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Ear fibroblasts derived from Taiwan yellow cattle are more heat resistant than those from Holstein cattle

Hung-Yi Wu[a], Shao-Yu Peng[b], Hung Li[b], Jai-Wei Lee[c], Piyawit Kesorn[c], Hsi-Hsun Wu[b], Jyh-Cherng Ju[d,e,f,g,*], Perng-Chih Shen[b,*]

[a] Department of Veterinary Medicine, National Pingtung University of Science and Technology, Neipu, Pingtung 912, Taiwan
[b] Department of Animal Science, National Pingtung University of Science and Technology, Neipu, Pingtung 912, Taiwan
[c] Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Neipu, Pingtung 912, Taiwan
[d] Graduate Institute of Biomedical Sciences, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan
[e] Core Laboratory for Stem Cell Research, Medical Research Department, China Medical University Hospital, Taichung 40447, Taiwan
[f] Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan
[g] Department of Animal Science, National Chung Hsing University, Taichung 40227, Taiwan

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ABSTRACT

The objective of this study was to compare the thermotolerances of ear fibroblasts derived from Holstein (H) and Taiwan yellow cattle (Y) and their apoptosis-related protein expressions with (1, 3, 6, 12, and 24 h) or without heat shock treatment. The results showed that the vaginal temperatures of Y (38.4–38.5 °C) were ($P < 0.05$) lower than that of H (38.8 °C) during the hot season. The apoptotic rates of ear fibroblasts derived from Y (6 h: 1.1%; 12 h: 1.6%; 24 h: 2.6%) were lower ($P < 0.05$) than those of cells derived from H (6 h: 1.8%; 12 h: 4.0%; 24 h: 6.9%), respectively, after heat shock (42 °C). The expression level of apoptosis inducing factor (AIF) in ear fibroblasts derived from H was higher ($P < 0.05$) than those derived from Y after the heat shock treatment for 6 h and 12 h, respectively. The level of cytochrome c of ear fibroblasts derived from H was higher ($P < 0.05$) than those derived from Y after the heat shock treatment for 1–12 h, respectively. The abundances of Caspase-3, Caspase-8 and Caspase-9 of ear fibroblasts derived from H were higher ($P < 0.05$) than those of cells derived from Y after 12 h and 24 h of heat shock, respectively; the Bcl-2/Bax ratios of ear fibroblasts derived from H were lower ($P < 0.05$) than those from Y-derived fibroblasts after heated for 1–24 h. The expression level of HSP-70 of Y-derived ear fibroblasts was also higher ($P < 0.05$) than that from H after the same duration of heat shock treatments. Taken together, the thermotolerance of ear fibroblasts derived from Taiwan yellow cattle was better than that of cells derived from Holstein cattle.

1. Introduction

The two major commercial bovine breeds are *Bos indicus*, such as Brahman, Nelore, Boran, Tuli, Gir, Gyr and Taiwan yellow cattle, and *Bos taurus*, such as Holstein, Angus and Hereford. The breeds in Taiwan are mainly Holstein (H) for milk and Taiwan yellow cattle (Y) for meat and breeding purposes.

It has been reported that Y cattle and Chinese southern yellow cattle share the same ancestor. Immigrants have brought them to Taiwan from southern China in the sixth century (Rouse, 1970), when they started multiplying successfully. Taiwan yellow cattle have been used for daily activities since seventeenth century. Therefore, Y cattle have adopted to this environment for more than four hundred years in Taiwan. Although Indonesian and Indian cattle were also brought to Taiwan during the periods of Dutch and Japanese colonizations, they contributed insignificant influences to the Y bloodlines (Li et al., 2007). Analyses of mitochondrial DNA D-loop polymorphisms (Lee et al., 2016) and microsatellite markers (Tu et al., 2014) have also verified that the Y cattle are of *Bos indicus* origin. Physiologically, they have fully adopted to the subtropical and tropical circumstances in Taiwan (Tu et al., 2014).

