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The herbal extract deriving from aerial parts of Scutellaria baicalensis shows anti-inflammation and anti-hypoxia responses in cultured fin cells from rabbit fish

Yi-Teng Xia\(^{a,b,c}\), Wei-Hui Hu\(^{a,b,c}\), Qi-Yun Wu\(^{a,b}\), Tina Ting-Xia Dong\(^{a,b,c}\), Ran Duan\(^{a,b,c}\), Jian Xiao\(^{b,d}\), Shao-ping Li\(^{f}\), Qi-Wei Qin\(^{c}\), Wen-Xiong Wang\(^{a,c,e}\), Karl Wah-Keung Tsim\(^{a,b,c,e}\)

\(^{a}\) Shenzhen Key Laboratory of Edible and Medicinal Bioresources, HKUST Shenzhen Research Institute, Nanshan, Shenzhen, China  
\(^{b}\) Division of Life Science and Centre for Medicinal Sciences, The Hong Kong University of Science and Technology, Clear Water Bay Road, Hong Kong, China  
\(^{c}\) Joint Laboratory of Guangdong Province and Hong Kong Region on Marine Bioresource Conservation and Exploitation, College of Marine Sciences, South China Agricultural University, Guangzhou, China  
\(^{d}\) Shanxi Key Laboratory of Phytochemistry, College of Chemistry and Chemical Engineering, Baaji University of Arts and Sciences, Shaanxi, China  
\(^{e}\) School of Energy and Environment, City University of Hong Kong, Kowloon, Hong Kong, China  
\(^{f}\) Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau

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A B S T R A C T

A new cell line derived from dorsal fin of rabbit fish Siganus fuscescens was developed and characterized. The cell line was isolated from the dorsal fin, named as rabbit fish fin (RFF) cell line, and which was sub-cultured for 50 cycles since the development. This cell line was tested for growth in different temperatures and serum concentrations, and the best growing condition was at 20% serum at 28 °C. In cultured RFF cells, amplification of 18S rRNA from genomic DNA and immunostaining of cellular cytokeratin confirmed the proper identity of S. fuscescens fish. After 30th passage of cultures, the cells were exposed to challenge of inflammation, triggered by LPS, and hypoxia, mimicked by CoCl\(_2\). Cultured RFF cells showed robust sensitive responses to inflammation and hypoxia in directing the expressions of cytokines and hypoxia inducible factor-1α (HIF-1α). The water extract of aerial part of Scutellaria baicalensis (SBA) has been shown in rabbit fish to prevent inflammation. Here, we extended this notion of testing the efficacy of SBA extract in the developed cultured RFF cells. Application of SBA extract inhibited the expression of LPS-induced inflammatory cytokines, i.e. IL-1β, IL-6, as well as the signaling of NF-κB. The application of CoCl\(_2\) in cultured RFF cells triggered the hypoxia-induced cell death and up regulation of HIF-1α. As expected, applied SBA extract in the cultures prevented the hypoxia-induced signaling. Our results show the established RFF cell line may be served as an ideal in vitro model in drug screening relating to inflammation and hypoxia. Additionally, we are supporting the usage of SBA herbal extract in fish aquaculture, which possesses efficacy against inflammation and hypoxia.

1. Introduction

Siganus fuscescens, known as grey rabbit fish, is wildly cultured and consumed in China, Australia and Indonesia [1,2]. However, this fish is very fragile when facing high temperature and hypoxia condition [3]. Additionally, diseases caused by pathogenic microbes and inflammation are leading to the death of S. fuscescens. These problems of aquaculture usually cause large number of fish’s death and economic lost to fishmen. The marine environment of aquaculture farm is very complex and hard to control. The in vivo experiment in fish farm not only time costing but also expensive. Fish cell line is an important model system to study fish biology, e.g. probing the efficacy of targeted drug or feeding [4–7].

Fish skin is the first barrier interacting with outer environment, and therefore which is considered as the biggest immune organ [8]. The skin cell is defending the pathogenic challenge by producing mucus and anti-microbial peptides [9]. In addition, fin cells are highly sensitive to low oxygen, and therefore which is a sensor for survival [10]. Cell line from S. fuscescens fish has not been established, which hinders the drug screening procedure for this fish species. In accord to the need, a cell line deriving from fin of S. fuscescens fish was established and characterized.

