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Epigenetic and Transcriptional Regulators of Mammary Gland Involution: A Multi-Omics Perspective

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Abstract

Mammary gland involution is a highly coordinated, reversible-to-irreversible process that transitions the lactating gland back to a non-lactating state. This regression involves cell death, immune activation, extracellular matrix (ECM) remodeling, and stromal reorganization. A growing body of evidence demonstrates that epigenetic modifications, transcriptional networks, and chromatin-restructuring events govern the pace and architecture of involution. Using a multi-omics lens—integrating transcriptomics, epigenomics, proteomics, and single-cell sequencing—this paper reviews core regulators of involution, including STAT3, NF-κB, C/EBP family members, HDACs, DNMTs, histone methyltransferases, and long non-coding RNAs (lncRNAs). We highlight the temporal two-phase model of involution, define epigenetic signatures of epithelial cell fate, and explore links between aberrant involution and postpartum breast cancer risk. Additionally, we propose an integrated systems biology roadmap for future translational research.

Keywords: Mammary Gland Involution, Epigenetic Programming, Transcriptional Regulation, Multi-Omics Integration, Apoptosis and Tissue Remodeling.

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Research Highlights

Reveals a multi-omics regulatory architecture integrating transcriptomics, epigenomics, proteomics, and metabolomics to map molecular events during mammary gland involution.

Identifies STAT3 as the central transcriptional driver orchestrating epithelial apoptosis, lysosomal-mediated cell death, and immune recruitment during early involution.

Highlights dynamic epigenetic remodeling, including DNA methylation shifts, histone mark redistribution (H3K27ac loss, H3K9me3/H3K27me3 gain), and enhancer accessibility changes that regulate involution-specific gene networks.

Demonstrates non-coding RNAs as critical regulators, with miRNAs and lncRNAs modulating STAT3, cell death pathways, and ECM remodeling signals.

Shows how NF-κB, C/EBP, and hormone-responsive transcription factors synergize with chromatin modifiers to shape the two-phase reversible-to-irreversible involution model.

Provides new insights into ECM degradation and immune cell infiltration, driven by coordinated transcriptional activation of MMPs and cytokine pathways.

Links aberrant involution signaling to postpartum breast cancer, highlighting inflammation-associated epigenetic scars and tumor-promoting microenvironmental changes.

Proposes a systems biology framework for integrating spatial transcriptomics, ATAC-seq, and single-cell data to advance future

mechanistic research.

Introduction

Mammary gland involution is a dynamic post-lactational process that requires precise regulation of epithelial apoptosis, immune cell recruitment, extracellular matrix (ECM) degradation, and tissue remodeling. Historically viewed as a passive regression process, involution is now recognized as an active, transcriptionally driven, and epigenetically modulated biological phenomenon.

Emerging omics tools—particularly ATAC-seq, ChIP-seq, scRNA-seq, and spatial transcriptomics—have revolutionized the understanding of the cellular and molecular events underlying involution. These approaches unravel how hormonal withdrawal, cytokine surges, and epigenetic rewiring orchestrate epithelial fate decisions.

This paper synthesizes insights from multi-omics analyses to delineate key transcription factors, chromatin modifiers, and regulatory RNAs that shape mammary involution.

The transition from a secretory, milk-producing organ back to a non-pregnant state is one of the most dramatic examples of physiological tissue remodeling in the adult mammal. This process, known as **mammary gland involution**, involves a highly orchestrated cascade of programmed cell death (apoptosis), immune cell infiltration, and stromal adipogenesis.

While the hormonal triggers—primarily the withdrawal of lactogenic hormones like prolactin—are well-

documented, the underlying molecular "control room" is far more complex. Modern biological inquiry has shifted from looking at single genes to a **multi-omics perspective**, recognizing that involution is governed by a tight interplay between the physical structure of DNA and the actual readout of the genetic code.

The Dual Layers of Control

To understand how the mammary gland "switches off" milk production and "switches on" tissue clearance, we must look at two distinct but intertwined regulatory layers:

Transcriptional Regulation: This involves the immediate recruitment of transcription factors (such as **STAT3**, the "master regulator" of involution) to specific gene promoters. These factors act as the gas and brake pedals for genes involved in cell death and tissue inflammation.

