PHYSIOLOGY OF LACTATION: OLD QUESTIONS, NEW APPROACHES

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Introduction

Questions regarding the mechanisms of growth, differentiation and function of the mammary gland have been raised a long time ago. Progress in endocrinology, biochemistry and morphology have contributed to improve knowledge of mammary gland physiology [1]. The rapidly expanding tools of biotechnologies and the systematic identification of the genes being transcribed in mammary tissues will now facilitate a genomic approach. While more and more genes responsible for mammary differentiation and function are identified, functional analysis of the complex networks leading to the integrated response of the mammary cell to hormones is now made possible.

Hormonal control of development, differentiation and function

The different phases of organisation of the mammary gland along the life of the female and during the reproductive period are set in motion by hormonal levels. Oestrogens and progesterone, with glucocorticoids and growth hormone and locally acting growth factors are the essential hormonal factors regulating the elongation and bifurcation of the primary ducts and the side branching and alveolar bud development during the oestrus cycles. The lobulo alveolar development during pregnancy is under the control of oestrogen, progesterone, placenta lactogen (PL) and/or prolactin (PRL). PRL and oxytocin are essential hormones for maintenance of a full milk secretion during lactation.

One of the major advances in the study of endocrine regulation of the mammary gland has been the development of techniques to obtain defined mutations in mice [2]. It is also possible to generate chimeric glands composed of tissues from knockout and wild-type animals. The use of mice deficient in hormones, growth factors, receptors or transcription factors now allow to dissect hormonal pathways. Exploiting these knockout animals has greatly aided to elucidate the specific role of the epithelium and the stroma. Thus, the use of progesterone receptor knockout mice in combination with mammary gland transplantation techniques has provided strong support for understanding of the functional involvement of epithelial rather than stromal progesterone receptors in mediating morphogenic response to progesterone [3]. The use of mice deficient in prolactin (PRL) and growth hormone (GH) receptors associated with surgical techniques such as transplantation of epithelial cells into cleared fat pads of recipient mice has permitted to show that stromal GHR but not epithelial GHR is required for functional mammary development. In contrast epithelial PRLR is required for development and milk protein gene expression during pregnancy [4].

Gene expression profiling and mammary function

Growth differentiation and function of the mammary tissue are set in motion by hormonal levels. Each phase corresponds to a patterned set of responses corresponding to changes in gene expression. To answer to the old questions raised by physiologists, the study of temporal changes of global gene expression at each physiological stage is now possible by utilizing microarrays to profile pattern of expression of genes. This approach has been successful in demonstrating the huge number of genes that are differentially expressed in the murine and bovine mammary epithelial cells during pregnancy, lactation and involution [5,6,7]. These approaches, in addition to providing confirmation of previous findings related to changes in gene expression have provided an intricate view of the signalling pathways involved during the different physiological stages.

Hormonal regulation and cellular physiology

Expression profiling provides a tool to identify the genes transcribed in the mammary tissue. However, to have a good grasp of the control of cell functioning, regarding the differentiation and the secretory function of mammary tissue, the temporal and spatial assignment of gene function has now to be considered.

The field of proteomics driven by technological advances such as two-dimensional (2D) gel system and mass spectrometry is highly productive. Separated proteins from subcellular fractionation such as Golgi complex and milk fat globule membranes have been compared between pregnant and lactating animal [8,9].

Although these new technologies, notably microarray technology and proteomic analysis, will continue to provide systematic discovery tools, they cannot explain the mechanisms of coordinated links between gene expression and final secretory function of this tissue. Cellular biology approaches are necessary to describe the post-translational events occurring in the cell and leading to changes in protein function. Regulatory function of prolactin is an example of these complex cellular events.

In the mammary gland, in addition to playing a central role in development and differentiation, PRL is also an important modulator of gene expression and of metabolic processes occurring in milk secretion. One intriguing question is how these numerous functional specificities are regulated at the molecular level.

The 23-kDa PRL binds to its basal membrane receptor and is internalised in lactating mammary epithelial cells then carried to the lumen [10]. By following intracellular transport of labelled PRL in the different intracellular organelles involved in cellular transport it was possible to show that the integrity of Golgi region is required for PRL transcytosis. By analysing the state of activation of the different signals, involved in the different effects of PRL, in lactating mammary cells it was possible to discriminate between prolactin-induced actions that are dependent or independent on the integrity of the Golgi apparatus [11]. These results show that the signalling to gene expression does not require the transcytosis of PRL whereas signalling to the milk protein secretion requires it. This example, showing that multiple cell sites of signal transduction after PRL binding to its receptor may exist, underlines the importance of considering the spatio-temporal aspects to identify cell compartments that receive and execute these signals.

Conclusion

For answering to questions regarding the roles of hormones on growth, development and differentiation and on the mechanism of secretory functioning, the arrival of the global analysis such as gene expression profiling and proteomics, provides a systematic number of data. These non-hypothesis driven technologies may now be used to detect specific protein-based regulatory events of physiological relevance.

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