[^1]: Corresponding author. Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, Hong Kong, China.  
E-mail address: botsim@ust.hk (K.W.-K. Tsim).

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production of medicinal herbs. To encourage the recycle of wasted materials deriving from noids, i.e. baicalein, baicalin, scutellarin and wogonin, are the major ities, including anti-virus, anti-microbial and anti-inflammation [11]. Chemical and pharmacological analyses have suggested that the flavonoids, i.e. baicalein, baicalin, scutellarin and wogonin, are the major active ingredients responsible for anti-microbial functions [12]. Having identification of active ingredients, we have revealed the aerial parts of S. baicalensis (SBA) contained reasonable amounts of these active fla-30 noids [13]; however, this aerial part was being disposed during the production of medicinal herbs. To encourage the recycle of wasted materials deriving from S. baicalensis, the SBA extract has been proposed as feeding for fish aquaculture. The intake of SBA by S. fuscensces fishes greatly improved the fish survival, as well as its inflammatory response to microbial [13]. Because of the character of low toxicity and low cost, many TCM with anti-inflammation and anti-hypoxia effects have already been applied in aquaculture feeding [14].

Having the established cell line from rabbit fish fin, named as RFF cell line, we therefore determined the efficacy of SBA in against inflammation and hypoxia. Besides, the signaling, induced by SBA, in NF-κB translocation during inflammation and hypoxia inducible factor-1α (HIF-1α) expression under hypoxia were illustrated here.

2. Materials and methods

2.1. Culture of fin cells

The isolation of fin cells was followed by reported protocol with minor modification. Two healthy S. fuscensces fishes (approximately 15 g in weight) were collected from an aquaculture farm (Shenzhen, China): the fishes were maintained in an aquarium equipped with seawater recirculation system. The fishes were anesthetized with 2-phenoxyethanol (1:10,000) and then washed with diluted bleach (1:100), wiped with 70% ethanol, to remove surface contamination. The fishes were decapitated, and the fins were subsequently removed and placed in HBSS medium with antibiotics (penicillin, 100 U/mL; streptomycin, 100 μg/mL; amphotericin-B 0.01 μg/mL) (Thermo Fisher Scientific, Waltham, MA) for washing. Then, the fins were minced into small pieces (approximately 2 mm³) using surgical scissors. Tissue pieces were put into DMEM (FBS free) (Thermo Fisher Scientific) with antibiotics, then 5 mL collagenase A (0.4 mg/mL; Sigma-Aldrich, St Louis, MO) was added. The tissue mixture was kept in an incubator with orbital shaking at 28 ℃ at speed of 90 rpm for 60 min. The solution was centrifuged and washed with DMEM to remove collagenase. Then, 5 mL of 0.25% trypsin-EDTA solution was added and maintained for 20 min at 37 ℃. Three mL FBS (Thermo Fisher Scientific) was added for trypsin neutralization and centrifuged at 200 X g for 10 min. The cell pellet was resuspended in DMEM and filtered with 180 μm-nylon to remove un- digested tissues. Then, centrifuged at 200 X g for 10 min. Cell pellets were suspended and seeded into 25-cm² flasks and cultured in 3 mL of DMEM (supplemented with 20% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, amphotericin-B 0.01 μg/mL; pH 7.4) in an incubator at 28 ℃ with 5% CO₂. Afterwards, the medium was replaced with fresh medium by half every 2–3 days. The subculture was carried out at 1:2 split subsequently by trypsinization when primary cell cultures grew to 90–100% of confluence. The culture was designated RFF cell line.

2.2. DNA extraction and PCR analysis

Total genomic DNA were extracted from cultured RFF cells (at passage 30) or fin tissues of S. fuscensces using microElute Genomic DNA kit (Omega Bio-tek, Norcross, GA). The quantitation and quality of isolated DNA was tested by NanoDrop™ 2000 spectrophotometers (Thermo Fisher Scientific). Fragments of S. fuscensces 18S ribosomal RNA (~700 bp) were amplified using primers 3′-CCA TTG GTT CAT TCG GAT TA-5′ and 3′ TTG GAT GTT TTA GTG AGG TC-5′. The PCR products were sequenced, and the obtained sequences were aligned against known S. fuscensces 18S rRNA sequences from NCBI database (GenBank Accession No. AB276986.1).

