Understanding Epigenetic Mechanisms of Lactation Failure

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Introduction
Breastfeeding protects infants against the onset of childhood obesity and reduces the risk for type 2 diabetes later in life. In mothers, exclusive lactation for the first six months is associated with lower weight retention, weight issues 15 years later, and incidence of type 2 diabetes. Despite the importance of breastfeeding to the mother and infant, the mechanisms that control the initiation of lactation are not well understood. For example, obese mothers have inadequate breast milk production. This problem is directly related to the hormone prolactin, which normally stimulates milk production by breast cells. But we do not currently understand exactly how prolactin initiates milk production in breast cells.

Human peptidylarginine deiminase (PAD) enzymes are highly expressed in the lactating breast cells. PADs regulate the structure and function of other proteins through a reaction termed citrullination. For example, PAD enzymes can turn the expression of genes on by modifying histones, which organize DNA. Understanding how PADs regulate the expression of genes in the breast cells may allow us to target these enzymes to increase breast milk production in obese women.

Objectives
The goal of this study is to determine how prolactin regulates PAD expression and if mammary proteins are citrullinated during lactation.

Materials and Methods
Experiments are being conducted in the University of Wyoming’s Biological Sciences Building. These studies utilize a mouse mammary epithelial cell line termed CID-9 cells. Studies also use mouse mammary glands collected on lactation days two and nine. All animals are housed and cared for following approved guidelines by the UW Institutional Animal Care and Use Committee.

Results and Discussion
Prolactin is critical to initiate lactation. It binds the prolactin receptor on breast cells, which stimulates the expression of lactation-related genes to produce breast milk. If prolactin initiates epigenetic mechanisms (i.e., changes in gene expression that do not involve changes in the underlying gene DNA sequence) to control milk production is unclear. To investigate this question, we used CID-9 cells and lactating mice. First, CID-9 cells treated with prolactin for 48 hours show an increase in PAD3 expression; however, this response is blocked in the presence of an inhibitor (SD-1029) (Figure 1). Next, we examined if citrullinated proteins are present in lactating mouse mammary glands. On lactation days two (L2) and nine (L9), we collected mammary glands and examined the levels of citrullinated proteins. Our results show that multiple proteins are citrullinated including histones suggesting that PADs regulate the expression of genes (Figure 2).

Our upcoming studies will use DNA sequencing to determine which genes are regulated by PAD enzymes in the lactating mammary gland. Our overall goal is to determine how prolactin acts through PADs to regulate lactation. We believe that our results will increase understanding of the molecular mechanisms controlling lactation and provide novel treatments for obese women with milk production problems.

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Figure 1. Prolactin induces PAD3 protein expression. (DMSO is short for dimethyl sulfoxide.)

Figure 2. Multiple proteins are citrullinated in the lactating mammary gland including histones. (A) Citrullinated proteins are present in L2 and L9 mammary glands. (B) L2 and 9 lysates contain citrullinated histones.