the c-kit protooncogene receptor and its ligand stem cell factor (scf) signal pathway play a crucial role in interstitial cells of Cajal (ICCs) development and maintenance of their phenotype. We aimed to determine the expressions of scf mRNA and scf protein in the gallbladders in guinea pigs fed on high cholesterol diet (HCD). Methods: The gallbladder and serum samples from 20 guinea pigs of HCD and from 20 guinea pigs of standard diet (StD) were used for this study. Serum lipid analysis was performed using standard laboratory procedure. Expression of scf mRNA was detected by reverse transcription polymerase chain reaction (RT-PCR), and expression of scf protein was detected by Western blot analysis. Results: Laboratory results showed serum total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride (TG) concentrations were significantly higher in the HCD group than in the StD group of guinea pigs ( $P < 0.001$ , respectively). Decreased expression of scf mRNA and protein were demonstrated in the HCD group compared with the StD group ( $P < 0.05$  respectively). Conclusion: The data indicates that the expression of scf mRNA and c-kit protein is significantly decreased in the gallbladders in guinea pigs of HCD.

**Keywords:** Gallbladder, stem cell factor, interstitial Cajal-like cells

## Introduction

Recently, the understanding on gastrointestinal physiology of smooth muscle system has been greatly changed due to the finding of interstitial cells of Cajal (ICCs). ICCs are the pacemaker cells of gut motor activity. Some evidence has suggested that ICCs generate slow waves in phasic gastrointestinal muscles, actively propagate slow waves, and mediate or transduce neural inputs from enteric motor neurons to smooth muscles [1-3]. It was well recognized that ICCs express the proto-oncogene c-kit, which encodes a membranous receptor tyrosine kinase. Sufficient evidence suggests that the c-kit and its ligand stem cell factor (scf) signal pathway plays a crucial role in ICC development and maintenance of their phenotype. ICCs can also be found in multiple organs other than intestine where they are called Interstitial Cajal-like cells (ICLCs).

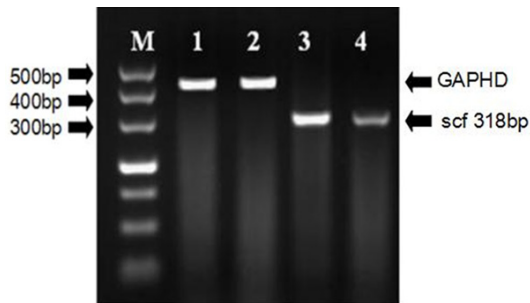
ICLCs were recently discovered in the wall of the human and guinea pig gallbladder. And these ICLCs may be involved in generating

rhythmic electrical activity in the guinea pig gallbladder musculature [4-6]. Recent studies have showed the reduction of ICLCs in the gallbladders in guinea pigs fed on high cholesterol diet (HCD) and gallstone patients [7]. Could we hypothesize that the reduction of ICLCs resulted from an abnormal c-kit/scf signal pathway? In 2009, Hu et al. found the expression of c-kit mRNA and c-kit protein significantly decreased in the gallbladders in guinea pigs of HCD [8]. To date, no studies have examined the expression of scf mRNA and protein from gallbladder tissues in guinea pigs. We used reverse transcription-polymerase chain reaction (RT-PCR) and Western blot techniques to determine the expression of scf mRNA and protein in the gallbladders of HCD guinea pigs.

## Materials and methods

### *Animals and diet*

40 male guinea-pigs (4 weeks, 120-125 g) were obtained from the Animal Research Center in



**Figure 1.** The mRNA expression of scf in guinea pig gallbladder. Compared with StD (control) group, the mRNA expression of scf was decreased in HCD group. M: marker; lanes 1 and 3, StD (control) group; lane 2 and 4, HCD group.

Shengjing Hospital of China Medical University, China and randomly assigned to two groups. The StD group (n = 20) was fed a standard diet (StD). The HCD group (n = 20) was fed a high cholesterol diet (HCD) (2% cholesterol). All animals were raised in standard lab condition (12 hour light cycle change, temperature 21-24°C, moisture 50-55%). The Animal Care Committee of Shengjing Hospital of China Medical University approved all protocols for these animal studies.

## Gallbladders and blood samples preparation

20 animals in each diet group were fasted for 16 hours and anesthetized after 8 weeks of feeding. The animals were weighed and then underwent laparotomy and cholecystectomy. Blood was aspirated from the heart and spun at 15,000 rpm for 5 min to separate serum. All gallbladders and blood samples from the two groups were frozen to -80°C.

## Serum lipid analysis

In all animals, serum total cholesterol (TC), low (LDL) and high (HDL) density lipoprotein cholesterol, and triglyceride (TG) concentrations were assessed with the use of a standard laboratory procedure.