Epigenetic Regulation: This is the "infrastructure" of the genome. Through DNA methylation and histone modifications, the cell can lock or unlock entire regions of chromatin, making them accessible or inaccessible to the transcriptional machinery.

Why a Multi-Omics Approach?

Historically, researchers focused solely on the **transcriptome** (the RNA levels). However, looking at RNA alone is like reading a script without knowing if the stage was set or the actors were allowed in the building. By integrating various "omics," we get a 360-degree view:

Epigenomics (ATAC-seq/ChIP-seq): Identifies which areas of the genome are physically "open" for business.

Transcriptomics (RNA-seq): Measures which genes are actually being expressed in real-time.

Proteomics: Confirms if those messages are being translated into functional proteins that drive the remodeling process.

Key Concept: Mammary gland involution is not merely a "dying off" of cells; it is an active, genetically programmed re-engineering of the tissue environment that mirrors many pathways found in wound healing and, significantly, breast cancer progression.

This review explores how the epigenetic landscape is reshaped during the transition from lactation to involution. We will examine how specific chromatin modifiers and transcription factor networks collaborate to ensure that the mammary gland resets itself efficiently, while also discussing how "mistakes" in this regulatory crosstalk can create a pro-tumorigenic environment.

Review of literature

The mammary gland is one of the few organs that undergoes repeated cycles of large-scale tissue remodeling, functional differentiation, and programmed cell death throughout adult life. Involution, the process by which the lactating gland returns to its pre-pregnancy state, is a highly orchestrated transition characterized

by the massive apoptosis of secretory epithelial cells and extensive extracellular matrix remodeling (Ivanova et al., 2021; Xuan et al., 2022). Recent multi-omics approaches have begun to unravel the complex interplay between epigenetic modifications and transcriptional networks that govern this physiological "reset."

Epigenetic Landscapes: From Poised to Repressed

Epigenetic mechanisms, including DNA methylation and histone modifications, serve as the foundational regulatory layer that "primes" or "locks" gene expression patterns during the transition from lactation to involution.

DNA Methylation and Demethylation: During the transition to the non-lactating (dry) period, global and local changes in DNA methylation occur. While open chromatin marks at milk protein gene promoters are established during pregnancy and lactation, these marks are often lost during involution, leading to the silencing of biosynthetic pathways (Rijnkels et al., 2013). This process is mediated by the coordinated activity of DNA methyltransferases (DNMTs) and TET family proteins (Ivanova et al., 2021).

Chromatin Remodeling: Proteins such as **BPTF** (a subunit of the NURF complex) and **DEK** have been identified as critical for maintaining chromatin accessibility. BPTF, in particular, is essential for the survival and differentiation of mammary epithelial cells; its depletion leads to an arrest in development and an upregulation of apoptotic pathways, mirroring aspects of the involution process (Frey et al., 2017).

Post-transcriptional Modifications (m6A): Emerging evidence highlights the role of RNA-level epigenetics. For instance, **m6A RNA methylation** has been shown to negatively regulate gene networks associated with lactation, effectively acting as a "brake" to facilitate the physiological shift toward involution (Wang et al., 2024).

Transcriptional Networks and Master Regulators

The rapid shift in the mammary gland's transcriptome is driven by a hierarchy of transcription factors that sense local signals like milk stasis and systemic hormonal changes.

The STAT3/MYC Axis: STAT3 is widely regarded as the "master death switch" in the mammary epithelium. Its upregulation during early involution triggers the acute-phase response and initiates apoptosis (Piantoni et al., 2010). Concurrently, **MYC** expression facilitates the cell cycle exit and enhances the apoptotic program (Piantoni et al., 2010).

TGF-β Signaling: The **TGF-β** pathway acts as a critical paracrine signal during involution, promoting tissue remodeling and the breakdown of the basement membrane (Song et al., 2000; Piantoni et al., 2010).

