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Dynamic Extracellular Matrix Remodeling and Immune Reprogramming in the Postpartum Mammary Gland: Implications for Maternal Health and Breast Cancer Risk

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ABSTRACT

The mammary gland is a uniquely dynamic organ, undergoing profound structural and functional transformations throughout the pregnancy-lactation-involution cycle. These changes are orchestrated by a complex interplay between systemic hormones and the local microenvironment. Recent studies identify the extracellular matrix (ECM) and the innate immune landscape as critical determinants of both physiological tissue homeostasis and pathological progression. Postpartum breast cancer (PPBC), defined as breast cancer diagnosed within ten years following childbirth, represents a distinct clinical entity characterized by increased aggressiveness, higher metastatic potential, and poor prognosis. This review provides a comprehensive synthesis of recent updates in mammary gland biology, focusing on the triphasic pattern of collagen regulation, the role of matrix metalloproteinases (MMPs) and lysyl oxidase (LOX) in tissue remodeling, and the emergence of an immunosuppressive "wound-healing" environment during involution. We explore molecular regulatory nodes, including microRNAs and calpains, and the impact of environmental exposures on breast tissue composition. By integrating data derived from high-resolution spatial profiling and single-cell transcriptomic analyses, this article aims to provide an understanding of how the postpartum mammary microenvironment supports tumorigenesis, highlighting potential biomarkers and therapeutic targets to improve maternal health outcomes.

Introduction

Breast cancer (BC) remains the most prevalent malignancy and the primary cause of cancer-related mortality among women worldwide[1]. A well-established epidemiological paradox exists regarding parity: while a full-term pregnancy confers long-term protection against lifetime breast cancer risk, it is accompanied by a transient but significant increase in risk in the immediate years following childbirth, a phenomenon known as the "postpartum paradox"[2]. This window of vulnerability is increasingly critical in modern clinical practice as trends in delayed childbearing continue to rise, with the average age of first-time mothers frequently extending into the late thirties and early forties[3].

Postpartum breast cancer (PPBC) is defined variably across studies, but most consensus guidelines use a window of 5 to 10 years following the most recent childbirth; some studies restrict the postpartum interval to 2–5 years, while others extend it to 10 years. This variability reflects differences in the latency of involution-associated risk and the study populations examined. Regardless of the exact interval, PPBC is no longer viewed merely as a temporal classification. It is now recognized as a biologically distinct and clinically aggressive subtype[4]. Studies comparing BC in parous women to age-matched nulliparous cohorts reveal that PPBC is associated with more aggressive pathologic features, a higher incidence of triple-negative breast cancer (TNBC), and a significantly higher rate of distant metastasis[5]. The aggressive phenotype of PPBC is thought to be largely driven by the pro-tumorigenic microenvironment that arises during the transition from a lactating to a post-weaning state, a process known as mammary involution. However, the vast majority of evidence linking involution to tumor progression derives from rodent models; direct causal evidence in humans remains limited, and the translational relevance of findings from acute, synchronized weaning models to the more gradual, heterogeneous human involution process requires cautious interpretation[6].

During involution, the mammary gland undergoes one of the most dramatic physiological remodeling events in adult mammalian biology[7]. Upon weaning, the cessation of milk removal triggers massive programmed cell death of the secretory epithelium, coupled with a total architectural reorganization of the stroma[8]. This process involves the recruitment of specialized immune cells and extensive extracellular matrix (ECM) remodeling that closely mirrors a chronic wound-healing response[9]. Emerging evidence suggests a model in which an “involution-like” ECM signature and an immunosuppressive, exhausted T-cell milieu act as dual drivers of PPBC progression; this remains a leading hypothesis that requires further validation in human cohorts[10, 11]. This review will explore these dynamics in depth, providing an expert-level synthesis of how reproductive history shapes the landscape of maternal health and cancer risk.

Physiological States and the Triphasic Mammary Cycle

The mammary gland is characterized by its extraordinary plasticity, undergoing orchestrated cycles of proliferation, differentiation, and regression[7]. This triphasic cycle is governed by systemic endocrine signaling and reciprocal local crosstalk within the stroma (Table 1).

Pregnancy (The Expansion and Preparation Phase): During pregnancy, the mammary epithelium receives signals from systemic hormones, primarily estrogen and progesterone, to proliferate rapidly[12]. This phase, termed alveologenesis, is characterized by extensive branching and the formation of secretory lobuloalveolar units[13].

