In-Vivo Insights: ACTB Expression in Animal Models of Breast Cancer – Profiling, Metastasis, Pharmacodynamics, and AI-Integrated Imaging

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Abstract

β-actin (ACTB) is a key cytoskeletal protein increasingly recognized for its multifaceted role in cancer biology, including cell migration, invasion, and resistance to therapy. This review synthesizes evidence from in-vivo breast cancer models, focusing on ACTB expression profiling across mouse xenograft and orthotopic platforms. The relationship between ACTB modulation and metastatic progression to the lungs, bone, and liver is systematically examined. Further, the article integrates pharmacodynamic data connecting ACTB knockdown or inhibition with tumor regression, highlighting its potential as a predictive biomarker. A dedicated section explores advances in non-invasive imaging, particularly the AI-powered Swalife HistoAnalysis tool, which refines quantification of ACTB in tissue contexts and links imaging phenotypes with molecular datasets. The review advocates for standardized protocols and collaborative AI-imaging platforms to bridge the translational gap from animal studies to clinical innovation, outlining future research priorities.

Keywords: ACTB, breast cancer, xenograft models, metastasis, pharmacodynamics, non-invasive imaging, AI quantification

Introduction

β-actin (ACTB) is a highly conserved cytoskeletal protein fundamental to the structure and function of eukaryotic cells. As a key component of the actin cytoskeleton, ACTB plays an essential role in maintaining cell shape, enabling motility, and facilitating intracellular transport. Over recent decades, research has increasingly uncovered ACTB's dynamic involvement in cancer biology, particularly in breast cancer, where it influences critical processes such as cell migration, invasion, proliferation, and resistance to therapy.[1]

The actin cytoskeleton, with ACTB as a principal constituent, is crucial for epithelial cell plasticity a feature that cancer cells exploit during invasion and metastasis. Dysregulation or altered expression of ACTB has been linked to the epithelial-mesenchymal transition (EMT), a process by which epithelial cells acquire mesenchymal properties, enhancing their motility and invasiveness. ACTB's role extends to modulating invadopodia formation, essential cellular structures that degrade extracellular matrix and facilitate tumor cell dissemination. Moreover, ACTB interacts with signaling pathways governing cell survival and proliferation, underscoring its multifaceted role in breast cancer pathophysiology.[2]

Animal models, particularly murine systems, serve as indispensable tools to probe these complex biological processes in vivo. While in-vitro studies have provided foundational insights, they cannot fully replicate the complexities of tumor-stromal interactions, immune modulation, and metastatic progression encountered in living organisms. Thus, in-vivo models are critical for studying the spatiotemporal dynamics of ACTB expression and function within a more physiological context.[3]

Two major types of in-vivo breast cancer models dominate preclinical research: xenografts and orthotopic models. Xenograft models introduce human breast cancer cells subcutaneously or into non-mammary sites in immunocompromised mice. While this approach enables rapid tumor growth and experimental manipulation, it

notably lacks the native tumor microenvironment.[4] Orthotopic models, where breast cancer cells are implanted into the mammary fat pad of mice, better mimic the native tissue architecture and microenvironment, thereby providing a more clinically relevant platform to study tumor growth, local invasion, and metastasis.

Rationale for employing these models lies in their ability to recapitulate key aspects of human breast cancer progression, including heterogeneity of tumor subtypes and metastatic organotropism. Importantly, these models facilitate longitudinal studies for evaluating pharmacodynamic responses to targeted interventions, including those that perturb ACTB expression or function.[5]

This review aims to synthesize the current state of knowledge regarding ACTB expression in breast cancer in-vivo models, focusing on expression profiling in xenograft and orthotopic systems, ACTB's role in metastasis to critical organs (lungs, bone, and liver), and pharmacodynamic correlations between ACTB modulation and tumor regression.[6] A unique emphasis is placed on the integration of advanced non-invasive imaging modalities and artificial intelligence (AI)-driven analyses, especially utilizing digital pathology platforms such as Swalife HistoAnalysis. These technologies represent the frontier of quantitative oncologic research by enabling precise, reproducible measurement of ACTB in tissue specimens and real-time tracking of tumor dynamics.[7]