It is known that the thermotolerance of *Bos indicus* is superior to *Bos taurus* (Kamwanja et al., 1994; Gaughan et al., 1999). Holstein cattle belong to *Bos taurus* and are the breed of world's highest milk production and of the largest bovine population of dairy cattle. When these cattle are raised in tropical and subtropical area, the pregnancy rate (Collier et al., 2006 ; Schuller et al., 2014) and milk production (Silanikove et al., 2009; Bernabucci et al., 2010) are reduced signifi-

* Correspondence to: Department of Animal Science, National Pingtung University of Science and Technology, No. 1, Shuefu Road, Neipu, Pingtung 912, Taiwan.
E-mail addresses: jcju@dragon.nchu.edu.tw (J.-C. Ju), pcshen@mail.npust.edu.tw (P.-C. Shen).

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cantly during the hot seasons which causes a tremendous economic loss. During the hot seasons, the elevated temperatures mainly cause an imbalance of their endocrine system of these animals, leading to subfertility with either low pregnancy rates or high abortion rates. However, these phenomena are rarely noticed in *Bos indicus* (Wolfenson et al., 2000).

In terms of thermal sensitivity, embryos of *Bos taurus* are more vulnerable to heat stress than those of *Bos indicus* (Rocha et al., 1997; Monteiro et al., 2007; Silva et al., 2013). At the cellular level, the apoptotic rates of lymphocytes in the Holstein and Angus cattle are dramatically increased compared to the Brahman after heat shock treatment (Kamwanja et al., 1994; Paula-Lopes et al., 2003). It is also reported that the peripheral blood mononuclear cells (PBMCs) of Sahiwal (a thermotolerance breed in India) share a higher level of heat shock protein 70 (HSP-70) expression and a better survival rate than those from the crossbreed of Frieswal cattle (Sahiwal and Holstein hybridization) under heat stress (Bhanuprakash et al., 2016). These studies revealed that the somatic cells of *Bos indicus* (such as Brahman and, Sahiwal) are more resistant to heat shock than those of *Bos taurus* (such as Holstein and Angus).

Under heat shock conditions, whether the cell becomes apoptotic or not is mainly attributed by pro-apoptotic and anti-apoptotic factors (Arya et al., 2007; Kumar et al., 2015). The increased expression of pro-apoptotic factors, such as apoptosis inducing factor (AIF), cytochrome c, B-cell lymphoma 2-associated X protein (Bax), and cysteine-aspartic proteases (Caspases-3, −8, and −9) prompt the cells undergoing apoptosis. On the contrary, elevated expression level of anti-apoptotic factors, such as B-cell lymphoma 2 (Bcl-2) and heat shock proteins (HSP-27, HSP-60 and HSP-70) may play protective roles in preventing apoptosis (Arya et al., 2007; Kumar et al., 2015).

There are multiple factors that contribute to thermotolerance; therefore, it is extremely complicated to study the underlying mechanisms and to evaluate the thermotolerance of the animal. However, our previous studies have shown that thermotolerance of cloned cattle could be transmitted to the somatic cells of their offspring (Kesorn et al., 2017). It can be postulated that somatic cells are able to be used as an indicator for investigating the thermotolerance of animals. It has been well-documented that *Bos indicus* is more heat-resistant than *Bos taurus*. However, the molecules related to thermal tolerance at the cellular level remain unclear. In the present study, the thermotolerances of somatic cells (ear fibroblasts) from Taiwan yellow cattle (*Bos indicus*, a heat-resistant breed) and Holstein cattle (*Bos taurus*, a heat-sensitive breed) were compared. In addition, the expressiones of apoptosis-related proteins (AIF, cytochrome c, Caspases-3, Caspases-8, Caspases-9, Bax, Bcl-2, and HSP-70) under various heat shock conditions were analyzed.

2. Materials and methods

2.1. Animals

A total of 12 female cattle (2–3 years old, 6 H and 6 Y) were used in this study and the animals were raised in a conventional barn. All the animal protocols were under the supervision of the Institutional Animal Care and Use Committee (IACUC; approval number: NPUST-IACUC-104-092) of National Pingtung University of Science and Technology, Taiwan.

