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Cellular thermotolerance is inheritable from Holstein cattle cloned with ooplasts of Taiwan native yellow cattle

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ABSTRACT

We have previously demonstrated that the somatic cells from cattle cloned with Holstein (H) donor cells and Taiwan native yellow cattle (Y) ooplasm (Yo-Hd) had better thermotolerance than those from cattle cloned with both Holstein donor cells and ooplasm (Ho-Hd). The present study aimed to investigate whether the cellular thermotolerance of these cloned cattle is transmissible to their offspring (Ho-Hd-F1 and Yo-Hd-F1). Thermotolerance of ear fibroblasts derived from these cloned cattle and their offspring were analyzed. Polymorphisms in mitochondrial DNA (mtDNA) D-loop of ear fibroblasts derived from Yo-Hd and Yo-Hd-F1 indicated that the cytoplasm is originated from *Bos indicus* (Y). After heat shock, the apoptotic rates, B-cell lymphoma 2-associated X protein/B-cell lymphoma 2 ratios, and relative expression levels of cysteine-aspartic proteases (caspases)-3, -8, and -9 of ear fibroblasts with Y-originated cytoplasm (including Y, Yo-Hd, and Yo-Hd-F1) were lower ($P < 0.05$) than those of ear fibroblasts with H-originated cytoplasm (including H, Ho-Hd, and Ho-Hd-F1). In contrast, the relative level of HSP-70 was higher ($P < 0.05$) in ear fibroblasts with Y-originated cytoplasm than that of with H-originated cytoplasm. Based on our results, thermotolerance of ear fibroblasts derived from Yo-Hd and Yo-Hd-F1 cattle is better and can be transmitted, at least at the cellular level, to their offspring.

1. Introduction

Elevated ambient temperatures and humidity cause reduced reproductive efficiencies [1,2] and production traits [3–5] in farm animals, which could be even worse due to the global climate changes [6]. In 2015, for instance, during the hot seasons in southern Taiwan, the ambient temperature ranges from 23 °C to 36 °C, plus the high humidity index (51%–99%), which results in an extremely stressful environment for animal production. In animal industry, the deleterious effects on animal performance are caused by multiple factors such as suboptimal environment, management, age, and breeds [7]. Heat stress is one of the major factors responsible for reduced reproductive performance in domestic animals in tropical and subtropical areas. It is well known that *Bos indicus* cattle are more thermotolerant than *Bos taurus*. Several *in vitro* studies also

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found that *Bos indicus* embryos are less sensitive to heat shock in comparison with *Bos taurus* embryos [8–10]. It has been reported that heat shock may induce apoptosis in many cell types, including preimplantation embryos [11,12], peripheral blood mononuclear cells (PBMCs) [13,14], and mammary epithelial cells [15]. However, the level of thermotolerance varies in different cells types. In addition, the blastocyst rate, a quick indicator of developmental potential, is significantly reduced in the embryos derived from oocytes of less thermotolerant breeds (*Bos taurus*) but not in embryos from oocytes of more thermal resistant breeds (*Bos indicus*) after heat shock treatment [16]. Our previous studies indicated that the thermotolerance in cloned embryos [17] and ear fibroblasts of cloned cattle reconstructed with heat-tolerant Taiwan native yellow cattle (Y; *Bos indicus*) ooplasm and heat-sensitive Holstein (H; *Bos taurus*) donor cells (Yo-Hd) were better than those cells of cloned cattle reconstructed using H ooplasm and H donor cells (Ho-Hd) [18]. These results clearly suggested that the ooplasm plays a pivotal role in thermotolerance at the cellular level.

Apoptosis is a critically regulated mechanism for eliminating cells that are unnecessary, damaged, nonfunctional, abnormal, or misplaced [7,19]. Heat stress may initiate a spectrum of apoptosis-related gene expressions and biochemical mechanisms that are responsible for the thermotolerance of the host [7,20,21]. The extrinsic receptor-mediated and the intrinsic mitochondrial apoptosis pathways have been illustrated [22]. Proapoptotic factors, including B-cell lymphoma 2-associated X protein (Bax), endonuclease G (Endo-G), apoptosis-inducing factor (AIF), and cysteine-aspartic proteases (caspases-3, -8, and -9), are upregulated to facilitate cell death. In contrast, antiapoptotic factors, such as heat shock proteins (HSP-27, HSP-60, and HSP-70), and B-cell lymphoma 2 (Bcl-2), can liberate the cells from the early stage of apoptosis [23,24]. Therefore, whether cells undergoing apoptosis or not largely depends on the homeostatic status between antiapoptotic and proapoptotic factors [23].