Recent high-resolution proteomics and single-cell RNA sequencing (scRNA-Seq) have reshaped our understanding of this phase. While fibroblasts were traditionally viewed as the primary architects of the extracellular

Table 1. Triphasic Regulation of Collagen and Extracellular Matrix Composition Across the Mammary Cycle

Phase	Collagen/ECM Status	Key Features	Functional Consequence	Ref
Pregnancy (Expansion & Preparation)	Fibrillar collagens (types I, III) upregulated; high flexibility, low cross-linking	– Collagen also produced by lymphoid/myeloid immune cells – Enrichment of type VI collagen and SPARC	Accommodates rapid alveologenesis without premature stiffening	[14, 15]
Lactation (Suppression)	Collagen synthesis nearly undetectable around acini	– Basement membrane (laminin, type IV collagen) serves as the primary support Adipocytes undergo lipolysis	Prevents stiff matrix from inhibiting acinar expansion/contraction during milk ejection	[15-18]
Involution (Remodeling)	Massive transient deposition of "involution collagen"; structurally distinct	– Linearized, aligned, and radially oriented fibers (TACS-3) – High LOX activity → increased cross-linking and stiffness – Elevated pro-tumorigenic proteins: osteopontin, tenascin-C, laminin-α1	Creates a pro-invasive ECM scaffold; promotes tumor cell migration and metastasis; NSAID treatment partially reverses these changes	[15, 33-35]

matrix (ECM), emerging data reveal an ectopic upregulation of fibrillar collagens (Types I and III) by lymphoid and myeloid immune cells, likely facilitating rapid tissue expansion[14]. The pregnancy-associated ECM is characterized by high flexibility and low cross-linking, mediated by Type VI collagen and SPARC, optimized to accommodate burgeoning alveoli without the mechanical stiffness often associated with malignancy[15]. Enrichment of type VI collagen and the secreted protein acidic and rich in cysteine (SPARC) further suggests a matrix designed for elasticity [15].

Lactation (The Suppression Phase): Lactation represents a state of terminal differentiation where the mammary epithelium is specialized for milk secretion. During this phase, the microenvironment is characterized by a unique metabolic and immune profile that generally suppresses tumorigenesis, although the high metabolic demands of the tissue create a complex metabolic landscape that is still being elucidated. In a remarkable functional shift, collagen synthesis becomes nearly undetectable around the mammary acini during active lactation[15]. This suppression is likely a functional requirement for milk production[15]. As the mammary acini must cycle between expansion and contraction during nursing, a dense, stiff collagen matrix would likely inhibit these rapid morphological changes[16].

During this phase, the basement membrane (BM) remains the primary structural support, enriched with laminins and type IV collagen, which are essential for maintaining mammary epithelial cell (MEC) polarity and lactation performance[17]. Additionally, adipocytes in the mammary fat pad undergo significant lipolysis to satisfy the high energy demands of milk production, resulting in a reduction of lipid content and a shift in stromal composition[18].

Involution (The Remodeling Phase): Involution is the process by which the lactating gland returns to its non-secretory, quasi-nulliparous state[19]. The triphasic regulation of collagen and ECM composition across pregnancy, lactation, and involution is summarized in Table 1. This process occurs in two distinct phases[20]. Stage 1 (reversible) is triggered by milk engorgement and the accumulation of milk proteins, and is characterized by the phosphorylation and activation of signal transducer and activator of transcription 3 (STAT3) in mammary epithelial cells, leading to lysosomal-mediated cell death[21]. In rodents, if the suckling young are returned within approximately 48 hours, this phase can be reversed and lactation restored[22].

Stage 2 (irreversible) ensues if weaning continues; the gland enters an irreversible phase marked by the activation of proteases and extensive structural remodeling[23]. This phase involves the breakdown of the basement membrane by matrix metalloproteinases (MMPs) and the clearance of billions of apoptotic mammary epithelial cells by professional phagocytes[24]. The ECM changes during this second phase are the most dramatic and pro-tumorigenic of the entire cycle, with a massive, transient deposition of "involution collagen" that is structurally distinct from the collagen of pregnancy[15]. These changes include the alignment and linearization of collagen fibers, increased lysyl oxidase-mediated cross-linking that elevates tissue stiffness, and the enrichment of pro-invasive proteins such as osteopontin and tenascin-C, collectively generating a microenvironment that facilitates tumor cell invasion.