Bridging basic research and clinical application requires robust, quantitative tools capable of linking molecular insights with phenotypic outcomes. The adoption of AI-powered imaging analysis not only enhances data reproducibility but also empowers predictive modeling and personalized therapeutic strategies.[8]

This review synthesizes evidence derived from mouse models to elucidate the multifaceted role of ACTB in breast cancer progression, metastasis, and therapeutic response. Additionally, it highlights the transformative potential of AI-integrated imaging tools for enhancing quantitative accuracy and translational relevance. Through critical assessment and identification of knowledge gaps, this work lays a foundation for future research aimed at accelerating clinical translation and improving patient outcomes.

Expression Profiling of ACTB in Xenograft and Orthotopic Mouse Models

Overview of Models:

Preclinical breast cancer research heavily relies on murine models to study tumour biology in vivo. Two principal model systems dominate: xenograft models and orthotopic models. Xenograft models typically involve subcutaneous implantation of human breast cancer cell lines into immunocompromised mice, offering ease of tumour monitoring and rapid tumour establishment. These models facilitate investigation of tumour biology in a relatively controlled environment, although they lack the native mammary microenvironment. In contrast, orthotopic models implant cancer cells directly into the murine mammary fat pad, better recapitulating the breast tissue milieu.[9]

Orthotopic implantation provides superior mimicry of the tumor microenvironment, enabling interactions with native stromal, vascular, and immune components. These interactions critically influence tumor architecture, gene expression, and metastatic potential, making orthotopic models more clinically representative. Subcutaneous xenografts, while simple and reproducible, often fail to capture this complexity, especially concerning stromal crosstalk and organ-specific metastatic patterns.[10]

Key Findings on ACTB Expression:

ACTB expression has been extensively profiled across these mouse models to elucidate its role in breast tumor progression. Multiple studies have consistently found that ACTB is upregulated at both mRNA and protein levels in aggressive breast cancer subtypes, particularly triple-negative breast cancer (TNBC), characterized by poor prognosis and high metastatic potential.[11]

In xenograft models using TNBC cell lines like MDA-MB-231, ACTB mRNA and protein levels are elevated compared to less invasive subtypes such as luminal A (e.g., MCF-7). This heightened expression correlates with enhanced migratory and invasive behavior in vitro and accelerated tumor growth and metastasis in vivo. Orthotopic models confirm these trends, with ACTB expression displaying spatial heterogeneity within tumors higher levels are found at invasive fronts and metastatic lesions, underscoring its relevance to tumor dissemination.[12]

Transcriptomic analyses via RNA sequencing in orthotopic models reveal dynamic regulation of ACTB during tumor progression. ACTB expression often increases in parallel with epithelial-to-mesenchymal transition (EMT) gene signatures, linking it to cytoskeletal remodeling, cell adhesion, and motility pathways critical for metastasis.[13]

Methodological Insights:

Several molecular techniques underpin these profiling studies. Quantitative PCR (qPCR) accurately measures ACTB mRNA levels in tumor tissues, enabling model and treatment comparisons. Western blotting complements this by quantifying protein abundance and post-translational modifications that influence ACTB function.[14]

Immunohistochemistry (IHC) remains the gold standard for spatial localization of ACTB in tumor sections, allowing visualization of heterogeneous expression patterns. When combined with digital pathology and AI-driven image analysis tools like Swalife HistoAnalysis, IHC achieves enhanced objectivity, reproducibility, and precision in quantification, especially in complex murine tumor histology.[15]

High-throughput RNA sequencing technologies enable unbiased genome-wide transcriptional profiling, identifying co-regulated gene networks alongside ACTB during breast cancer progression. This approach has also uncovered differential ACTB isoform expression and splice variants, which may contribute to functional diversity in tumor contexts.[16]

Challenges and Gaps:

Despite advances, several limitations hamper expression profiling of ACTB. Biological heterogeneity among mouse models and cell lines contributes to variability in expression patterns and tumor phenotypes. The immunocompromised status of xenograft hosts limits investigation into ACTB's role in immune interactions and the tumor microenvironment.[17]