2.3. Growth curves

The effects of FBS concentration and culture temperature on cell growth were investigated as previously described [15] with modification. RFF cells at a density of 3 x 10⁵ cells per well were seeded into 96-well plates and incubated at different temperatures (28 ℃ and 37 ℃) in DMEM containing different FBS (10–30%). After cultured for 24 h, the non-adherent cells were removed by PBS washing. RFF cells were cultured over 10 days, and the medium was changed every 2 days. Every day, viability assay was applied onto the cells, and the value of OD₅₇₀nm was used to measure the relative cell number.

2.4. Cell viability assay

MTT assay was employed for revealing cell viability. In brief, cells were seeded in 96-well plate. After the treatment for 1 day, the final concentration of 0.5 mg/mL of MTT (Thermo Fisher Scientific) solution was applied, the production of purple crystal was dissolved in DMSO. The optimized absorbance was set at 570 nm.

2.5. DNA construction and transfection

The vector, pGL4.32 [luc2P/NF-kBRE/Hygro], contains five copies of a NF-κB response element (NF-kB, 5′-GGG AAT TTC CG-3′) that drives transcription of the luciferase reporter geneLuc2P (Promega Corporation, Madison, WI), namely as pNF-kB-Luc. The vector, pGL3-basic, contains an interleukin-6 (IL-6) promoter sequence from seabream that drives transcription of the firefly luciferase sequence. The vector pBI-GL (BD Biosciences Clontech, San Jose, CA) contains six hypoxia responsive elements (HRE: 5′- GTG ACT ACG TGTCG CTA G-3′) having a downstream reporter of firefly luciferase gene; this vector was named as pHRE-Luc. RFF cells (at passage 30) were seeded onto a 24-well cell culture plate at a density of 1 x 10⁶ cells per well. After being cultured for 24 h at 28 ℃, RFF cells were transfected with plasmid pNF-kB-Luc or pIL-6-Luc or pHRE-Luc by Lipofectamine™ 3000 transfection kit (Invi- trogen, Carlsbad, CA). Briefly, 1 μL of Pl 3000™ reagent and 0.5 μg plasmid were diluted by 25 μL of Opti-MEM™ medium. Then, the diluted plasmid was added to a tube containing diluted Lipofectamine™ 3000 reagent (0.75 μL in 25 μL of Opti-MEM™ medium) and mixed well. After incubation for 10–15 min at room temperature, 50 μL mixture was added to each well. The cells were treated at 28 ℃ for 6 h, then the transfecting regimen was removed. The cells were pre-treated with various concentrations of drugs for 4 h, then challenged with an inflammatory response by lipopolysaccharide (LPS), or with hypoxia response by applying cobalt (II) chloride (CoCl₂), for 24 h. The medium was aspirated, and the cultures were washed by PBS, twice. The cells were lysed by a buffer containing 0.2% Triton X-100, 1 mM dithiothreitol (DTT) and 100 mM phosphate buffer (pH 7.8) at 4 ℃. Followed by centrifugation at 13,200 rpm at 10 min, the supernatant was collected and used to perform luciferase assay (Tropix, Bedford, MA). The luminescent reaction was quantified in a Tropix TR717TM Microplate Luminometer, and the activity was expressed as absorbance (up to 595 nm) per mg of protein.