Postpartum Breast Cancer (PPBC): A Deep Dive into a Distinct Phenotype

PPBC is increasingly recognized as a unique clinical entity with a biology that distinguishes it from other BCs[4].

Definition and Epidemiology: Historically, PPBC was grouped under the broader category of pregnancy-associated breast cancer (PABC). However, recent evidence suggests that BCs diagnosed after the cessation of lactation have a significantly worse prognosis than those diagnosed during pregnancy[25]. Current expert consensus defines PPBC as a cancer diagnosed within five to ten years of the most recent childbirth[4]. It is estimated that PPBC may account for up to 50% of BC cases in women under the age of 40[26].

Clinical Characteristics and Aggressive Phenotype: The clinical hallmark of PPBC is its high metastatic potential. Patients diagnosed in the early postpartum period (within five years of birth) are nearly three times more likely to develop distant metastasis resulting in death compared to age-matched nulliparous controls[27]. PPBC is characterized by a higher proportion of triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2)-enriched subtypes, larger tumor size at diagnosis, and a higher incidence of lymph node involvement[5]. A distinctive feature of PPBC is its propensity for hepatic (liver) metastasis, with an approximately three-fold increase in liver metastasis observed in postpartum breast cancer patients compared to controls[28]. Furthermore, these aggressive features translate into significantly worse disease-free and overall survival rates, particularly in node-positive disease[5].

T-Cell Exhaustion and the "Cold" Tumor Microenvironment: A "cold" tumor microenvironment is characterized by poor infiltration and/or functional inactivation of anti-tumor effector immune cells, particularly $CD8^+$ T cells, resulting in an inability to mount an effective immune response against the tumor[29]. Exhausted T cells, which progressively lose their proliferative capacity, cytokine production, and cytolytic function, are a central feature of such cold tumors and contribute directly to immune evasion and disease progression. Recent studies using GeoMx Digital Spatial Profiling (DSP) have provided a high-resolution view of the PPBC microenvironment. Tumors diagnosed shortly after birth are enriched with markers of T-cell exhaustion, such as Cytotoxic T-lymphocyte-associated protein 4 (CTLA4), Programmed cell death protein 1 (PD-1), and Granzyme B (GZMB)[10]. This transcriptomic signature of non-responsive T-cells suggests that the involution microenvironment actively inhibits the host's ability to orchestrate an effective anti-tumor immune response[30]. Furthermore, the immune checkpoint B7 homolog 4 (B7-H4), which is upregulated by progesterone during pregnancy to maintain fetal tolerance, may be hijacked by emerging breast tumors to evade surveillance[31].

How Physiological Phases Prime Pathogenesis

The high aggressiveness of PPBC is not an intrinsic property of the cancer cells alone, but

a result of the cells "mimicking" or hijacking the normal developmental programs of involution[11]. The key ECM components, enzymatic drivers, and their pro-tumorigenic consequences are detailed in Table 2.

The Involution ECM as a Scaffold for Invasion: In rodent models, "involution collagen" deposited after weaning may serve as a structural "highway" facilitating cancer cell migration. Unlike the wavy fibers of the

Table 2. Extracellular Matrix Dynamics and Pro-Tumorigenic Remodeling in the Postpartum Mammary Gland

