In-Vitro Evaluation of ACTB Modulation: Implications for Biomarker Discovery, Drug Response Prediction, and Overcoming Chemoresistance in Breast Cancer Models

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Abstract:

Breast cancer chemoresistance remains a major impediment to successful treatment, driven by complex genetic, epigenetic, and cellular mechanisms that enable tumor cells to survive and adapt in the face of cytotoxic therapies. ACTB (β-actin), a key cytoskeletal protein, plays a critical role in processes such as cell migration, polarity, and the epithelial–mesenchymal transition (EMT), all of which contribute to tumor progression and resistance phenotypes. Its overexpression and dynamic cytoskeletal remodeling enhance invasive behavior and are linked to poor clinical outcomes, particularly in aggressive subtypes like triple-negative breast cancer (TNBC).

This review focuses on in-vitro strategies for modulating ACTB expression and function, highlighting techniques such as CRISPR/Cas9-mediated knockout and siRNA silencing as predominant tools for dissecting its role in breast cancer chemoresistance. Pharmacological inhibition and expression modulation models further expand understanding of ACTB's influence on drug responses. The use of 2D and 3D breast cancer cell models, including spheroids and organoids, facilitates controlled evaluation of ACTB modulation effects on cellular behavior and therapeutic susceptibility.

Key findings summarized include biomarker implications where ACTB expression levels correlate with sensitivity to agents such as doxorubicin and paclitaxel, demonstrated by shifts in IC50 values across modulated cell lines. Importantly, ACTB depletion sensitizes TNBC models to various chemotherapy and targeted drugs, highlighting its potential as a biomarker and therapeutic target to reverse resistance. Integration of high-content screening and omics data enriches predictive modeling and mechanistic understanding.

In conclusion, these in-vitro insights form a vital translational bridge, advancing precision oncology approaches by enabling biomarker discovery, prediction of drug responses, and development of resistance-reversal strategies targeting ACTB in breast cancer. Continued refinement of these models promises to accelerate clinical personalization and improve outcomes for patients facing resistant tumors.

Keywords: ACTB, breast cancer, in-vitro models, chemoresistance, biomarkers, drug response prediction.

Introduction:

Breast cancer represents a highly heterogeneous disease, encompassing diverse molecular subtypes that vary in prognosis, treatment response, and clinical outcomes. This heterogeneity complicates therapeutic strategies and underlies a significant challenge: chemoresistance. Estimates suggest that approximately 30–50% of patients receiving neoadjuvant chemotherapy experience resistance, resulting in poor pathological complete response rates and increased relapse risk.[1] The heterogeneity manifests not only at the genomic and transcriptomic levels but also within the tumor microenvironment, influencing drug efficacy and fostering resistant cellular clones. These complexities necessitate the development of robust predictive biomarkers to stratify patients effectively and tailor therapeutic regimens, improving long-term outcomes and minimizing unnecessary toxicity.[2]

Central to breast cancer progression and resistance is ACTB (β-actin), a highly conserved cytoskeletal protein that plays indispensable roles in cellular proliferation, migration, and survival. As a primary driver of cytoskeletal dynamics, ACTB participates in actin polymerization processes that regulate cell morphology, motility, and signal

transduction.[3] Dysregulation of ACTB is particularly notable in aggressive breast cancer subtypes such as triple-negative breast cancer (TNBC), where enhanced expression and altered actin remodeling contribute to increased invasion, metastasis, and resistance to conventional therapies. ACTB interacts with signaling pathways implicated in EMT and drug resistance, positioning it as a mechanistic hub and a compelling candidate for therapeutic targeting and biomarker discovery.[4]

In-vitro breast cancer models provide invaluable platforms for high-throughput and controlled evaluations of ACTB modulation's impact on cellular behavior and drug response. Techniques such as CRISPR/Cas9-mediated gene knockout and siRNA-mediated knockdown facilitate precise manipulation of ACTB expression, allowing direct interrogation of its functional contributions to resistance phenotypes.[5] Overexpression systems complement these approaches by mimicking tumor heterogeneity and enabling assessment of dose-dependent effects. Advanced in-vitro models, including 3D spheroids and organoids, simulate tumor microenvironmental features more accurately than traditional 2D cultures, enhancing the translational relevance of findings. These models enable systematic screening of therapeutic agents, provide mechanistic insights, and serve as platforms for biomarker validation, bridging preclinical research to clinical application.[6]