2.6. Quantitative real-time PCR

In order to reveal the inflammation and hypoxia response in cultured RFF cells, the expression levels of inflammation cytokines, IL-1β, IL-6, TNF-α, induced by LPS, and HIF-1α, induced by CoCl₂, were
measured by real-time PCR. Total RNA from cultured cells was isolated by RNeasy reagent (Molecular Research Center, Cincinnati, OH) and then reverse transcribed into cDNAs by using MMLV (Moloney Murine Leukemia Virus) reverse transcriptase according to the manufacturer's instructions (Invitrogen). Real-time PCR was employed here by using FastStart Universal SYBR Green Master (ROX) according to the manufacturer’s instructions (Roche Applied Science, Mannheim, Germany). The primers were as followed: 5′-GTG GTA TTA TTC ATG AGC TT-3′ (sense primer, S) and 5′-TTT CAT CAC ACA GAG CAG GTA-3′ (antisense primer, AS) for rabbit fish HIF-1α. For fish inflammatory cytokines the primers were as followed: 5′-ACC ATC TGG CTT GGG GAA C-3′ (S) and 5′-GAA TGA GTC GTG TGG TCT GGA AG-3′ (AS) for fish IL-6; 5′-AGC CAA TCT GGC AAG GAT CA-3′ (S) and 5′-GAT GAA GAA CCA GTT GTT GT-3′ (AS) for fish IL-1β; 5′-CTT CAC GCT CAA CAA GTC TCA G-3′ (S) and 5′-AAA GCC TGG TCC TGG TTC ACT C-3′ (AS) for fish TNF-α; β-actin was used as an internal control, and its primer sequences were 5′-TTA TGA ACG G TG CCT GCC-3′ (S) and 5′-TGA AGG AGT AGC CAC GCT CTG T-3′ (AS). SYBR green signal was revealed by ABI 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). Transcript levels were quantified by using ΔCt value method, and the values of target genes were normalized by β-actin in the same sample for comparison. PCR products were analyzed by gel electrophoresis and melting curve analysis, as to confirm the specific amplification.

2.7. Herbal material preparation

The raw material of SBA (the aerial part of S. baikalensis) was obtained from Hebei Province in 2017, which was authenticated by Dr. Tina Dong, one of the authors. The voucher specimen (SBA#2018-10-20) was deposited at Center for Chinese Medicine at HKUST. The raw material of SBA was smashed into powder then weighed and sonicated with water for 30 min, twice: the volume was 15 times and 10 times, respectively. The extract was frozen dry by Labconco Free Zone Freeze Dry System. The dried extract was dissolved in DMSO to get final concentration of 100 mg/mL. A Waters 2695 series system (Waters, Milford, CT), equipped with a degasser, a binary pump, an auto-sampler, and a thermo-stated column compartment was employed for HPLC analysis of SBA water extract. A Grace prevail select C18 column (particle size 5 μm, 4.60 mm × 250 mm) was used. Acetonitrile (as Solvent A) and 0.1% formic acid (as Solvent B) were utilized as mobile phase, at 1.0 mL/min at room temperature. Gradient elution was as: 0–25 min, 15–21% of solvent A; 25–40 min, 21–28% solvent A; 40–50 min, 28–30% of solvent A; 50–60 min, 30–35% solvent A; 60–65 min, 35-15% of solvent A. A DAD detector at an absorbance of 276 nm was used.

2.8. Immunofluorescent staining

Cells were grown on glass coverslip per-coated by lysine for 24 h. After PBS wash, cells were fixed with 4% paraformaldehyde (PFA) for 15 min. Cells were incubated with 0.2% Triton X-100 in PBS for 10 min and then blocked by 5% BSA for 1 h. Cultures were separately stained with anti-cytokeratin antibody (ab94894, Abcam, Cambridge, UK) or anti-NF-xb p65 antibody (sc-7151, Santa Cruz Biotechnology, Dallas, TX) at 1:100 at 4 °C overnight, followed with the FITC-conjugated anti-rabbit antibodies (AP037F, Sigma-Aldrich). Samples were mounted with ProLong™ Gold Antifade Mountant with DAPI (Thermo Fisher Scientific). Samples were then examined by Leica SP8 Confocal Microscope. The fluorescent intensity was then quantitated by selecting the nuclear and cytoplasmic regions in different fields using the LAX 5 imaging software.

2.9. Statistical analysis and other assays

Protein concentration was measured by a kit from Bio-Rad (Hercules, CA). Each result was presented as the mean ± SD, calculated from 3 to 5 independent samples, with triplicated. Comparisons of the mean for untreated control cells and treated cells were analyzed using one-way analysis of variance (ANOVA) and Student’s t-test. Significant values were represented as *p < 0.05, **p < 0.01.