Orthotopic models, though more representative, are technically complex and require advanced imaging and molecular tools to longitudinally monitor ACTB. Furthermore, lack of standardization in tissue processing, RNA/protein extraction, and staining across laboratories complicates cross-study comparisons. There is a critical need for consensus protocols and reporting standards to improve reproducibility and clinical relevance.[18]

Additionally, most studies analyze bulk tumor tissue, masking cell type–specific ACTB expression in heterogeneous microenvironments composed of cancer cells, stromal fibroblasts, immune cells, and endothelial cells. Emerging single-cell transcriptomics and spatial proteomics hold promise for resolving these details.[19]

ACTB's Role in Metastasis to Lungs, Bone, and Liver

Mechanistic Overview:

Metastasis is the primary cause of mortality in breast cancer, involving a multistep cascade where cancer cells detach from the primary tumour, invade nearby tissues, enter the circulation, and colonize distant organs. Central to this process is the dynamic remodelling of the cytoskeleton, with β-actin (ACTB) playing a pivotal role in enabling the invasive and migratory properties of cancer cells.[20]

ACTB drives epithelial-mesenchymal transition (EMT), a phenotypic switch during which epithelial cells lose polarity and adhesion and gain mesenchymal traits conducive to motility. During EMT, cytoskeletal reorganization facilitates formation of specialized protrusions called invadopodia—actin-rich membrane extensions that degrade the extracellular matrix (ECM), allowing tumor cells to disseminate.[21]

Additionally, ACTB participates in ECM remodeling by regulating secretion and localization of matrix metalloproteinases (MMPs), enzymes essential for ECM degradation. The cycles of ACTB polymerization and depolymerization generate mechanical forces required for cellular translocation and invasion. These functions empower breast cancer cells to breach basement membranes and intravasate into vasculature.[22]

Site-Specific Evidence:

Lungs:
The lung is the most common site of breast cancer metastasis. Mouse models, including xenografts and orthotopic implants of human triple-negative breast cancer (TNBC) cell lines, faithfully replicate pulmonary metastasis. Studies show ACTB expression is markedly increased in circulating tumor cells (CTCs) derived from primary tumors. ACTB supports cytoskeletal flexibility that CTCs require to survive shear stress in circulation and extravasate into lung parenchyma.[23]

Pulmonary metastasis models using bioluminescence and fluorescence imaging demonstrate that ACTB-overexpressing cells have enhanced colonization and tumor formation in lung tissue. Mechanistically, ACTB activates survival pathways such as PI3K/Akt and MAPK, promoting resistance to apoptosis during metastatic transit

and colonization. ACTB also regulates integrin-mediated adhesion to lung endothelial cells, facilitating extravasation.[24]

Bone:
Breast cancer frequently metastasizes to bone, causing osteolytic lesions that lead to bone weakening and pain. ACTB is heavily involved in crosstalk between tumor cells and the bone microenvironment. Osteoclasts, bone-resorbing cells, depend on actin-rich podosomes to adhere to and degrade bone matrix. Tumor cells manipulate ACTB-actin dynamics to recruit and activate osteoclasts, creating a vicious cycle of bone destruction that supports tumor growth.[25]

Bone metastasis mouse models show increased ACTB expression in tumor cells within the bone niche and in osteoclasts. This amplifies matrix degradation and releases bone-derived growth factors that stimulate tumor proliferation. Advanced imaging techniques such as in-vivo micro-CT combined with ACTB-targeted fluorescent probes reveal spatial and temporal patterns of tumor-osteoclast interactions.[26]

Liver:
The liver is another frequent metastatic site. Breast cancer cells must resist anoikis (cell death induced by loss of ECM attachment) to survive in this organ. ACTB facilitates anoikis resistance by reorganizing the cytoskeleton to sustain survival signaling in detached cells.[27]