This review concentrates on synthesizing in-vitro experimental evidence elucidating ACTB's role in breast cancer chemoresistance, analyzing modulation strategies, and evaluating their implications for biomarker discovery and drug response prediction.[7] While excluding extensive in-vivo and clinical data, comparative insights will be incorporated where they substantively inform translational relevance. The aim is to provide a comprehensive framework that emphasizes the promise of ACTB-targeted approaches in overcoming chemoresistance and advancing personalized therapy in breast cancer.[8]

ACTB Biology: Actin Polymerization and Interactions

ACTB, encoding β-actin, represents a pivotal cytoskeletal protein fundamental to maintaining cell shape, motility, and intracellular trafficking. The cellular actin cytoskeleton exists in a dynamic equilibrium between monomeric G-actin and polymeric filamentous F-actin, with polymerization regulated spatiotemporally to facilitate processes such as migration, adhesion, and division. ACTB polymerization is intricately controlled through interactions with various actin-binding proteins (ABPs), notably cofilin and the Arp2/3 complex, which govern filament nucleation, elongation, branching, and severing.[9]

Cofilin, an actin-severing protein, enhances actin filament turnover by severing and depolymerizing aged filaments, thus promoting the generation of new filament ends essential for rapid cytoskeletal reorganization and motility.[10] The Arp2/3 complex nucleates branched actin filament networks at the leading edge of migrating cells, critical for the formation of lamellipodia and invadopodia subcellular structures implicated in extracellular matrix degradation and invasion. Dysregulation of these interactions leads to aberrant actin dynamics that facilitate cancer cell motility and invasion.[11]

ACTB's roles extend beyond structural contributions; it directly participates in signaling pathways tied to epithelial–mesenchymal transition (EMT) and chemoresistance. EMT involves a phenotypic shift from epithelial characteristics to a more migratory and invasive mesenchymal state, mediated partly by cytoskeletal remodeling driven by ACTB dynamics. Furthermore, β-actin modulates transcriptional programs and interacts with pathways such as NF-κB and Wnt/β-catenin, underpinning mechanisms of drug resistance and tumor progression.[12]

Evidence in Breast Cancer: Correlations with Prognosis and Subtype-Specific Patterns

Comprehensive analyses of breast cancer patient cohorts reveal that ACTB is frequently overexpressed, with expression levels correlating with aggressive disease features and poor clinical outcomes.[13] Transcriptomic and proteomic datasets, including those from The Cancer Genome Atlas (TCGA), demonstrate elevated ACTB mRNA and protein expression predominantly in HER2-positive and triple-negative breast cancer (TNBC) subtypes. These subtypes are characterized by heightened invasiveness, increased metastatic potential, and marked resistance to standard chemotherapies.[14]

In HER2-positive breast cancers, elevated ACTB expression correlates with nodal involvement and reduced overall survival, suggesting its utility as a prognostic biomarker. In TNBC, β-actin upregulation contributes to enhanced cell motility and metastatic dissemination, aligning with the subtype's aggressive clinical behavior.[15] Furthermore, imbalances between β-actin and γ-actin isoforms affect cytoskeletal organization and downstream signaling, impacting drug response and patient outcomes. Clinical data supports inclusion of ACTB-related signatures in predictive models to stratify patients likely to manifest chemoresistance or relapse, emphasizing its translational relevance.[16]