Non-Coding RNA Regulation: Multi-omics studies have identified specific microRNA (miRNA) clusters, such as the **miR-423-3p/IGF1R axis**, that target pathways involved in cell adhesion and the PI3K/Akt signaling pathway to promote remodeling during the dry period (Yu et al., 2025).

Multi-Omics Integration: A Holistic View

Integrating transcriptomics (RNA-seq) with epigenomics (MeRIP-seq or ATAC-seq) has revealed that involution is not merely a "shut-down" but a highly active metabolic and immunological state.

The transition to involution is governed by a loss of "open" chromatin states at secretory loci, coupled with a transcriptional surge of pro-apoptotic and pro-inflammatory factors (Piantoni et al., 2010; Rijnkels et al., 2013). This epigenetic memory may also prime the gland for subsequent lactation cycles, suggesting that involution is a "reprogramming" rather than just a regression (Holliday et al., 2018).

Table-1: Multi-Omics Integration Transcriptomics (RNA-seq)

Regulator Category	Key Factors	Primary Function in Involution

Transcription Factors	STAT3, MYC, PPARG	Induction of apoptosis and metabolic rewiring.
Epigenetic Marks	H3K27me3, DNAme	Silencing of milk protein genes (CSN2, LALBA).
Growth Factors	TGF-β1, IGFBP3	Extracellular matrix remodeling and cell death.
RNA Modifications	m6A Methylation	Post-transcriptional silencing of lactation genes.

Biological Overview of Mammary Gland Involution

The involution process occurs in **two phases**:

Phase I (Reversible Phase; 0–48 hours): Triggered by milk stasis, STAT3 activation via LIF (Leukemia Inhibitory Factor), Initiation of lysosomal-mediated programmed cell death, Immune infiltration: macrophages, neutrophils, Epigenetic priming of apoptotic pathways

Phase II (Irreversible Phase; >48 hours): Extensive epithelial apoptosis, ECM degradation by MMPs, Stromal remodeling, Expansion of phagocytic macrophages, Activation of chromatin-condensing enzymes and repressor complexes, This biphasic progression makes involution a powerful model for studying transcriptional and epigenetic regulation in adult tissue plasticity.

Transcriptional Regulators of Mammary Gland Involution

STAT3: Master Regulator of Involution

STAT3 drives: Lysosomal-mediated cell death, Suppression of milk protein genes, Upregulation of SOCS3, Recruitment of immune cells, Remodeling-associated genes (MMP3, MMP9),.

NF-κB Signaling

Activated by milk stasis, cytokines, Drives inflammatory transcriptional programs, Regulates acute-phase genes.

C/EBP Family (C/EBPβ, C/EBPδ)

Induce epithelial apoptosis, Regulate ECM remodeling genes, Modulate macrophage recruitment Glucocorticoid and Hormone-Responsive Transcription Factors,GR downregulation removes lactation-maintaining signals, PR and ER interactions modify chromatin accessibility.

Epigenetic Regulators in Mammary Involution

DNA Methylation Dynamics

Key DNMTs (especially **DNMT1 & DNMT3a**) regulate: Repression of lactation-specific genes, Activation of apoptotic genes, Cell fate transitions

Histone Modifications

Involution is associated with: **H3K27ac loss** at milk-protein genes, **H3K9me3/H3K27me3 gain** at survival pathways, **Increased HDAC1/2 activity, EZH2-mediated Polycomb**

repression

Chromatin Remodeling Complexes: SWI/SNF complexes reorganize enhancer accessibility, CHD family remodelers shape epithelial-to-mesenchymal transcriptional transitions

Non-Coding RNAs

lncRNAs: Regulate STAT3 signaling (e.g., Lnc-LIFR), Control epithelial survival genes

miRNAs: miR-21: promotes apoptosis, miR-424: regulates ECM genes

Multi-Omics Insights into Involution

Transcriptomics

Bulk and single-cell RNA sequencing reveal: Early upregulation of apoptosis and inflammation genes, Late-phase induction of ECM-degrading enzymes, Distinct transcriptional states in luminal vs. basal cells

Epigenomics

ATAC-seq studies show: Chromatin closing at milk synthesis genes, Opening of enhancers for immune and remodeling genes.

