Commentary

Evolving views of involution

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Abstract

The developmental preparation of the mammary gland for milk production that occurs during pregnancy is followed by an equally dramatic process of involution as the gland returns to its prepregnancy state. Two detailed, microarray-based surveys reported in this issue extend our understanding of the nature and timing of molecular and cellular events in involution that underlie these developmental changes.

Keywords: development, involution, mammary gland, microarray, mouse

Introduction

The mammary gland provides a unique window into fundamental developmental processes utilized by higher organisms. In preparing to perform its critical role in milk synthesis, the mammary gland must implement a coordinated program involving proliferation and differentiation of the secretory epithelium, utilization of fat stores within the surrounding adipose tissue, and production of milk proteins. The results of this postnatal developmental process provide critical support for neonatal mammals, and maternal investment in this process is critical for the survival of the species. Once the young have been weaned, however, it is no longer necessary to expend such extensive resources on milk production. In a remarkable reversal of the developmental transition that occurs during pregnancy, the mammary gland undergoes an equally coordinated process of involution that returns it to a state superficially resembling that of the prepregnant gland.

Two papers in this issue of *Breast Cancer Research* [1,2] provide new insights into the cellular and molecular details of postlactational involution within the murine mammary gland. Both groups utilized the ability of DNA microarray technology to measure transcript abundance simultaneously from a substantial fraction of the genome. Previously reported large-scale gene expression surveys of mammary development spanning puberty, pregnancy, lactation, and involution have been mined to identify not only individual transcripts of known significance but also coordinately regulated groups of genes from which functional pathways of importance in mammary gland physiology could be inferred [3–5] (for a more detailed review of previous microarray studies of murine mammary gland development, see that by Master and Chodosh [6]). These new contributions from the laboratories of Gusterson [1] and Watson [2] both survey a greater number of genes during mammary development and provide significantly greater temporal resolution during involution than previous microarray studies. In addition to providing satisfying confirmation of previous findings related to changes in the physiology and cellular composition of the mammary gland during involution, these studies provide a more intricate view of the molecular details by which this remodeling takes place.

Gene expression patterns during involution

Broadly speaking, mammary involution is known to proceed in at least two stages, within which distinct, but temporally overlapping, morphogenetic programs can be identified [7,8]. The first involves the widespread apoptosis of alveolar epithelial cells, thereby reversing the dramatic expansion in this cellular compartment that occurs as a consequence of pregnancy-induced lobuloalveolar development. The second stage involves removal of the

resulting apoptotic debris as well as remodeling of the extracellular matrix. Additionally, adipocytes regain their substantial lipid stores such that the former morphologic state of the gland is approximated.

Consistent with this biphasic model, a variety of gene expression patterns during involution that reflect these distinct morphogenetic stages have been described. Strange and coworkers [7] provided the first detailed description of gene expression changes in the involuting mammary gland, including the induction of genes implicated in stress response, tissue remodeling, and apoptotic cell death. Subsequent microarray studies performed nearly a decade later identified discrete clusters of genes that are upregulated exclusively at day 2 or day 7 of involution, as well as clusters of genes that are upregulated at both stages [4]. The former group included several genes that had previously been associated with apoptosis during early involution, such as *Clu*, *Stat3*, *Igfbp5*, and *Cebpd* [7,9,10]. The latter group included gene expression clusters that correspond to the degradation and remodeling of the extracellular matrix that occurs during the second phase of mammary gland involution. These consisted of changes in the expression of several matrix metalloproteinases with previously described roles in involution, as well as lysosomal hydrolases [4,8]. Microarray approaches also revealed marked differences in the expression of specific cathepsin isoforms between day 2 and day 7 of involution [4]. Finally, using nondirected computational approaches to identify associations between clustered developmental expression profiles and prospectively acquired functional gene annotation, Master and coworkers [4] previously demonstrated statistically significant overlap between genes upregulated during early involution and thiol proteases, zymogens, hydrolases, lysosomal enzymes, major histocompatibility complex components, and genes within the complement pathway.

Despite these early insights, prior forays into gene expression profiling of this important developmental transition generally lacked the temporal resolution required to extend the undoubtedly primitive two-stage model of mammary involution. The groups of Watson [2] and Gusterson [1] now elegantly remedy this situation by exploring in detail the temporal complexities of gene expression and functional pathways that may be activated during this period of profound morphologic change. Although the broad outlines of the classic two-stage model are supported by these new contributions, their remarkable level of detail reveals a more intricate regulation of cellular and molecular processes during involution. For example, Clarkson and coworkers [2] note a general trend in gene expression by which death receptor pathway components are upregulated at the earliest time points studied, whereas proapoptotic and antiapoptotic components that influence the intrinsic mitochondrial contribution to apoptosis (such as *Bax*, *Mcl1*, *Bcl2l*, and *Apaf1*) are more highly expressed later in involution. This suggests, at the very least, that transcriptional programs influencing the early apoptotic stage of alveolar regression are not all activated simultaneously. Similarly, both groups describe the expression of genes indicative of an acute phase response; moreover, Clarkson and coworkers go on to distinguish between an early class II acute phase response and a later class I response that correlates with *Stat3*, *Cebpb*, and *Cebpd* expression.

Although the detailed significance of these findings is as yet unclear, they nevertheless suggest that each of the broad stages of involution may ultimately be divided into a number of discrete substages. Experimental manipulation and analysis of these substages will probably yield further insights into critical regulatory watersheds that exist in the global program of involution as the gland reaches an irreversible 'point of no return'.

