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The effects of cell death-inducing DNA fragmentation factor-α-like effector C on milk lipid synthesis in mammary glands of dairy cows

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ABSTRACT

Adequate lipid synthesis by the mammary gland during lactation is essential for the survival of mammalian offspring. Cell death-inducing DNA fragmentation factor-α-like effector C (CIDEC) is a lipid droplet-associated protein and functions to promote lipid accumulation and inhibit lipolysis in mice and human adipocytes. However, the function of CIDEC in regulation of milk lipid synthesis in dairy cow mammary gland remains largely unknown. In this study, 6 multiparous Holstein cows (parity = 3) in early lactation were allocated to high-fat milk (milk yield 33.9 ± 2.1 kg/d, milk fat >3.5%, n = 3) and low-fat milk (milk yield 33.7 ± 0.5 kg/d, milk fat <3.5%, n = 3) groups according to their milk fat content. Lactating cows were slaughtered at 90 d in milk and mammary tissues were collected to detect CIDEC localization. Immunofluorescence staining of sections of lactating mammary glands with high- and low-fat milk showed that CIDEC was expressed in the cytoplasm of epithelial cells and localized to lipid droplets. Lipid droplets and CIDEC protein were also detected in isolated lactating mammary epithelial cells of dairy cows. Immunostaining of CIDEC in isolated mammary epithelial cells also confirmed its presence in the nucleus. The knockdown of *CIDEC* in cultured bovine mammary epithelial cells decreased milk lipid content and reduced expression of genes associated with mammary de novo fatty acid synthesis, short- and long-chain intracellular fatty acid activation, triacylglycerol synthesis, and transcription regulation. These genes included those for acetyl-CoA carboxylase (ACC, −60%), fatty acid synthase (FASN, −65%), acyl-CoA synthetase short-chain family member 2 (ACSS2, −50%), acyl-CoA synthetase long-chain family member 1 (ACSL1, −30%), diacylglycerol acyltransferase 1 (DGAT1, −60%), sterol regulatory element-binding protein 1 (SREBP1, −45%), and SREBP cleavage activating protein (SCAP, −66%). Conversely, in cells overexpressing *CIDEC*, triacylglycerol content was increased, and transcription of those genes involved in milk lipid synthesis was coordinately upregulated. These results suggest that CIDEC plays an important role in regulating milk lipid synthesis in dairy cow mammary gland via a mechanism involving gene expression, which provides further insight into the mechanisms regulating mammary lipogenesis in ruminants.
Key words: dairy cow, mammary gland, milk lipid, cell death-inducing DNA fragmentation factor-α-like effector C

INTRODUCTION

Lipids, primarily triacylglycerols (**TAG**), are major milk constituents of most mammals, providing a large percentage of the calories, essential fatty acids, and bioactive lipids required for neonatal growth and development (McManaman, 2009). Lactating mammary gland synthesizes large amounts of TAG from fatty acids (**FA**) derived from the blood and from de novo lipogenesis (Emery, 1973). In cows, about half of the milk FA are derived from de novo synthesis (Bauman and Griinari, 2003).

Milk lipid synthesis is a complicated process in vivo and is highly regulated by gene expression. Cell death-inducing DNA fragmentation factor-α-like effector C (**CIDEC**) is a lipid droplet-associated protein (Tan et al., 2014). It is highly expressed in both white and brown adipose tissues and is strikingly upregulated during adipogenesis in mice (Matsusue et al., 2008; Nishino et al., 2008; Toh et al., 2008). Cell death-inducing DNA fragmentation factor-α-like effector C has the ability to enhance neutral lipid accumulation not only in adipocytes but also in many nonlipogenic cells (Puri et al., 2007). In mice, *CIDEC* overexpression leads to promotion of the formation of intracellular lipid droplets through TAG accumulation in hepatocytes in vitro

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and in vivo, whereas knockdown of *CIDEC* results in loss of hepatic lipids (Matsusue et al., 2008). In 3T3-L1 adipocytes, *CIDEC* depletion significantly stimulates lipolysis and reduces the size of lipid droplets (Keller et al., 2008). The mammary gland is one of the major lipid-synthesizing organs in dairy cows. However, little is known about the effect of CIDEC on milk lipid synthesis in the mammary gland of dairy cows.

