博 士 論 文 の 内 容 の 要 旨

氏名	Sharmin Mst Mamuna
学位名	博士（医工学）
学位授与年月日	2021 年 9 月 30 日
論文題目	Study on the intracellular mechanism related to milk yield of mammary epithelial cells （乳腺上皮細胞の乳量と関連する細胞内機構に関する研究）

（博士論文の内容の要旨）

Modern dairy cows are not well adapted with this higher amount of milk production. The cow starts to synthesis higher amount of milk after parturition, but suffers from physiological stresses due to negative energy balance (NEB). NEB is one of the major reasons for reduction of milk yield as well as lactation persistency during lactation. Therefore, establishment of moderated type of lactation persistency is necessary for sustainable milk production. Knowledge related to mammary gland physiology will be beneficial in this regard. The intracellular mechanisms for increase and decrease of milk yield during early lactation remains unknown. Milk yield is determined by the secretory activity and the number of mammary epithelial cells (MECs). Previous studies suggested that unfolded protein response (UPR) factor X-box binding protein 1 (XBP1) is involved in the synthesis of milk protein and that C/EBP homologous protein (CHOP) expression is negatively correlated with milk yield. However, the detailed mechanisms related to secretory activity and the loss of MECs under the context of UPR needs to be investigated. Therefore, the purpose of the study was to discover the intracellular mechanisms of enhancing the secretory activity and the loss of MECs in bovine MECs.

Firstly, I focused on endoplasmic reticulum (ER) biogenesis to search the mechanism behind the increase of milk production in early lactation. ER biogenesis in terms of ER-membrane bound phospholipid synthesis by associated genes is indispensable for the well-developed and abundant ER. Insulin like-growth factor-1 (IGF1) is the possible candidate for that, since its serum concentration increases in early lactation and is responsible for enhancing the protein synthesis of MECs. Therefore, I investigated whether XBP1s affects the ER biogenesis of bovine MECs and IGF-1 involves in this process or not. MAC-T (a cell line of bovine MECs) cells were treated with IGF-1, and the results showed that IGF-1 enhanced the expressions of *XBP1s* and ER biogenesis-associated genes, ratio of pIRE1α/IRE1α protein, staining of ER tracker dye. 4μ8C, potent inhibitor of IRE1 endonuclease activity, significantly inhibited the IGF-1-induced *XBP1s,* ER biogenesis-associated genes expression and the staining of ER tracker dye. Moreover, IGF-1-induced *XBP1s* and ER biogenesis related gene expressions were significantly reduced by rapamycin (small molecule inhibitor of mTORC1) treatment. Based on *in vitro* study, mammary gland biopsy samples of periparturient dairy cows were analyzed to know the status of ER biogenesis related genes expression under the natural physiological condition. It was found that the relatively low level of *XBP1s* expression in mammary gland tissue before parturition increased gradually from immediately after parturition through lactation. ER biogenesis-related genes and *IGF1R* expressions were also increased in mammary gland tissue during lactation. Therefore, IGF-1 increases *XBP1s* expression to induce ER biogenesis in bovine MECs to enhance the milk production. Thus, IGF-1 may play crucial role for increasing the milk yield.

Previous study found that the expression levels of *CHOP* is negatively correlated with milk yield and induces severe ER stress in MEC, but the triggering factors for *CHOP*-induced apoptosis was unknown. Fatty acids (FA) are the components of non-esterified fatty acids (NEFA), and the serum NEFA concentration also becomes higher during early lactation period in NEB condition. Therefore, the objective was to clarify the impact of FA on inducing ER stress in MECs. MAC-T cells were stimulated with various types of FA to measure the ER stress induced UPR-related genes expression and the viability of bovine MECs. Result showed that, palmitic (PA) and stearic acids induced severe ER stress and apoptosis with enhancing the CHOP expression at both mRNA and protein level. Unsaturated long-chain FA did not induce CHOP expression to reduce the cell viability, marking those FA as moderate ER stress inducer.

Therefore, saturated fatty acids play important roles in MEC viability by inducing severe ER stress. Thus, the reduction of number of MECs by saturated FA can decreases the milk yield and reduces the lactation persistency.

Based on the detrimental effect of PA, prevention was necessary to conserve number of MECs for proper milk yield. 5-Aminolevulinic acid (5-ALA) acts as antioxidant, was speculated to ameliorate the PA-induced cell death due to its anti-apoptotic properties. Therefore, I examined the efficiency of 5-ALA in inhibiting ER stress-induced apoptosis of MECs in response to PA. Result showed that pretreatment of 5-ALA reduced *CHOP* expression to decrease the PA-induced apoptosis of MECs. The apoptosis-related genes *BCL2* and *BAX* were increased and decreased respectively. Moreover, 5-ALA pretreatment elevated the expression of antioxidant gene, heme oxygenase-1. Therefore, 5-ALA has the potential to ameliorate PA-induced severe ER stress in MECs. It can be used to prevent the PA mediated severe ER stress induced cell loss to increase milk yield.

In summary, present study indicates that UPR has the significant role for increasing the ER biogenesis in terms of secretory activity and decreasing the number of MECs. IGF-1 enhances ER biogenesis via IRE-1-XBP1 axis, while excessive amount of saturated FA increases the apoptosis of MECs through PERK-ATF4-CHOP pathway. In addition, 5-ALA down regulated PA-induced *CHOP* expression to decline the bovine MECs death. These findings will be beneficial to understand the regulation of increase and decrease of milk yield at cellular level during early stage of lactation and thus will be helpful to establish moderate type of lactation persistency for sustainable milk yield.