Orthotopic mouse models show upregulation of ACTB in liver metastatic lesions. ACTB interacts with focal adhesion kinase (FAK) and other cytoskeletal proteins to maintain integrin signaling and activate anti-apoptotic pathways, ensuring tumor cell survival and colonization in hepatic microenvironments.[28]

Experimental Data:
In-vivo imaging has been pivotal in tracking ACTB's role in metastasis. Bioluminescent imaging of luciferase-expressing breast cancer cells with manipulated ACTB expression quantifies metastatic burden over time in lungs, bone, and liver.[29]

Fluorescence intravital microscopy visualizes ACTB dynamics at single-cell resolution during invasion and extravasation, highlighting its role in modulating tumor cell shape, motility, and stromal interactions in real time.[30]

Therapeutic Implications:
Targeting ACTB-mediated pathways shows promise in mitigating metastasis. Small molecules like cytochalasin D and latrunculin inhibit actin polymerization, reducing invadopodia formation, cell migration, and invasion in preclinical models.[31]

Novel inhibitors targeting upstream regulators of ACTB polymerization, including Rho GTPases and formins, have demonstrated efficacy in curbing metastatic spread in experimental breast cancer. However, selective targeting remains challenging due to ACTB's ubiquitous role in normal cellular functions, underscoring the need for precision therapies that disrupt pathological ACTB dynamics in tumors.[32]

Emerging research suggests combining ACTB pathway inhibitors with immune checkpoint therapies to enhance anti-tumor immunity and prevent metastasis.

Pharmacodynamic Correlation Between ACTB Modulation and Tumor Regression

Modulation Strategies:

β-actin (ACTB) is crucial in breast cancer progression, prompting extensive research into strategies to modulate its expression or function in preclinical models. Two main approaches prevail: genetic modulation and pharmacological intervention.[33]

Genetic techniques include targeted knockdown or knockout of ACTB using RNA interference (siRNA/shRNA) and CRISPR/Cas9 genome editing. siRNA-mediated silencing transiently reduces ACTB mRNA levels, enabling study of downstream effects such as impaired cell motility and invasion. CRISPR/Cas9 offers more permanent disruption, allowing investigation of long-term impacts on tumor growth and metastasis in vivo.[34]

Pharmacological strategies focus on actin polymerization inhibitors like cytochalasin D and latrunculin A/B, which bind actin monomers or filaments to prevent polymerization or induce depolymerization. These agents diminish cancer cell motility and invasion by disrupting the dynamic actin cytoskeleton. Additionally, selective small molecule inhibitors targeting regulatory proteins upstream of ACTB (e.g., Rho-family GTPases, formins, Arp2/3 complex) are under active development.[35]

Correlation Evidence:
Dose-response studies in mouse models reveal strong correlations between ACTB modulation and tumor regression. Genetic knockdown of ACTB in xenograft and orthotopic breast cancer models consistently reduces tumor volume and metastatic burden. For example, siRNA-mediated ACTB silencing in MDA-MB-231 xenografts yielded a dose-dependent tumor growth decrease accompanied by impaired vascular invasion.[36]

Pharmacological inhibition with cytochalasin D similarly reduces primary tumor size and metastatic lesions in lungs and bone. Longitudinal bioluminescent imaging demonstrates sustained tumor suppression following treatment.[37]

Survival studies show that ACTB knockdown or inhibition prolongs overall survival and delays metastasis onset in aggressive breast cancer models. Moreover, combination therapies pairing ACTB modulators with conventional chemotherapeutics produce additive or synergistic decreases in tumor burden and improved survival.[38]

Biomarker Potential:
ACTB's consistent modulation following therapeutic intervention supports its role as a valuable pharmacodynamic (PD) biomarker. Quantitative evaluation of ACTB expression through immunohistochemistry (IHC), Western blotting, or RNA assays enables monitoring of treatment efficacy in preclinical and possibly clinical settings.[39]

Emerging AI-driven digital pathology tools, such as Swalife HistoAnalysis, facilitate high-throughput, reproducible quantification of ACTB in tumor tissues, enhancing objective PD assessment. These tools link molecular changes to phenotypic outcomes, aiding treatment optimization and patient stratification.[40]