Phase / Context	ECM Component / Feature	Key Regulation / Enzyme	Pro-Tumorigenic Consequence	Ref
Pregnancy	Fibrillar collagens I & III (also produced by immune cells)	Upregulated by estrogen/progesterone; also secreted by lymphoid/myeloid cells	Low cross-linking and high elasticity accommodate alveologenesis without premature stiffening	[14, 15]
	Type VI collagen, SPARC	Enriched in pregnancy-associated ECM	Supports matrix flexibility and secretory unit expansion	[15]
Lactation	Basement membrane proteins: laminin, type IV collagen	Collagen synthesis is suppressed; BM remains the primary structural support	Maintains MEC polarity and lactation performance; prevents stiff matrix from inhibiting acinar cycling	[15-18]
Involution (physiological)	"Involution collagen" (fibrillar)	Transient massive deposition; structurally distinct from pregnancy collagen	Acts as a scaffold for tumor cell migration ("highway"); linearized, aligned, radially oriented fibers (TACS-3)	
	Collagen packing density and fiber alignment	SHG imaging reveals higher packing density; TACS-3 signature	TACS-3 is an independent poor prognostic factor that facilitates collective cell invasion	[15]
	Lysyl oxidase (LOX)	Peak expression during mid-involution	Increases collagen cross-linking and tensile strength → matrix stiffness linked to malignant transformation and therapy resistance	
	Osteopontin, tenascin-C, laminin-α1	Upregulated in involuting ECM (proteomics)	Fail to support normal ductal organization; promote tumor cell invasion	[33, 34]
	Proteolyzed collagen fragments	Increased fibrillar collagen proteolysis	Act as chemoattractants for macrophages, amplifying the tumor-promotional milieu	[9]
Involution (functional consequence)	Whole ECM from the involuting gland	Isolated ECM promotes invasiveness in vitro	In vivo: increases metastasis to the lung, liver, and kidney compared to nulliparous ECM	[34]
Enzymatic remodeling	MMP-9	Highly sensitive to reproductive state; degrades basement membrane; produced by macrophage–adipose stromal cell interactions	Creates a "leaky" microenvironment that supports angiogenesis and tumor cell proliferation	[23, 36] [37]
Therapeutic modulation	NSAIDs (ibuprofen)	Reduce collagen fibrillogenesis; decrease tenascin-C and laminin levels	Attenuate the tumor-promotional attributes of involuting ECM; evidence for prophylactic intervention	[33, 35]
Environmental exposures	Collagen I, laminin (DES)	In utero DES → early collagen depletion followed by pubertal surge	Increased matrix density and altered fiber orientation → permanently susceptible microenvironment	[45]
	Water and collagen content (PAHs)	PAH–tobacco smoke interaction during pregnancy	Altered breast tissue composition may influence mammographic density and lifetime cancer risk	[47]

nulliparous gland, involution-associated collagen is linearized and aligned, with fibers that are straighter and more tightly packed, as demonstrated by second harmonic generation (SHG) imaging, revealing higher collagen packing density during involution compared to nulliparous and lactating glands[15]. These fibers are radially oriented, with many positioned perpendicular to the mammary epithelium, a signature known as Tumor-Associated Collagen Signature-3 (TACS-3); an architecture that in human breast cancer facilitates collective cell invasion and is a well-established poor prognostic factor[15]. Additionally, in rodent involuting glands, tissue stiffness increases significantly during mid-involution due to peak expression of lysyl oxidase (LOX), which mediates collagen cross-linking and is associated with increased levels of cross-linked collagen[15]. Increased matrix stiffness has been associated with malignant transformation and therapeutic resistance in experimental models[32]. Proteomic analyses of rodent involuting ECM have further identified compositional changes in the involuting ECM, including increases in pro-tumorigenic proteins such as osteopontin, tenascin-C, and laminin-α1, which collectively contribute to a microenvironment that fails to support normal ductal organization while actively promoting tumor cell invasion[33, 34]. Functionally, ECM isolated from involuting mammary glands promotes invasiveness in tumor cells and increases metastasis to the lung, liver, and kidney in vivo compared to ECM from nulliparous glands[34]. The ECM remodeling process also involves increased proteolysis of fibrillar collagen, generating bioactive fragments that act as chemoattractants for macrophages, which in turn contribute to the tumor-promotional milieu[9]. Importantly, in rodent models, nonsteroidal anti-inflammatory drug (NSAID) treatment during involution has been shown to reduce collagen fibrillogenesis, decrease tenascin-C and laminin levels, and attenuate the tumor-promotional attributes of the involuting ECM, providing preclinical evidence for potential therapeutic intervention targeting this matrix-driven pathway[33,35]. Together, these coordinated changes in collagen architecture, alignment, cross-linking, composition, and stiffness create a uniquely permissive ECM that facilitates invasion and metastatic dissemination in the postpartum mammary gland, providing a mechanistic explanation for the high rate of metastases observed in PPBC (Table 1 and 2)[15,34].

Enzymatic Drivers and the "Leaky" Environment: The enzymatic restructuring required to regress the lactating gland can inadvertently facilitate tumor spread. In rodent mammary glands, MMP-9, which is highly sensitive to the reproductive state, is essential for degrading the basement membrane during involution[23]. In bovine models, exogenous MMP-9 administration accelerates involution[36]. In *in vitro* co-culture models using human macrophages and adipose stromal cells, elevated MMP-9 levels may contribute to a microenvironment that favors angiogenesis and tumor cell proliferation (Table 2)[37].