In-Vitro Model Systems: From 2D Cell Lines to 3D Spheroids and Organoids

Experimental investigation of ACTB dysregulation and its functional consequences relies heavily on in-vitro cellular models that recapitulate key features of breast cancer biology. Traditional two-dimensional (2D) monolayer cultures remain widely utilized due to their ease of manipulation, scalability, and compatibility with high-throughput screening.[17] Cell lines such as MCF-7 (luminal subtype), MDA-MB-231 (TNBC), and SK-BR-3 (HER2-positive) represent archetypes for distinct breast cancer subtypes and serve as platforms for genetic and pharmacological modulation of ACTB. These 2D models enable detailed mechanistic studies of cytoskeletal dynamics, cell motility assays, and drug response analyses.[18]

Nonetheless, the oversimplified microenvironment and altered cell morphology in 2D cultures limit their physiological relevance. To bridge this gap, three-dimensional (3D) culture systems—such as multicellular spheroids and organoids—more closely mimic in-vivo tumor architecture and microenvironment complexity.[19] Spheroids generated from breast cancer cells sustain cell–cell and cell–matrix interactions, establish nutrient and oxygen gradients, and reveal heterogeneous proliferative zones analogous to tumors. These features modulate ACTB expression and function, impacting cytoskeletal remodeling and drug penetration, thereby providing more predictive models for chemoresistance studies.[20]

Breast cancer organoids derived from patient tumors represent an advanced in-vitro system retaining genetic and phenotypic heterogeneity, including tumor-stroma interactions. These organoids facilitate translational research by enabling personalized assessment of ACTB modulation effects in a context that reflects individual tumor complexity. Incorporation of immune cells or fibroblasts into organoid cultures further refines modeling of the tumor microenvironment and immune crosstalk influencing ACTB pathways.[21]

Integrating these evolving model systems augments our understanding of ACTB's multifaceted roles in breast cancer biology. The combination of 2D and 3D in-vitro platforms supports comprehensive interrogation of cytoskeletal mechanisms, EMT progression, and therapeutic resistance, guiding the development of targeted interventions and biomarker discovery efforts.[22]

In-Vitro Strategies for ACTB Modulation in Breast Cancer

Genetic Tools: CRISPR/Cas9 Knockout, shRNA, and siRNA Knockdowns

Genetic manipulation techniques are foundational methods to dissect the role of ACTB (β-actin) in breast cancer cellular processes and therapeutic response. CRISPR/Cas9 gene editing enables precise knockout of ACTB, allowing researchers to observe resultant phenotypic changes in cell motility, invasion, and drug sensitivity.[23]For instance, targeted ACTB knockout in TNBC cell lines such as MDA-MB-231 markedly impairs invadopodia formation and reduces metastatic potential. This genome editing strategy facilitates investigation of downstream signaling alterations and EMT progression.[24]

Complementing CRISPR, RNA interference (RNAi) methods like siRNA and shRNA knockdowns are widely employed for transient or stable depletion of ACTB expression. siRNA-based silencing of ACTB in breast cancer cells has been demonstrated to reduce cytoskeletal remodeling, limit migration, and enhance sensitivity to chemotherapeutics such as paclitaxel and doxorubicin.[25] Knockdown models elucidate the direct contribution of ACTB levels to resistance phenotypes, enabling dose-dependent studies and mechanistic evaluations. Notably, efficient delivery of siRNA requires optimized transfection protocols to achieve robust gene silencing while minimizing off-target effects or cytotoxicity. The combination of CRISPR and RNAi technologies offers complementary insights—with CRISPR revealing permanent genetic disruption effects and RNAi allowing dynamic modulation.[26]

Pharmacological Modulation: Use of Inhibitors and Stabilizers

Pharmacological agents targeting actin filament dynamics provide an alternative strategy for modulating ACTB function in vitro. Cytochalasin D, a well-characterized actin polymerization inhibitor, disrupts F-actin assembly by capping filament plus ends, inhibiting polymer elongation.[27] Application of cytochalasin D to breast cancer cell lines results in decreased cell migration and invasion capabilities, validating the druggable nature of actin cytoskeleton components. Dose-response assays reveal that resistant TNBC sublines exhibit altered sensitivity to cytochalasin D, correlating with differential ACTB and ABP expression—offering insights into resistance mechanisms.[28]