3. Result

3.1. Characterization of established RFF cell line

The cell line RFF was derived from dorsal fin tissue of healthy S. fuscescens (rabbit fish). The culture reached full confluence in 3 days at 28 °C. The cells were sub-cultured at a split ratio of 1:2 for every 1–2 days. Fibroblast-like cells and epithelial-like cells were recognized in the first few passages of cultured RFF cells, and thereafter keratinocyte-like cells became predominant, starting from 5 cycles of passage (Fig. 1A). A strong immunostaining signal in the cells, incubated with anti-cytokeratin antibody, was identified (Fig. 1B). The RFF cells could be sub-cultured over 50 times without much change in cell morphology and identity. To confirm the origin of RFF cells, a ~700-bp PCR product flanking 18S rRNA was amplified from cultured RFF cells or dorsal fin of S. fuscescens (Supplement Fig. 1). DNA sequencing and comparative analysis showed that the partial 18S rRNA sequence. The sequence amplified from genomic DNA isolated from cultured RFF cells showed ~99% identity with S. fuscescens tissue, and both had ~99% identity to known S. fuscescens 18S rRNA sequence from NCBI database (GenBank Accession No. AB276986.1). Thus, the identity of derived RFF cells was confirmed here.

RFF cells at 30th passage was analyzed for cell growth. RFF cells could only grow under the temperature of 28 °C (Fig. 2). RFF cells did not survive under 37 °C and ~50% of cells were dead after 24 h of culture. The growth rate of RFF cells was different with various concentrations of FBS (from 10 to 30%) in DMEM at 28 °C, showing maximum growth rate at 20% FBS (Fig. 2A). The growth was at peak after 6 days of culture. Serum at 10% or 30% in the medium could not support the proper growth of RFF cells. Thus, 20% FBS in medium was employed for culture at 28 °C, and which should be used with 6 days of...
3.2. The effect of SBA extract to relieve inflammation

In order to test inflammatory response of cultured RFF cells, LPS was applied here to induce inflammation. Application of LPS (1000 ng/mL) in cultures induced the mRNA expression of pro-inflammatory cytokines, i.e. IL-1β, IL-6, TNF-α, to 2–4 folds (Fig. 3A): this induction was in a dose-dependent manner. To demonstrate the stimulation of transcriptional rate of NF-κB and IL-6, the luciferase reporter plasmids, i.e. pNF-xB-Luc and pIL-6-Luc, were employed here for cell transfection. In transfected RFF cells, the luciferase activities, driven by pNF-xB-Luc and pIL-6-Luc, were increased ~25 folds and 2.5 folds, respectively, when challenged with LPS (Fig. 3B). In line to mRNA expression, this induction was in a dose-dependent manner.

The developed cell could be used for drug screening. The water extract of aerial part of S. baicalensis (SBA) was proposed as a feeding for rabbit fishes for anti-inflammation [13]. A HPLC chromatogram of the extract of SBA was shown (Supplement Fig. 2A). Besides, the amounts of scutellaran and baicalin were 1.083% and 0.123%, respectively. The aforesaid requirements guaranteed the repeatability of assays thereafter, as quality control parameters. In cultured RFF cells, different concentrations of SBA extract were applied to test the cytotoxicity. Up to 0.5 mg/mL of SBA extract, the cultured REF cells did not show significant cell death (Supplement Fig. 2B). Here, SBA extract was applied for 4 h before the challenged of LPS. The pre-treatment with SBA extract in the cultures, dose-dependently, suppressed the LPS-induced expressions of mRNAs encoding IL-1β, IL-6 and TNF-α (Fig. 4A). Under treatment of 0.05–0.1 mg/mL of SBA extract, the suppression of cytokine expression by cultured RFF cells was almost reaching maximum. Dexamethasone served as a positive control suppressing the LPS-induced mRNA level to completion (Fig. 4A). The maximal suppression triggered by SBA was as good as that of dexamethasone.