Macrophage Re-education and Immune Suppression: In the rodent model, the massive influx of F4/80-expressing macrophages during involution is necessary to clear billions of apoptotic MECs through efferocytosis (the phagocytic clearance of apoptotic cells)[30]. However, this intensive wound-healing activity re-educates macrophages toward an M2-like, alternatively activated phenotype[9]. These "involution macrophages" cooperate with adipose stromal cells to secrete pro-inflammatory and angiogenic cytokines, such as interleukin 6 (IL-6), C-X-C motif chemokine ligand 1 (CXCL1), C-X-C motif chemokine ligand 5 (CXCL5), and monocyte chemoattractant protein-1 (MCP-1)[37]. This milieu is believed to suppress anti-tumor immunity and supply growth factors that could support the expansion of occult tumor cells, at least in murine models[38].

Despite the compelling evidence linking postpartum involution to breast cancer

progression, not all studies have observed a uniform risk increase. Some population-based analyses found that the excess metastasis risk is largely confined to women diagnosed within 2-5 years of delivery and is less pronounced in certain molecular subtypes, suggesting that involution-associated risk may be time- and subtype-dependent[27]. Furthermore, prolonged lactation itself exerts a long-term protective effect against breast cancer, indicating that the reproductive cycle contains both transient adverse and lasting beneficial windows. These nuances highlight the need for further investigation into the factors that tip the balance from tissue repair to tumor promotion. In contrast, a pooled analysis of 15 prospective cohorts reported only a modest, transient elevation in breast cancer risk following childbirth, an effect substantially attenuated by prolonged breastfeeding[2]. These discrepancies likely reflect differences in postpartum window definitions, tumor subtype distributions, and the use of rodent models with acute synchronized involution versus the more gradual and heterogeneous human process.

Immune Remodeling as a Bridge to Tumor Progression: The postpartum involution microenvironment is now understood to orchestrate a coordinated immunosuppressive program that simultaneously disarms anti-tumor immunity and provides trophic support for malignant cells. Central to this program is the functional coupling between M2-like macrophages and exhausted T cells.

Involution-associated macrophages, reprogrammed toward an alternatively activated phenotype by efferocytosis and local cytokine cues, secrete IL-6, IL-10, transforming growth factor-beta (TGF-β), and C-C motif chemokine ligand 2 (CCL2), which not only suppress cytotoxic T-cell activity but also reinforce the exhausted T-cell state characterized by sustained PD-1, CTLA-4, and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) expression[9,10,30,37]. Concurrently, the loss of mature dendritic cell function and the predominance of regulatory T cells further dampen adaptive immune surveillance[30,38]. This immune landscape mirrors the "cold" tumor microenvironment described above, and is thought to provide a permissive niche for the outgrowth of occult tumor cells.

Preclinical evidence suggests that disrupting these immunosuppressive circuits can reduce postpartum tumor progression. In rodent models, NSAID-mediated dampening of the wound-healing inflammatory response reduces M2 macrophage recruitment and collagen remodeling[33,35]; Blockade of colony-stimulating factor 1 receptor (CSF-1R) signaling depletes involution macrophages and attenuates metastasis[6]; and immune checkpoint inhibition (anti-programmed death 1 [PD-1]/anti-programmed death-ligand 1 [PD-L1]) partially restores T-cell function in postpartum mammary tumors[6]. Although these strategies remain confined to experimental models, they collectively identify macrophage re-polarization, cytokine neutralization (such as anti-IL-6), and checkpoint blockade as rational targets for future clinical investigation in postpartum breast cancer.

Molecular Regulatory Nodes and Environmental Programming

Expert-level understanding of this field requires examining the fine-tuned molecular switches that govern these transitions.

MicroRNAs as Bioreporters and Regulators: MicroRNAs (miRNAs) serve as critical post-transcriptional regulators of mammary function. In terms of lactation performance, human milk levels of let-7g-5p have been identified as bioreporters, with high levels associated with low milk supply and early cessation of breastfeeding, likely through downregulation of the prolactin receptor (PRLR)[39]. Furthermore, miRNAs such as miR-148a and the miR-200 family directly regulate prolactin signaling and epithelial differentiation during lactation; miR-148a suppresses PRLR expression, while miR-200