ACTB PD biomarker utility extends to therapies targeting cytoskeletal dynamics, epithelial-mesenchymal transition, and metastatic pathways. Tracking ACTB modulation may predict therapeutic response or resistance emergence, offering insights for adaptive treatments.[41]

Limitations:
Despite encouraging correlations, variability in pharmacodynamic responses to ACTB modulation exists across models. Factors such as tumor subtype, genetic background, and tumor microenvironment influence outcomes. Differences in delivery efficiency and off-target effects of genetic tools and pharmacological agents complicate interpretation.[42]

Since ACTB is ubiquitously expressed in normal tissues, systemic toxicity concerns arise when targeting its pathways. Off-target cytoskeletal disruptions in non-malignant cells highlight the need for tumor-selective modulation.Moreover, compensatory mechanisms within the actin cytoskeleton network may offset ACTB inhibition effects, indicating the necessity for combination or multi-target therapies.[43]

Standardized PD assays and validation in clinically relevant models remain critical challenges. Integrating longitudinal imaging, molecular analyses, and functional assays is essential to fully capture ACTB-modulation therapeutic impact.[44]

Integration of Non-Invasive Imaging and AI-Driven Quantification (Swalife HistoAnalysis)

Non-Invasive Techniques:

The study of breast cancer progression and the molecular dynamics of pivotal proteins like β-actin (ACTB) has been profoundly enhanced by the advent of non-invasive imaging technologies. These modalities allow researchers to observe biological processes in live animal models in real time without disturbing physiological states, offering insights that static or endpoint analyses cannot provide.[45] Among the foremost techniques utilized are magnetic resonance imaging (MRI), positron emission tomography combined with computed tomography (PET/CT), and ultrasound imaging, each providing unique advantages for tracking ACTB expression and tumor behavior in vivo.[46]

MRI remains a gold standard for soft tissue imaging due to its high spatial resolution and excellent contrast between normal and tumor tissues. Advanced MRI methodologies such as diffusion-weighted imaging (DWI) capture changes in tumor cellularity, reflecting alterations in tissue density and integrity associated with ACTB-mediated cytoskeletal remodeling. Dynamic contrast-enhanced MRI (DCE-MRI) further offers functional assessments by visualizing tumor vascularity and perfusion, which are often influenced by ACTB-driven actin dynamics impacting angiogenesis and invasive cell motility.[47]

PET/CT imaging delivers the power of combining functional metabolic data with anatomical localization. Radiotracers like fluorodeoxyglucose (FDG) allow assessment of glucose metabolism, a surrogate for tumor activity tightly linked to ACTB-mediated processes like invasion and proliferation. PET/CT can detect early metastatic sites

and quantify metabolic heterogeneity within tumors, providing a direct functional correlation to molecular changes in ACTB expression.[48]

High-frequency ultrasound serves as a cost-effective and versatile method for longitudinal tumor tracking. It enables precise measurement of tumor volume and vascularity changes over time. Doppler ultrasound, in particular, measures blood flow kinetics, which may reflect alterations in angiogenesis and the invasive capacity of tumor cells, both modulated by ACTB-related cytoskeletal rearrangements.[49]

AI Integration (Swalife HistoAnalysis):

While these imaging techniques provide invaluable macroscopic data, delineating molecular and cellular details such as ACTB expression patterns within tissue architecture demands high-resolution histopathological analysis. This is where Swalife HistoAnalysis—a cutting-edge AI-powered digital pathology platform—plays a transformative role by converting immunohistochemistry (IHC) slides into precise, quantitative datasets.[50]

Swalife employs deep learning, specifically convolutional neural networks (CNNs), trained on large annotated image datasets to perform robust segmentation of ACTB-stained tissues at the cellular and sometimes subcellular levels. This segmentation discriminates tumor cells from stromal and immune populations based on staining intensity and morphological features. Automated algorithms then quantify ACTB expression by calculating staining intensity distributions, percentage positivity, and spatial relationships.[51]