2.2. Chemicals and reagents

The chemicals and reagents used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) unless otherwise specified.

2.3. Measurement of ambient temperatures and body temperature of cattle

Ambient temperatures and body core temperature of cattle during the hot season (August 2013) were measured based on the procedure described by Shiao et al. (2011). For the ambient temperature, an OBO Pro RH/Temp meter (Onset Computer Corp., Bourne, MA) was used. The device was set at 2 m above the neck clamp and the cattle bed, and any changes of temperature and humidity in the barn were recorded automatically every 3 days with 3 replicates. The recorded temperature and humidity data at 03:00 and 13:00 were selected for analysis. During this trial, the vigina of each cow was probed with a temperature measurement device (HOBO water temp pro, Bourne, MA), which was placed in the vagina of cattle along with CIDR (controlled internal drug release) without progesterone (P4) for temperature recording once per minute.

2.4. Calculation of the temperature-humidity index (THI)

The temperature (T) and humidity (RH) index (THI) was calculated according to the method of NOAA National Oceanic and Atmospheric Administration (1976) by using the formula described as follows:

$$THI = 9/5 \times T + 32 - 00.55 \times (1 - RH) \times (9/5 \times T - 26)$$

where THI < 72 is defined as no thermal stress; 72 < THI < 78 is defined as mild thermal stress; 78 < THI < 84 is defined as moderate thermal stress; 84 > THI is defined as severe thermal stress.

2.5. Collection and culture of ear fibroblasts

Ear fibroblasts were collected from all animals and processed as previously mentioned (Shen et al., 2008). In brief, ear tissues were acquired from the animal and cultured in the medium (DMEM, Gibco, Grand Island, NY, USA) containing 10% (v:v) fetal bovine serum (FBS, Gibco, 10270-106) and 1% (v:v) penicillin/streptomycin (Gibco, 15140-122) in an incubator at 38.5 °C with 5% CO_2 and humidified atmosphere in air. Subsequent to the removal of massive tissue chunks, ear fibroblasts were continuously cultivated to passage 3 and then were cryopreserved in FBS with 10% (v:v) dimethyl sulfoxide (DMSO).

2.6. Evaluation of cellular thermotolerance

2.6.1. Heat shock treatment

Ear fibroblasts were heat-shocked as described by Lee et al. (2016). Cryopreserved ear fibroblasts were thawed at 37 °C for 25 s, and then cultured in DMEM with 1% penicillin/streptomycin and 10% FBS at 38.5 °C (5% CO_2 in air) for 24 h (passage 4). Afterwards, the ear fibroblasts were trypsinized and cultured (1×10^6/mL) in DMEM supplemented with 10% (v:v) FBS (passage 5) for 24 h before cell harvest. The culture condition for the non-heat shock ear fibroblasts (control group) was 38.5 °C and 5% CO_2 with saturated humidity in air for 24 h. The condition of heat-shock treatment was 42 °C and 7% CO_2. Different durations of heat shock treatment was applied before cell harvest (24 h). Protein extraction of ear fibroblasts were performed after cell harvest; Cellular apoptotic rates of the control group and certain heat-shock groups (heat shock treatment for 6, 12 and 24 h) were analyzed.

2.6.2. Detection of apoptosis

Apoptosis of cells was investigated by terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) by using *In Situ* Cell Death Detection Kit® (Roche, Indianapolis, IN, USA; Lee et al., 2016). Ear fibroblasts were suspended in Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline (DPBS, Gibco, Grand Island, NY, USA) and fixed in 4% (v:v) paraformaldehyde for 1 h. After two washes with Ca^{2+}/Mg^{2+}-free DPBS, the cells were water-bathed in 1 mL 0.1% (w:v) sodium citrate and 0.1% (v: v) Triton X-100 (in DPBS) for 30 min. The cells were then rinsed with DPBS and adjusted to 2×10^6 cells/mL prior to adding TUNEL reaction solution at 37 °C for 1 h in the dark. Finally, the cells were resuspended in Ca^{2+}/Mg^{2+}-free DPBS for the detection