We have recently demonstrated that heat-shocked ear fibroblasts from purebred Y and Yo-Hd cattle have significantly lower proapoptotic factors (Bax, cytochrome c, and caspases) and higher antiapoptotic factors (HSPs and Bcl-2) compared with those from purebred H and Ho-Hd cattle, which resulted in a reduced apoptosis rate [18]. It would be interesting to know if the thermotolerance of ear fibroblasts from Yo-Hd cattle can be transmitted to their offspring. Therefore, we investigated the differential expressions of apoptosis-related proteins between ear fibroblasts of previously cloned cattle (Ho-Hd and Yo-Hd) and their F1 offspring produced by insemination with Holstein semen (Ho-Hd-F1 and Yo-Hd-F1) in response to heat shock conditions. These results are fundamental to understand the transmissibility of thermotolerance in cloned animals.

2. Materials and methods

2.1. Animals

A total of 12 cattle, including two cloned cows (one Ho-Hd and one Yo-Hd) produced in our previous study [25], were used. Moreover, these clones were all possessed donor nuclei from the same Holstein cow (i.e., donor nuclei with identical genetic backgrounds). The two cloned cows were superovulated and inseminated with the same Holstein semen. At 7 days after artificial insemination, blastocysts were collected by embryo flushing, and then transferred to recipient females of purebred Holstein (heat-sensitive breed), which gave birth to four cloned offspring, Ho-Hd-F1 ($n = 2$) and Yo-Hd-F1 ($n = 2$), respectively. In addition, three purebred Taiwan native yellow cattle (Y, heat-tolerant breed) and three purebred Holstein (H, heat-sensitive breed) were randomly selected as the control. All experimental protocols regarding animal use were approved by the Institutional Animal Care and Use Committee (IACUC; approval number: NPUST-IACUC-101–083) of National Pingtung University of Science and Technology, Taiwan.

2.2. Chemicals and reagents

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

2.3. Preparation of ear fibroblasts and culture

Ear fibroblasts were prepared as previously described [26]. Briefly, ear tissues (3-mm^2 per piece) were obtained from each animal (<3-month of age) and cultivated with the whole piece of tissue in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 1% (v:v) penicillin/streptomycin (Gibco, 15140-122), and 10% (v:v) fetal bovine serum (FBS, Gibco, 10270-106) at 38.5 °C with 5% CO_2 in air. After discarding the lumps of ear tissue, fibroblasts were continuously cultured till passage 3 and then were stockpiled in FBS containing 10% (v:v) DMSO in liquid nitrogen until use.

2.4. Verification of cytoplasm origin

Total DNA, comprising mitochondrial DNA (mtDNA), was extracted from the ear fibroblasts of each animal by exploiting a commercially available DNA Isolation kit (Qiagen, Limburg Province, Netherlands). Primers (forward primer 5′-CTCACCATCACCCCCAAAGCT-3′ and reverse primer: 5′-TCATCTAGCATTTTCAGTG-3′) specific for the DNA D-loop of mitochondria were used, and polymerase chain reaction (PCR) was implemented in accordance with the subsequent procedures. Four steps were performed: step 1 is 94 °C for 1 minute; step 2 is denaturation at 94 °C for 30 seconds followed by annealing at 47 °C for 40 seconds and extension at 72 °C for 30 seconds (step 2 was duplicated for 40 cycles); step 3 is at 72 °C for 2 minutes; and step 4 is 4 °C. The amplicons were cut by two restriction enzymes including *Dde* l (BioLabs, San Diego, CA, USA) and *BstN* l (BioLabs) followed by electrophoresis (2% agarose gel, 100 V, 20 minutes). DNA polymorphism of mitochondria was analyzed by using an image analysis system (Alphaimager HP; Alpha Innotech, San Diego, CA, USA) to identify the cytoplasmic origins (H or Y) of ear fibroblasts [27].