To further demonstrate potential NF-κB and IL-6 suppression mediating by SBA extract, the transfected RFF cells with pNF-xB-Luc or pIL-6-Luc were used here. In transfected RFF cells, the activities of pNF-xB-Luc and pIL-6-Luc were robustly induced by LPS, and which were suppressed markedly by pre-treatment of SBA extract (Fig. 4B&C). A maximal suppression was revealed at 0.1–0.2 mg/mL of SBA extract. Again, dexamethasone suppressed the LPS induction, robustly. The transcription of p65 subunit of NF-κB in LPS-induced RFF cells was probed here. As expected, application of LPS in cultures induced the nuclear translocation of NF-κB p65 subunit (Fig. 5A). Around 45% of NF-xB p65 subunit was translocated into nuclear (Fig. 5B). The translocation of NF-xB p65 subunit was inhibited by pre-treatment of SBA extract in a dose-dependent manner. With the application of SBA extract, only about 20%–25% of NF-xB p65 subunit was translocated into nuclear. As a control, dexamethasone suppressed the translocation completely. The translocation rate was about 20% (Fig. 5A&B). These results strongly suggested that SBA extract could relieve inflammation damage by...

Fig. 2. Growth curves of RFF cells at different temperatures and different serum concentrations. RFF cells were cultured under 28 °C or 37 °C in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with different fetal bovine serum (FBS) concentration (10%, 20%, 30%). MTT assay was applied on these cells every day. The cell number was calculated by MTT assay. Data are shown in percentage of change compared to control group (day 0 of culture), and in Mean ± SD, where n = 4, each with triplicate samples.

Fig. 3. The inflammation response of RFF cells. (A): Expression levels of pro-inflammation cytokines IL1-β, IL-6, TNF-α in cultured RFF cells were measured after treating with different concentrations of LPS for 24 h. Total mRNAs were extracted from RFF cells to perform real-time PCR analysis. (B): Two luciferase reporter plasmids contain NF-xB and IL-6 promoter sequences, named pNF-xB-Luc and pIL-6-Luc, were transfected in cultured RFF. The transfection efficiency was 50–60%, as indicated by vector containing GFP fluorescence protein. The transfected cells were treated with LPS for 24 h. Values are expressed as fold of increase to basal reading (as 1, no LPS added), and in Mean ± SD, where n = 4. Statistical comparison was made with the basal group; *p < 0.05; **p < 0.01.
preventing the signaling of NF-κB p65.

3.3. The effect of SBA extract to relieve hypoxia

Hypoxia and high temperature are well-known two lethal causes of fish farming. To mimic hypoxia condition in cultured REF cells, CoCl$_2$
could be used. Increase amount of applied CoCl₂ caused the cell to die in a dose-dependent manner (Fig. 6A). CoCl₂ at 100 mM caused ~50% cell death in cultured REF cells. The pre-treatment of SBA extract in cultures prevented the CoCl₂-induced cell death in a dose-dependent manner (Fig. 6B). Starting 0.3 mg/mL of SBA extract, the cell protection was fully achieved. Another lethal factor, temperature, was also tested here. At 37 °C, cultured REF cells were dead in accord to time by hours (Fig. 6C). Having 30% of the temperature-induced cell death, application of SBA extract protected the cell in dose-dependent manner (Fig. 6D). At low dose of SBA (0.1 mg/mL), the cell survival could be restored to over 80%.

To cope with hypoxia, HIF is the critical one, and which is requiring a binding between an inducible HIFα subunit (HIF-1α, HIF-2α and HIF-3α) and a constitutive HIFβ subunit (HIF-1β, HIF-2β and HIF-3β) [16]. During hypoxia, the activated HIF complex binds to hypoxia response element (HRE) domain, located in the enhancer region of the EPO gene, and therefore the complex promotes the transcription of EPO, as a result to increase the production of red blood cell, as well as oxygen uptake [17]. In RFF cultures, CoCl₂ was able to robustly induce the expression of mRNA encoding HIF-1α in a dose-dependent manner (Fig. 7A). In parallel to mRNA expression, the promoter activity of pHRE-Luc was also induced by CoCl₂ in the transfected REF cells (Fig. 7B). In the SBA pre-treated cells, the CoCl₂-induced HIF-1α expression was markedly suppressed: the maximal suppression was revealed at ~0.2 mg/mL of SBA extract (Fig. 7C). This suppression was in line by using pHRE-Luc construct in the transfected cells (Fig. 7D), suggesting the anti-hypoxia function of SBA in rabbit fish.