family members enforce the luminal epithelial phenotype, and their coordinated downregulation during weaning facilitates secretory de-differentiation. Regarding persistent risks, research using murine models shows that forced or abrupt weaning leads to a persistent downregulation of tumor-suppressive miRNAs, such as miR-143 and miR-145, which remain low even after full tissue regression[40]. This sustained suppression suggests that a lack of gradual weaning may maintain a pro-carcinogenic environment long-term[40]. Conversely, the miR-424(322)/503 cluster is transiently upregulated during the involution phase, where it targets key regulators of apoptosis and adipogenesis (including BCL2 and FGFR1), thereby coordinating epithelial clearance and stromal repopulation[41]; persistent dysregulation of this cluster may similarly contribute to the pro-tumorigenic remodeling of the postpartum gland. Together, these findings highlight a tightly orchestrated miRNA network that governs the successive phases of mammary remodeling, with perturbations in these circuits potentially tipping the balance toward postpartum breast cancer progression.

Calpain-2 and Inflammation: The murine model of weaning significantly impacts the local inflammatory response. Abrupt weaning induces a spike in Calpain-2 activity and acute inflammation, whereas spontaneous or gradual weaning maintains a milder inflammatory environment[40]. In murine models, higher Calpain-2 expression in the short lactation model persisted for up to 28 days postpartum, highlighting how weaning patterns can "program" the mammary gland[40]. The inflammation driven by abrupt weaning and calpain-2 activation may feed into multiple downstream pathways, including those controlled by C/EBPβ, a transcription factor that integrates inflammatory and developmental signals.

The LIP/LAP Ratio of C/EBPβ: The transcription factor C/EBPβ is a master regulator of mammary development and tissue remodeling[42]. It is translated into three isoforms: liver-enriched transcriptional activator protein 1 (LAP1), liver-enriched transcriptional activator protein 2 (LAP2), and liver-enriched transcriptional inhibitory protein (LIP). An aberrantly high LIP/LAP ratio, driven by pathways such as HER2 or TGFβ, is a hallmark of high-grade, hormone-receptor-negative breast cancers[43]. This ratio promotes the epithelial-to-mesenchymal transition (EMT) and matrix invasion, directly linking intracellular signaling to the pro-tumorigenic ECM[44]. In the context of PPBC, the involution microenvironment provides a rich source of TGFβ (liberated from the remodeled ECM and secreted by M2-like macrophages) that further amplifies LIP expression[43,44]. The resulting LIP-dependent EMT program synergizes with involution-associated ECM stiffening and aligned collagen fibers to enhance tumor cell motility and invasion, thereby contributing to the heightened metastatic burden, including the hepatic metastasis characteristic of PPBC. Thus, the LIP/LAP axis may represent a convergent node where developmental inflammatory signals and matrix remodeling co-opt a transcriptional switch that drives the uniquely aggressive biology of postpartum breast cancer.

Environmental Influences: Maternal health and BC risk are also shaped by environmental exposures during critical windows of susceptibility. In the case of endocrine-disrupting compounds, a mouse study showed that in utero exposure to the synthetic estrogen diethylstilbestrol (DES) can reprogram the mammary gland. This occurs through an early depletion of fibrillar collagens, followed by a subsequent surge in collagen I and laminin during puberty. These alternations lead to greater matrix density and changes in fiber orientation by adulthood, ultimately generating a tissue microenvironment that remains more susceptible to later pathological changes[45].

Similarly, gestational and lactational exposure to bisphenol A (BPA) has been shown to induce spontaneous mammary carcinomas in aged gerbils, strengthening the association between developmental endocrine disruption and malignancy driven by tissue remodeling[46]. Regarding ambient polycyclic aromatic hydrocarbons (PAHs), exposure to these air pollution-derived compounds during pregnancy may interact synergistically with tobacco smoke, leading to changes that significantly affect water and collagen content and influence mammographic density and lifetime cancer risk (Table 2)[47].

Clinical Perspectives: The Path Forward for Maternal Health

ECM Remodeling, Translational Insights: The identification of the "involution collagen" signature (linearized, aligned fibers, LOX-mediated cross-linking, and TACS-3 architecture) provides a structural rationale for the high metastatic propensity of PPBC. Most of this evidence derives from rodent models and ex vivo human tissue imaging[15,34]; direct demonstration that these ECM changes alone drive human metastasis is still lacking. Nonetheless, the finding that NSAIDs can attenuate collagen fibrillogenesis and reduce tenascin-C and laminin levels in involuting rodent glands[33,35] has generated considerable interest. It is important to emphasize that evidence for NSAID prophylaxis remains preclinical; no human trial has evaluated the safety or efficacy of NSAIDs during involution for breast cancer prevention, and NSAID use during lactation must consider potential risks to the infant. In parallel, the time since last birth (TSLB) is emerging as a robust, readily available prognostic biomarker in premenopausal breast cancer, supported by multiple observational cohort studies[10], although its formal integration into risk-stratification tools requires prospective validation.