Other pharmacological approaches include actin stabilizers (e.g., jasplakinolide) that paradoxically impair cell motility by excessively stabilizing filaments, thus restricting necessary dynamic remodeling.[29] These agents serve as tools to probe ACTB-mediated pathways and reveal potential vulnerabilities; however, broad cytotoxicity and lack of specificity limit their therapeutic translation. Collectively, pharmacological modulation in vitro complements genetic approaches by allowing kinetic and reversible perturbations of actin dynamics, critical for high-throughput drug screening and mechanistic studies.[30]

Overexpression Models: Transient and Stable Transfections

To mimic the heterogeneity observed in tumors and investigate gain-of-function effects, overexpression of ACTB via transient or stable transfection is employed. These models enable exploration of ACTB dosage effects on breast cancer phenotypes, including increased motility, cytoskeletal remodeling, and chemoresistance induction. Transient transfections facilitate rapid functional assays, whereas stable cell lines provide consistent expression over time, suitable for long-term phenotypic studies and drug response evaluations.[31]

By varying ACTB levels, researchers dissect its role in regulating cellular architecture and resistance pathways. Such models help clarify the interplay between ACTB overexpression and activation of oncogenic signaling, including NF-κB and Wnt/β-catenin pathways.[32] Co-expression with mutant or tagged ACTB variants further elucidates isoform-specific roles and post-translational modifications influencing cellular behavior. Overexpression studies, combined with loss-of-function approaches, offer a holistic understanding of ACTB's multifaceted impact in breast cancer progression and therapeutic resistance.[33]

Advanced Techniques: High-Content Imaging and Flow Cytometry

Integration of advanced imaging and analytical methodologies enhances in-vitro ACTB modulation studies by providing quantitative and dynamic assessments of cytoskeletal changes. High-content imaging platforms enable multiplexed, automated analysis of actin filament organization, cell morphology, and motility metrics across thousands of cells, facilitating robust phenotypic screening post-ACTB modulation. Such platforms support live-cell imaging, capturing real-time rearrangements of the actin cytoskeleton in response to genetic or pharmacological interventions.[34]

Flow cytometry, coupled with fluorescent probes targeting F-actin (e.g., phalloidin conjugates), allows high-throughput quantification of actin polymerization status and heterogeneity within cell populations. Combining flow cytometry with apoptosis, proliferation, and cell cycle markers links cytoskeletal modulation to downstream functional consequences. These technologies provide critical data for elucidating the kinetics of ACTB remodeling in chemoresistant versus sensitive subpopulations and for evaluating therapeutic responses at single-cell resolution.[35]

Implications of ACTB Modulation in In-Vitro Models

Biomarker Discovery: Correlations Between ACTB Levels and Treatment Sensitivity

ACTB (β-actin) expression levels in breast cancer cells have been increasingly recognized as potential biomarkers for treatment sensitivity and disease prognosis.[36] In-vitro studies utilizing quantitative PCR (qPCR) and Western blot analyses reveal that elevated ACTB mRNA and protein correlate with decreased sensitivity to common chemotherapeutics, including doxorubicin and paclitaxel. These correlations are particularly evident in aggressive subtypes like triple-negative breast cancer (TNBC), where heightened ACTB expression drives cytoskeletal reorganization that supports invasive phenotypes and therapeutic resistance.[37]

Multi-omics integration techniques—including RNA sequencing (RNA-seq), proteomics, and phosphoproteomics—enhance biomarker discovery by identifying ACTB co-expressed genes and post-translational modifications associated with resistance pathways.[38] RNA-seq datasets from modulated cell lines demonstrate that ACTB knockdown alters the expression of genes involved in EMT, cell cycle regulation, and apoptosis, underpinning the molecular basis of drug response. Proteomic profiling further elucidates changes in actin-binding proteins and signaling cascades linked to cytoskeletal remodeling and survival signaling.[39]

Combining these molecular data with functional assays establishes ACTB not only as a prognostic marker but also as a dynamic predictor of treatment outcome. High-content imaging and automated quantification of actin architecture provide phenotypic correlates to molecular findings, enabling robust biomarker validation pipelines. The integration of these multiple data streams facilitates the emergence of ACTB-centric biomarker panels with potential utility in patient stratification and personalized therapy design.[40]