Extending the biphasic model

More evidence for the complexity of involution is provided by Clarkson and colleagues [2] through direct clustering of gene expression profiles. They note three broad patterns of change in gene expression during this time: a rapid response within 12 hours after weaning, a moderately delayed response at around 24 hours, and a slow increase or decrease in gene expression over 3–4 days. Interestingly, these patterns were observed both for increases and decreases in gene expression. These data demonstrate that, viewed from the perspective of gene expression, one could reasonably argue for a conceptual expansion from two to three phases of involution. Additional intricacies were noted in the long-term kinetics of gene expression; for example, some genes in the rapidly responding (12-hour) group exhibited only transient changes in gene expression, whereas a smaller but significant group exhibited altered expression levels through 96 hours. The authors then tested for statistically significant associations between these patterns of gene expression and prospective annotation derived from the Gene Ontology consortium [11]. The 'intracellular signal' category, for example, was only significantly associated with expression profiles that showed a rapid, transient change in early involution. These types of analyses provide additional support for the notion that the observed gene expression profiles represent distinct cellular and metabolic processes. Comparison with gene expression data reported by Stein and coworkers [1] suggests that examining the longer term expression kinetics during involution may further expand the classification of these profiles.

Immune cell recruitment in the mammary gland

Analysis of these new gene expression data sets has confirmed the striking contribution of various immune cells to

gene expression profiles during involution. Both groups noted the expression of cytokines that may play a role in recruiting immune cells to the gland. Both groups also describe the presence of a substantial macrophage population in later involution, as was previously inferred from microarray studies [4] and demonstrated by direct observation [12]. These cells are thought to play an important role in the elimination of apoptotic debris [12]. However, current reports suggest that the immunologic component of the involuting gland is substantially more diverse than debris-clearing monocytes or macrophages. For example, Stein and coworkers [1] noted a marked increase in the number of plasma cells between days 2 and 4 of involution, which is consistent with a previous description by D'Cruz and colleages [5] of increased κ light chain and immunoglobulin heavy chain expressing cells at day 2 after weaning in FVB mice. The large increase in immunoglobulin gene expression, which was noted by both groups, was correlated by Stein and coworkers with direct, morphologic observations of plasma cells migrating from the lymph node. Interestingly, although the presence of these invading cells during involution might be presumed to be transient, infiltrating immunoglobulin-producing cells have been shown to be a persistent feature of the parous, involuted gland that may be related to the well known protective relationship between parity and breast cancer risk [5,13,14]. The cytokines identified by Stein and Clarkson and coworkers may now shed further light on the basis for this permanent change.

One discrepancy between this pair of studies relates to the role of neutrophils in early involution. Both groups found increased expression of *Cxcl1*, which encodes a neutrophil attractant chemokine [15], early in involution. Consistent with a functional role for this chemokine, Stein and coworkers [1] noted a corresponding increase in *LRG* (leucine-rich α_2 glycoprotein) expression, presumably indicating the presence of a neutrophil population [16]. This observation was directly confirmed at the histologic level, although the peak number of morphologically identified neutrophils appeared in the gland at a time when *LRG* expression was already decreasing. Thus, Stein and colleagues make a strong case for a neutrophilic component in the immune response of early involution, which is consistent with observations in a variety of other species [17]. In contrast, despite confirming the early expression of *Cxcl1*, Clarkson and coworkers [2] did not observe increases in any of a variety of neutrophil-specific transcripts. As such, their array results suggest an alternative model in which the marked involutional upregulation of uterocalin – a known inducer of neutrophil apoptosis – suppresses an increase in acute inflammatory cells despite *Cxcl1* signaling [18].

Several explanations may account for this difference. First, these results may simply reflect a strain-specific difference between Balb/c (as studied by Stein and coworkers [1]) and C57Bl/6 (as studied by Clarkson and coworkers [2]) mice. In this case, the data from Clarkson and colleagues would demonstrate that a neutrophilic infiltrate is not functionally required for involution to proceed. However, because Clarkson and colleagues base their interpretation on the lack of an increase in neutrophil-specific gene expression, it remains a formal, if unlikely, possibility that neutrophils are indeed recruited to the gland but exhibit atypical transcriptional profiles. The issue of neutrophil flux during involution will most easily be settled by quantitative, histologic examination of other mouse strains in a manner similar to that performed by Stein and colleagues. Nevertheless, these studies highlight an important caveat for interpreting microarray studies of complex tissues. That is, putative cell-specific markers – particularly those derived from reports in the literature in other tissues or contexts – need not always behave as expected. This point is made most forcefully the observation by Stein and colleagues that CD14 – a 'monocyte-specific' marker – is actually expressed by the mammary epithelium. As such, these papers highlight the critical importance of correlating spatial and morphologic information with gene expression data derived from homogenized complex tissues.

Conclusion

The enormous potential for microarray studies to generate biologically relevant, mechanistic hypotheses of development is beautifully confirmed by studies such as these [1,2]. For example, manipulation of the chemokine signaling pathways highlighted in these studies should allow a direct assessment of their role in normal involution. A better understanding of such details may help us to unravel not only involution but also the behavior of mammary epithelial cells during carcinogenesis. In addition to the contribution that these studies make to our understanding of the complexities of mammary gland involution, the availability of these and previous data sets for use by the broader scientific community will undoubtedly facilitate further insights and future work. In this regard, both groups are to be commended for their efforts toward placing these data sets in the public domain. Indeed, it is the proliferation of data sets and analyses such as these that will ultimately provide a truly molecular understanding of the evolving complexities of mammary gland development.

Competing interests

None declared.

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