Increased milking frequency in dairy cows results in greater milk production (Hillerton et al., 1990). Microarray analysis of the bovine mammary gland has shown that *CIDEC* expression is upregulated by increased milking frequency (Connor et al., 2008). This result suggests that CIDEC may participate in milk lipid synthesis. In mice and human adipocytes, CIDEC localizes to lipid droplets (Puri et al., 2007). However, no study directly investigating CIDEC expression in mammary gland of lactating cows has been reported. Peroxisome proliferator-activated receptor-γ (**PPARγ**) belongs to the nuclear receptor superfamily and is expressed in numerous types of cells, including adipocytes, macrophages, and mammary epithelial cells (Kang et al., 2015). In bovine and dairy goat mammary glands, PPARγ is considered to be a central regulator of lipid metabolism (Bionaz and Loor, 2008b; Kang et al., 2015). Gene network analysis in bovine mammary tissue shows that expression of PPARγ and its putative target genes is upregulated during lactation, suggesting a role for this nuclear receptor in the regulation of milk lipid synthesis (Bionaz and Loor, 2008b). The *CIDEC* has been identified as a direct target gene of PPARγ, and its upregulation can elevate TAG levels in mouse hepatocyte (Matsusue et al., 2008). Whether CIDEC participates in regulating milk lipid synthesis in lactating dairy cow mammary gland has not been well established.

The objective of this study was to reveal the specific role of CIDEC in regulating milk lipid synthesis in the mammary gland of dairy cows. To meet this objective, 6 lactating Holstein cows in the third parity were allocated to high-fat milk (milk yield 33.9 ± 2.1 kg/d, milk fat >3.5%, n = 3) and low-fat milk (milk yield 33.7 ± 0.5 kg/d, milk fat <3.5%, n = 3) groups. Lactating cows were slaughtered at 90 DIM and mammary tissues were collected to detect CIDEC expression. To evaluate the role of CIDEC in mediating milk lipid synthesis in the dairy cow mammary gland, mammary epithelial cells were isolated from lactating mammary tissues of dairy cows with high-fat milk. The expression of genes encoding enzymes and transcription factors involved in milk lipid synthesis were examined in cultured mammary epithelial cells by quantitative real-time PCR (**qPCR**) when *CIDEC* was knocked down or overexpressed.

MATERIALS AND METHODS

Animals and Tissue Collection

The Northeast Agricultural University Animal Care and Use Committee (Harbin, China) approved all procedures involving dairy cows. Six multiparous Holstein cows (in the third parity, calving at 52 to 54 mo of age) in early lactation were used in this study. Amplification of the polymorphism *DGAT1* K232A by tetra-primer amplification refractory mutation system PCR (Steinberg et al., 2009) showed that all cows carried the AA genotype of *DGAT1* (Supplemental Figure S1; https://doi.org/10.3168/jds.2016-11549). All animals were offered grass silage ad libitum supplemented with concentrates. Animals had access to a constant supply of fresh water and were milked twice daily at 0800 and 1530 h.

Lactating cows were allocated to 2 groups (3 cows per group), the high-fat milk (milk fat >3.5%) and low-fat milk (milk fat <3.5%, milk fat decreased likely due to diet-induced milk fat depression) groups, according to their milk fat content (Table 1). Milk yield of lactating cows with high-fat milk was 33.9 ± 2.1 kg/d. Milk yield of lactating cows with low-fat milk was 33.7 ± 0.5 kg/d. Somatic cell count was <50,000 cells/mL for all cows. The 6 lactating cows were slaughtered at 90 DIM. Immediately after exsanguination, several parenchyma samples were aseptically removed from the core of the mammary gland. Tissue samples for quantitation of protein and RNA were frozen immediately in liquid nitrogen and stored at −80°C until assay. Mammary tissues for cell culture were collected in 50-mL conical centrifuge tubes with Hank's Balanced Salt Solution (14170, Life Technologies Corporation, Grand Island, NY), transported, and preserved at 4°C until cell isolation and culture.

Table 1. Milk components of lactating Holstein dairy cows

Milk component	Dairy cows with high-fat milk	Dairy cows with low-fat milk
Milk protein (%)	3.27 ± 0.04	2.89 ± 0.02
Milk fat (%)	4.17 ± 0.01	3.20 ± 0.06
Lactose (%)	4.84 ± 0.03	4.52 ± 0.09
DM (%)	11.93 ± 0.01	10.89 ± 0.03