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Establishment and evaluation of a stable steroidogenic caprine luteal cell line

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Abstract

Many physiological, biological, pharmacologic, and toxicologic events and compounds affect the function of Saanen dairy goat luteal cells, resulting in implantation failure or early embryonic loss. Although primary luteal cell cultures have been used, their finite lifespan precludes assessment of long-term effects. In the present study, primary caprine luteal cells (CLCs) were immortalized through transfection of a plasmid containing the human telomerase reverse transcriptase (hTERT) gene. The expression of hTERT and telomerase activity were evaluated in transduced CLCs (hTERT-CLCs). In this study, these cells steadily expressed hTERT gene and exhibited higher telomerase activity at Passages 30 and 50. The hTERT-CLCs at Passages 30 and 50 expressed genes encoding key proteins, enzymes and receptors inherent to normal luteal cells, e.g., steroidogenic acute regulatory protein (StAR), cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), and LH-receptor (LH-R). In addition, immortalized caprine luteal cells produced detectable quantities of progesterone in response to 8-bromo-cAMP (8-Br-cAMP) or 22(R)-hydroxycholesterol (22R–HC) stimulation. Furthermore, this cell line appeared to proliferate more quickly than control cells, although no neoplastic transformation occurred either *in vivo* or *in vitro*. We concluded the immortalized CLCs by hTERT retained their original characteristics and may provide a useful model to study luteal cell functions.

Keywords: Immortalization; Corpus luteum; Luteal cells; Saanen dairy goat

1. Introduction

The corpus luteum (CL) is formed by a heterogeneous population of cells, including steroidogenic cells, fibroblasts and endothelial cells [1–3]. Steroidogenic cells are classified into two types: large luteal cells and small luteal cells [1,2]. There are many physiological, biological, pharmacologic and toxicologic events and compounds that can interfere with the normal function of luteal cells and cause abnormal luteal function, resulting in reproductive failure in mammals [4–7]. Normal luteal function is essential for establishment and maintenance of pregnancy in mammals, which is predominantly under the influence of progesterone, a principal steroid hormone synthesized and secreted from the mammalian CL [3,8–10]. Both types of luteal cells express 3β-HSD, a key enzyme for progesterone synthesis [10].

Previous *in vitro* studies used primary luteal cells to elucidate molecular mechanisms underlying regulation of progesterone secretion from the CL [11–13]. Although this approach can provide information regard-

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ing molecular mechanisms of normal luteal cell function, the ability of normal luteal cells to proliferate *in vitro* limits many studies, particularly those regarding long-term effects of physiological, biological, pharmacologic and, toxicologic events and compounds [5,12,13]. Like many normal somatic cells, normal luteal cells enter an irreversible state known as replicative senescence after a finite number of cell divisions [14]. During this process, luteal cells are characterized by a reduction in proliferative potential [15]. Meanwhile, there are irreversible changes in morphology, gene expression, and steroidogenic activity. Since it is difficult to generate reliable and reproducible results using different cell preparations or passages, developing a stable luteal lineage at early stages of pregnancy would be helpful for determining the characteristics and functions of caprine luteal cells on some aspects of reproductive processes.

Previous studies confirmed that the onset of replicative senescence is partly associated with shortening of telomeres [16]. In most normal somatic cells, telomere DNA will lose 10 to 200 bp at each mitosis. When a telomere is shortened to a certain threshold limit, it cannot protect the end of linear chromosomes from nuclease degradation, interchromosomal fusion and improper recombination, the cells become senescent [17,18]. However, cell senescence can be circumvented if telomere shortening can be prevented via induction of telomerase activity [19,20]. In that regard, overexpression of hTERT in telomerase-negative cells not only prevents telomere shortening, but also initiates telomerase activation and extends the life span of these cells [19–22].

The objective of the current study was to establish an immortalized caprine luteal cell line through transfection of a plasmid containing the hTERT gene.

2. Materials and methods

2.1. Luteal cell isolation and culture

Ovaries were collected (at a local abattoir) from healthy pregnant Saanen dairy goats (2–3 yrs old) in early (6–8 wk) stages of pregnancy. Stages of pregnancy were estimated by measuring fetal size, as described [23]. These ovaries were transported to the laboratory in PBS on ice within 30 min after slaughter.

All animals were clinically observed 2 wk before slaughter, and experiments were conducted according to institutional and ethical guidelines involving use of animals. Corpora lutea were dissected, washed thoroughly with cold PBS to remove excess blood, and placed immediately into culture medium (Dulbecco's Modified Eagle's and Ham's F-12, 1:1 (v/v) with 15 mmol/L HEPES, Gibco BRL, Carlsbad, CA, USA) containing 100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (Harbin Pharmaceutical Group, Ltd., Harbin, China). Tissue samples were cut into 1 mm^3 pieces and digested with collagenase II (2 mg/mL in DMEM/F12 media, Sigma, St. Louis, MO, USA) for 20 min at 37 °C in a shaking water bath. Supernatants were filtered through a 150 μm mesh to remove large matrix fragments and centrifuged (200×g for 10 min). Luteal cells were gently resuspended in DMEM/F12 medium supplemented with 10% fetal calf serum (Thermo Scientific HyClone, Beijing China), 100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin. The culture medium was refreshed every 2 to 3 days.

2.2. Transfections

Luteal cells at passage 2 were plated into six-well dishes and cultured in culture medium without antibiotics. When cells reached 90% confluence, the media was removed and cells were washed twice with serum-free DMEM/F12. Then, cells were transfected with pCI-neo-hTERT by Lipofectamine 2000 (Invitrogen TM, life technologies, USA) according to the manufacturer's instructions. After 24 h, transfected cells were selected by 450 μg/mL G418 (Sigma) in complete culture medium. The pCI-neo-hTERT was kindly provided by Y.P. Jin (College of Veterinary Medicine, Northwest A and F University, China). Approximately 2 to 3 wk later, drug resistant colonies were isolated with cloning rings. Three positive colonies were obtained and continually cultured in complete medium with G418 (250 μg/mL). Then, one of three clones was expanded by further culture and tested in the following studies (named hTERT-caprine luteal cells (CLCs)). The other two clones were frozen for long-term storage [24].

The transduced CLCs at Passage 30 were purified with a magnetic cell separation kit, according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). First, cells were trypsinized, centrifuged (200×g at 10 min) and washed twice with PBS. The pellet was resuspended and incubated with anti-LH receptor IgG (Rabbit IgG, Sigma) diluted one: 250 in 500 μL PBS (containing 1% bovine serum albumin, BSA; Sigma) for 15 min at 4 °C and gently mixed every 5 min. Then, cells were washed three times with PBS (containing 1% BSA) and incubated with goat anti-rabbit IgG microbeads (Miltenyi Biotec) diluted 1: 6 in 100 μL PBE [PBS containing 0.5% BSA, 2 mM EDTA] for 15 min at 4 °C and gently mixed every 5 min. Afterward, cells were washed