## RNA extraction and RT-PCR analysis

Following the manufacturer's instructions, total RNA was extracted from the muscular layer of the gallbladder tissues with TRIzol reagent (Invitrogen). cDNA was reverse-transcribed from 1 µg of total RNA and amplified for 35 cycles of denaturation (45 s at 94°C), annealing (45 s at 60°C), and synthesis (45 s at 72°C).

The primers of scf were 5' GCAGCATAATACCACG 3' (forward), and 5' AATACCATCATCCGTTC 3' (reverse), generating an amplified product of 318 bp. The primers of GAPDH were 5'-ACC-ACAGTCCATGCCATCAC 3' (forward) and 5'-TCC-ACCACCCTGTTGGGTA-3' (reverse), generating an amplified product of 452 bp. Thereafter, electrophoresis was applied to the PCR product with size markers on a 1.5% agarose gel stained with ethidium bromide. GAPDH gene was used as an internal control of the analysis of gene expression. The mRNA values are expressed as relative units calculated according to the following formula: density of the scf amplification product/density of the GAPDH amplification product.

## Protein extraction and western blot analysis

Total protein was extracted from gallbladder muscular layer tissues with RIPA lysis buffer. Protein concentrations were determined by Bradford assay. The electrophoresis of 10% SDS polyacrylamide gels was performed and then transferred to a nitrocellulose (NC) membrane (Pierce Biotechnology, Inc., USA). The membrane was then incubated with 5% non-fat milk overnight at 4°C to block nonspecific binding sites. The primary antibody of the anti-scf was applied for 1 h at room temperature. After washing with PBS, the secondary antibody of matching peroxidase conjugated was applied for 1 h. Specific protein bands were visualized with X-ray film using the chemiluminescence detection kit (ECL; Amersham). Optical density of the bands was analyzed with GIS-2020 (Tanon, China).

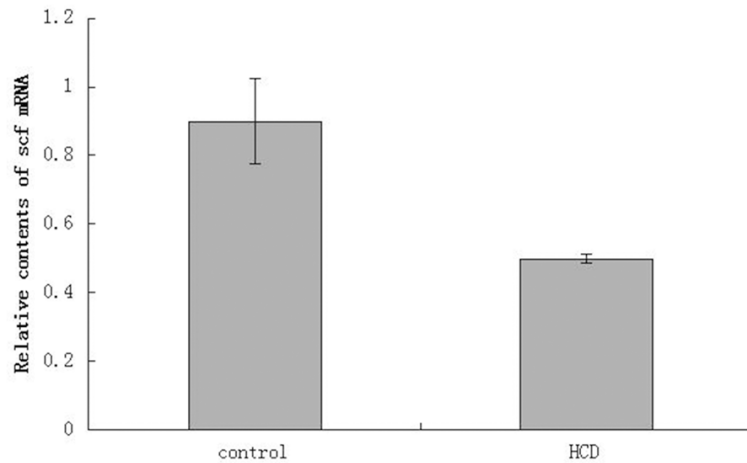
## Statistical analysis

All statistical analyses were performed using the SPSS11.5 software package. The data obtained was expressed as mean ± SD. Comparison between the different groups was performed using t-test, with P < 0.05 being considered an indicator of significance.

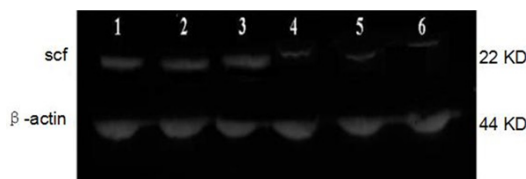
## Results

### Evaluation of guinea pig lithogenic model

No animal died in both groups. In the StD group, no gallstone or cholesterol monohydrate crystals were detected. The gallstone and cholesterol crystals had developed in all gallbladders in the HCD group at the 8th week (20/20).



**Figure 2.** The mean optical density of scf mRNA/GAPDH. Each bar represents the mean  $\pm$  SD (vertical line) with  $P < 0.05$ .



**Figure 3.** Expression of scf protein in gallbladder tissues from controls and guinea pigs with HCD evaluated by Western blot analysis. Lanes 1, 2 and 3 represent guinea pigs with StD and, lanes 4, 5 and 6 represent guinea pigs with HCD.

## Serum lipid analysis

Serum total cholesterol (TC) ( $36 \pm 4$  vs.  $110 \pm 18$  mg/dl), low density lipoprotein (LDL) cholesterol ( $22 \pm 4$  vs.  $74 \pm 9$  mg/dl), high density lipoprotein (HDL) cholesterol ( $2.2 \pm 0.2$  vs.  $7.2 \pm 1.4$  mg/dl), and triglyceride (TG) ( $55 \pm 6$  vs.  $112 \pm 18$  mg/dl) concentrations were significantly higher in the HCD group than in the StD group of guinea pigs ( $P < 0.001$ , respectively).