4. Discussion

In the present study, a cell line derived from the dorsal fin of rabbit fish was established and characterized, which was named as RFF cell line. The RFF cells grew well and could be sub-cultured for over 50th passages so far. The identity of RFF cells was originated from S. fuscescens, as confirmed by genomic sequencing and immunostaining. Cultured RFF cells showed maximal growth rate in medium having 20% FBS: this requirement was similar with other fish cell lines [5,18]. RFF cells could only grow at 28 °C. This temperature restriction was also reported from other established cell lines isolated from marine fish [7, 15]. Compared with the zebra fish fin cell line AB.9 (ATCC® CRL-2298™), RFF cells grew under similar temperature but required more serum in the medium, 20% in RFF vs 15% FBS in AB.9 cell. Moreover, the morphology of RFF cell was similar with that of AB.9 cell line [19].

Skin is the first barrier of fish facing various infection factors, and therefore which is highly sensitive to inflammation. When cultured RFF cells were challenged with LPS, the expression of pro-inflammation cytokine was induced, as well as the translocation of NF-κB and the
transcription of NF-κB-driven activity. The inflammatory responses of cultured RFF cells, induced by LPS, are similar to other fish cell lines that the expression of inflammatory cytokines, i.e. IL-1β, IL-6, TNF-α, increased robustly [20,21]. This signaling of inflammatory response is a classical mechanism of common inflammation [22]. Moreover, cultured RFF cells showed strong sensitive response to hypoxia. The expression of transcription factor HIF-1α and pHRE-Luc-driven luciferase activity were dose-dependently induced by CoCl₂, and this hypoxia response was reported similarly in other marine organisms [23,24]. These results therefore indicated that the developed RFF cell line could be a helpful tool in drug screening for anti-inflammation and/or anti-hypoxia effects.

Herbal products, in particular TCM, provide an abundant resource for drug and/or food development. Several Chinese herbal extracts have been applied in aquaculture feedings to strengthen fish’s immunity and to promote growth [25]. Scutellaria Radix (the dried root of S. baicalensis) has been used as a Chinese medicine for centuries in China and Asia, and this herb is well-known to have multiple pharmacological activities, including anti-inflammation [11] and anti-hypoxia [26]. Recently, the extract of Scutellaria Radix was shown to inhibit the infection of SARS-CoV-2 virus [27]. Indeed, this herb is considered as an expensive herb in the market. In contrast to other herbal extracts, we are using the waste product of Scutellaria Radix during their production line. The stems and leaves of S. baicalensis are being discarded as waste, but those active compounds, e.g. scutellarin and baicalin, are found there predominantly [12]. Scutellarin and baicalin are main functional flavonoids in Scutellaria Radix, as well as in our tested SBA extract. Baicalin was reported to have anti-inflammation effect on hypoxia/reoxygenation and TNF-α induced injury in cultural rat cardiomyocyte [28]. Besides, baicalin protected rat brain microvascular endothelial cells that injured by oxygen-glucose deprivation [29] and attenuated hypoxia-induced pulmonary arteriole remodeling on rat [30]. Scutellarin was reported with similar effect in protecting cardiomyocyte injury from hypoxia [31] and inflammation development in microglial [32]. Scutellarin and baicalin could enhance estrogenic activity and prevent cytotoxicity induced by Aβ (amyloid β-protein) [33]. Thus, the current result demonstrated the efficacy of SBA in cultured RFF cells, i.e. anti-inflammation and anti-hypoxia, could be triggered, at least partly, by scutellarin and baicalin within SBA extract.

5. Conclusion

A fin cell line from S. fuscescens (rabbit fish), designated as RFF, was established and characterized. The cells exhibited sensitive responses to inflammation and hypoxia. Therefore, RFF cell line can be an excellent in vitro system in drug/feeding screening. The water extract of aerial part of S. baicalensis protected the survival of cultured RFF cells in dose-dependent manners in fighting against inflammation, hypoxia and high temperature. For an economic reason, the aerial part of S. baicalensis is the waste product of Scutellaria Radix production.

Credit author statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of competing interest

The authors declare that there are no conflicts of interest.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsf.2020.07.050.

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