Immune Reprogramming, Translational Insights: The postpartum immune milieu (dominated by M2-like macrophages, exhausted T cells, and suppressive cytokines such as IL-6 and TGF-β) represents an attractive therapeutic target. In rodent models, NSAID-mediated dampening of the wound-healing response reduces M2 macrophage recruitment and collagen remodeling[33,35], while CSF-1R blockade depletes involution macrophages and attenuates metastasis[6]. Immune checkpoint inhibition (anti-PD-1/PD-L1) has shown partial restoration of T-cell function in postpartum mammary tumor models[6]. These strategies remain strictly experimental, and no clinical studies have yet evaluated immunomodulation specifically in the postpartum setting. Nevertheless, they highlight macrophage re-polarization, cytokine neutralization, and checkpoint blockade as rational areas for future clinical investigation.

Knowledge Gaps and Future Directions: Several critical questions remain unanswered. First, direct causal evidence linking involution-associated ECM remodeling and immune suppression to human breast cancer initiation or progression is limited; most findings originate from genetically engineered mouse models or observational cohorts. Second, the optimal biomarker panel for postpartum-specific risk assessment (potentially combining TSLB, circulating ECM fragments, and immune profiling) has not been defined. Third, the long-term impact of weaning practices (abrupt in comparison with gradual) and lactation duration on ECM and immune reprogramming in women is unknown and warrants a prospective study. Fourth, while preclinical data support NSAID intervention, the timing, dose, and safety in lactating women need formal evaluation. Finally, whether the LIP/LAP axis or MMP-9 can be therapeutically targeted in postpartum patients remains purely speculative.

In summary, the postpartum mammary gland is a unique biological landscape where normal tissue involution creates a transient pro-tumorigenic niche through aligned, stiffened ECM and an immunosuppressive

microenvironment. While preclinical models have illuminated the key cellular and molecular players (LOX-crosslinked collagen, M2 macrophages, exhausted T cells), their translation to human clinical practice remains in its infancy. The time since last birth already serves as a practical prognostic marker, and NSAIDs, CSF-1R blockade, and immune checkpoint inhibitors offer promising but unproven avenues for intervention. Future research must bridge the gap between rodent models and human biology through carefully designed prospective studies and clinical trials. By addressing these gaps, we can move toward personalized screening and therapeutic strategies that reduce the burden of postpartum breast cancer and safeguard maternal health.

Conclusion

The postpartum mammary gland is a landscape of profound biological change. Recent studies have elevated our understanding from a focus on the mammary epithelium to a holistic view of the extracellular matrix-immune cell-hormone axis. The involution process, through aligned and crosslinked collagen deposition driven by MMPs and LOX, together with the emergence of an M2-like macrophage and exhausted T-cell milieu, creates a uniquely permissive environment for tumor cell invasion and metastasis. The postpartum period is therefore a window of physiological vulnerability where the necessary processes of tissue regression may be hijacked by emerging tumor cells. Critical knowledge gaps remain: direct causal evidence linking involution-associated remodeling to human breast cancer is scarce, the optimal biomarker panel for postpartum-specific risk stratification is undefined, and the long-term impact of weaning strategies and lactation duration on immune and ECM reprogramming requires prospective human studies. From a translational perspective, the time since last birth represents a readily available prognostic biomarker, while the postnatal use of NSAIDs to dampen the wound-healing response and the targeting of the LIP/LAP or CSF-1R axes are promising therapeutic avenues that now warrant clinical evaluation. By recognizing that collagen is under reproductive control, that immune cells contribute to this matrix induction, and that involution triggers a state of immune exhaustion, clinicians and researchers can begin to solve the "postpartum paradox" through personalized screening and therapeutic innovation, ultimately safeguarding the health of mothers globally.

Conflict of Interest

Authors have no conflict of interests.

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Ethical Considerations

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Author's Contribution

Samane Heydari: Conceptualization, investigation, writing – original draft, writing – review & editing, visualization. Sadegh Safaei: Writing – review & editing.

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