## scf mRNA expression in the gallbladders of HCD guinea pigs

Reverse transcription polymerase chain reaction analysis showed the presence of scf mRNA in the gallbladders from the StD and HCD groups (**Figure 1**). As expected, a band sized 318 bp presented in the 1.2% agarose electrophoresis. The ratio of scf mRNA and GAPDH was  $0.899 \pm 0.124$  in the StD group and  $0.499 \pm 0.012$  in the HCD group. Decreased expression of scf mRNA was demonstrated compared

with the StD group ( $P < 0.05$ ) (**Figure 2**).

## scf protein expression in the gallbladders of HCD guinea pigs

The results of the Western blot and densitometric analysis of scf are depicted in **Figure 3**. The presence of scf protein in the gallbladder was revealed by Western blot analysis. Consistent with the findings of RT-PCR, a lower level of scf protein was demonstrated in the HCD group. The mean value of optical density was  $116.3 \pm 12.5$  in the StD group

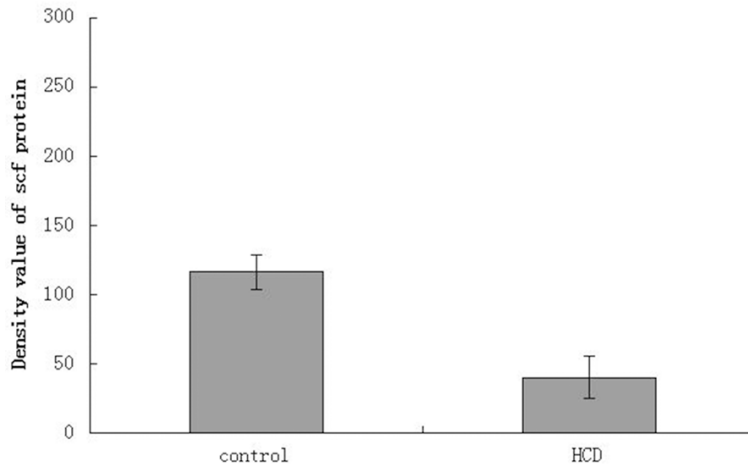
and  $40.2 \pm 15.2$  in the HCD group ( $P < 0.05$ ) (**Figure 4**).

## Discussion

Multiple factors are responsible for gallstone formation, among which decreased gallbladder motility is of importance [9]. Gallbladder motility involves multiple regulatory mechanisms, including smooth muscle and enteric nervous circuit activity as well as the recently described gallbladder ICLCs.

ICCs were first described by the Spanish neuropathologist, Ramón Santiago y Cajal (1852-1934) in 1889. ICCs have since been found in a wide range of tissues, mainly interposed between enteric nerves and smooth muscle cells, or in close proximity to smooth muscle externa [10, 11]. They are considered to be important in smooth muscle motility, electrically coupled to each other and to neurons and myocytes [12]. Their main roles are to initiate pacemaker activity; and their dysfunction has been linked to a variety of intestinal motility disorders [13]. The ICLCs in guinea pig gallbladder may play a similar role, possibly as neurohumoral factors regulating gallbladder smooth muscle motility. Recent studies have showed the reduction of ICLCs in the gallbladders in guinea pigs fed on high cholesterol diet (HCD) and gallstone patients.

Up to date, we do not know why ICCs decrease from human tissues in a variety of pathological conditions. In steel mutants with scf mutations,



**Figure 4.** The mean optical density of scf protein. Each bar represents the mean  $\pm$  SD (vertical line) with  $P < 0.05$ .

impaired development of interstitial cells and intestinal electrical rhythmicity were observed [14]. Scf was also important for ICCs culture. It has been reported that local presentation of scf increases expression of c-kit immunoreactive ICCs in culture and there is a dose-dependent and time-limited proliferation of cultured murine ICCs in response to scf [15, 16]. All these evidence suggests that scf is a key factor for ICCs development and maintenance of their phenotype. In this study, we used a guinea pig model to investigate scf expression during gallstone formation. Our results indicated that the scf expression decreased gradually in the gallbladder of high cholesterol diet-induced guinea pig. Together with the results of the study carried out by Hu et al [8], we think inhibition of c-kit/scf pathway plays an important role in the decreased number of ICLCs in gallbladders of guinea pig fed on HCD and c-kit/scf pathway maybe a target for prevention and treatment of gallstone disease. Much effort needs to be made to determine whether decreased expression of scf are caused by cholesterol.

Therefore, based on the information available, it can be concluded that the expression of scf mRNA and protein significantly decreased in the gallbladders of guinea pigs of HCD.

## Acknowledgements

This study was supported by National Natural Science Foundation of China (NSFC) (NO. 81000183), Shenyang Scientific and Technological Funding (NO. F11-264-1-24) and Natural

Science Foundation of Liaoning (NO. 2013021060).

## Disclosure of conflict of interest

None.

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