More than mere quantification, Swalife integrates predictive modeling to associate quantitative ACTB IHC metrics with biological outcomes. Machine learning classifiers analyze expression patterns across multiple samples, identifying features predictive of metastatic burden, response to therapy, or prognostic categories. This not only enables objective scoring but also aids in uncovering novel predictive biomarkers embedded within histological data.[52]

For instance, projects combining longitudinal in-vivo MRI with ex-vivo Swalife IHC analyses illustrate multidimensional data integration. MRI captures growth kinetics and vascular changes, while AI-driven tissue quantification exposes molecular underpinnings such as localized ACTB upregulation at invasive fronts or metastatic niches. This synergistic workflow enhances understanding of tumor biology across temporal and spatial scales.[53]

Similarly, PET/CT studies linked with Swalife analysis of excised tissues uncover metabolic heterogeneity correlated with ACTB expression variability, allowing researchers to stratify tumors not only by size or spread but by underlying cytoskeletal activity and invasiveness.[54]

Synergies and Future Potential:

The integration of non-invasive imaging with AI-powered histopathology exemplifies the evolving landscape of oncology research. Platforms like Swalife bridge in-vivo functional imaging and ex-vivo molecular detail, enabling longitudinal, multiscale understanding of breast cancer progression.[55]

Looking forward, the potential for AI extends to integrating multi-omics data—combining genomics, transcriptomics, and proteomics with imaging features to construct holistic, predictive models of tumor behavior. Such multidimensional analyses will fuel personalized therapeutic strategies by predicting patient-specific responses and resistance mechanisms grounded in ACTB biology.[56]

Furthermore, the growing availability of large, annotated histopathological datasets improves the training and robustness of AI algorithms, enhancing transferability across laboratories and clinical settings. Standardizing imaging acquisition and staining protocols will further improve data harmonization and cross-study comparability—critical for clinical translation.[57]

Challenges:
Despite these gains, several challenges persist. Harmonizing imaging acquisition parameters, IHC protocols, and AI annotation standards remains paramount to ensure reproducibility and reliability. Variability in tissue preparation, staining intensity, and sample quality introduces noise that complicates algorithm training and validation.[58]

Clinical adoption demands rigorous validation of AI models against gold-standard manual pathology and clinical outcomes, ensuring trust, regulatory compliance, and interpretability. AI systems must provide transparent, explainable insights to be accepted by pathologists and oncologists.

Conclusion:

β-actin (ACTB) has transcended its classical role as a ubiquitous cytoskeletal “housekeeping” protein to emerge as a pivotal player in the progression of breast cancer, particularly within preclinical in vivo models. The extensive

evidence synthesised in this review illuminates how ACTB expression correlates with aggressive tumour phenotypes, promotes epithelial-mesenchymal transition (EMT), drives metastatic dissemination to key organs like lungs, bone, and liver, and modulates cellular processes underlying invasion and survival. Its role is intricately linked to remodelling the cytoskeleton, facilitating invadopodia formation, and orchestrating extracellular matrix degradation—hallmarks of metastatic competency.

Preclinical mouse models, particularly xenograft and orthotopic systems, have been instrumental in elucidating ACTB's multifaceted contributions. Orthotopic models, by better recapitulating the native tumor microenvironment and metastatic niche, provide deeper insights into spatial and temporal ACTB expression changes that drive tumor progression and dissemination. Genetic (siRNA, CRISPR) and pharmacological interventions targeting ACTB in these models yield consistent evidence that ACTB downregulation reduces tumor burden, metastasis, and prolongs survival, establishing it as both a therapeutic target and pharmacodynamic biomarker.

In addition, the integration of state-of-the-art non-invasive imaging modalities—MRI, PET/CT, and ultrasound—with AI-powered digital pathology tools exemplified by Swalife HistoAnalysis represents a major leap forward in preclinical cancer research. These technologies provide unprecedented capabilities to quantitatively track ACTB dynamics longitudinally, correlate molecular alterations with tumor phenotypes, and reduce inter-observer variability inherent in manual histopathology. AI algorithms enable precise segmentation and quantification of ACTB in complex tissue specimens and integrate multi-omic datasets to predict metastatic risk and therapeutic responses.

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