ChIP-seq analyses map: STAT3 binding landscapes, Polycomb enrichment during regression

Proteomics

Proteome profiling identifies: MMP-driven ECM turnover proteins, Proteolytic regulators, Cytokine-driven immune modulators

Metabolomics

Involution alters lipid metabolism: Increase in lysosomal lipases, Changes in phospholipid turnover, Pro-inflammatory lipid mediators

Integration of Epigenetic & Transcriptional Networks

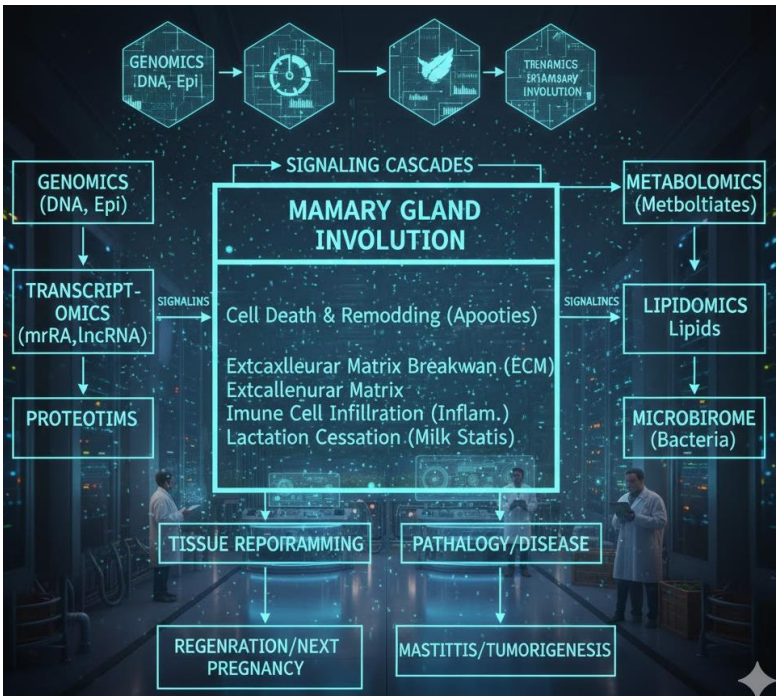

Figure 1. Multi-Omics Regulatory Model of Mammary Involution.

Table: Key Epigenetic & Transcriptional Regulators in Involution.

Regulator Type	Key Factors	Primary Role	Omics Evidence
Transcription Factor	STAT3	Apoptosis, lysosomal death	ChIP-seq, scRNA-seq

TF	NF-κB	Inflammation	ATAC-seq, RNA-seq
Epigenetic Enzyme	DNMT1/3a	DNA methylation shifts	WGBS
Histone Modifier	HDAC1/2, EZH2	Repression of lactation genes	ChIP-seq
ncRNA	miR-21, lnc-LIFR	Apoptosis & STAT3 modulation	RNA-seq

Implications for Postpartum Breast Cancer

Aberrant involution is linked to: Pro-inflammatory tumor-like microenvironment, Increased ECM remodeling → higher metastasis risk, Persistent STAT3/NF-κB activation, Epigenetic scars predisposing malignancy, Understanding involution mechanisms provides **therapeutic targets** for reducing postpartum breast cancer incidence.

Future Directions

Integration of spatial transcriptomics to map epithelial–immune interactions. Identification of epigenetic drug targets to modulate aberrant involution, Use of AI-based regulatory network modeling, and translational studies linking human involution biomarkers to cancer risk.

Conclusion

Mammary gland involution is a complex, multi-layered process regulated by an interplay of transcription factors, epigenetic

modifiers, and dynamic chromatin landscapes. Multi-omics approaches have uncovered hierarchical regulatory networks—dominated by STAT3 signaling, DNA methylation shifts, histone modifications, and non-coding RNAs—that orchestrate the transition from lactation to regression. A systems-level understanding of these processes holds tremendous translational potential for women's health and postpartum breast cancer prevention.

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