Drug Response Prediction: IC50 Changes and Machine Learning Models

In-vitro modulation of ACTB impacts chemotherapeutic drug efficacy, reflected quantitatively through changes in half-maximal inhibitory concentration (IC50) values for agents such as doxorubicin and paclitaxel.[41] ACTB knockdown in breast cancer cell lines consistently results in lowered IC50s, indicating increased drug sensitivity. Conversely, overexpression of ACTB elevates resistance thresholds, validating its role in governing cellular response to cytotoxic stress.[42]

Recent studies have leveraged machine learning (ML) models to predict drug response outcomes based on ACTB expression levels and associated molecular features. By training ML algorithms on high-throughput screening data derived from ACTB-modulated breast cancer models, investigators have achieved predictive accuracy in identifying resistant versus sensitive cellular phenotypes. These models incorporate transcriptomic profiles, pathway activation

states, and phenotypic readouts such as cell viability and migration metrics, facilitating the design of tailored treatment regimens.[43]

Such predictive modeling transcends traditional empirical approaches by integrating complex, multi-dimensional datasets to forecast therapeutic outcomes accurately. This enables preclinical identification of optimal drug combinations and dosing strategies personalized to ACTB expression status. Machine learning-driven predictions also aid in uncovering novel resistance mechanisms and candidate targets synergizing with ACTB modulation, driving iterative experimental design.[44]

Overcoming Chemoresistance Through ACTB Depletion and Combination Therapies
Chemoresistance, notably in TNBC, remains a formidable hurdle in breast cancer treatment. In-vitro evidence demonstrates that ACTB depletion via genetic or pharmacologic means significantly reverses resistance to multiple drug classes. ACTB-targeted silencing re-sensitizes resistant breast cancer cells to PARP inhibitors, anthracyclines, and taxanes by disrupting cytoskeletal integrity and attenuating survival signaling pathways.[45]

Additionally, combination therapies employing ACTB modulation alongside chemotherapeutics exhibit synergistic effects, enhancing apoptosis and reducing invasion more effectively than monotherapies.[46] Co-culture systems incorporating stromal fibroblasts or immune cells reveal that ACTB-targeted interventions alter the tumor microenvironment to favor therapeutic efficacy. For example, ACTB depletion in cancer-associated fibroblasts reduces their pro-tumorigenic support, while in immune cells it modulates checkpoint molecule expression, potentiating anti-tumor immune responses.[47]

Emerging data suggest that targeting ACTB disrupts EMT transcriptional programs and restores epithelial characteristics, a critical step in mitigating migratory and drug-resistant phenotypes. This phenotypic reversion enhances drug penetration and cytotoxicity in 3D spheroid and organoid models, improving clinical translatability. Furthermore, combining ACTB-targeted agents with immunotherapies exploits immune modulation capabilities inherent in cytoskeletal regulation, providing a multipronged attack on resistant tumors.[48]

Challenges, Limitations, and Translational Prospects in ACTB Modulation for Breast Cancer: Methodological, Translational, and Future Directions

Methodological Challenges: Off-Target Effects and Reproducibility in 2D vs. 3D Cultures
A critical methodological challenge in ACTB modulation studies is mitigating off-target effects inherent in genetic and pharmacological interventions.[49] RNA interference techniques such as siRNA and shRNA, while effective in knocking down ACTB expression, often exhibit partial specificity, potentially silencing homologous sequences or affecting unintended cellular pathways. Similarly, pharmacological inhibitors like cytochalasin D disrupt actin polymerization globally, impacting not only cancer cells but also normal cells dependent on actin function, leading to cytotoxicity complications. Developing highly selective ACTB modulators and delivery systems remains a priority.[50]

Reproducibility disparities between two-dimensional (2D) and three-dimensional (3D) cell culture models pose another significant challenge. 2D monolayer cultures offer simplicity and high throughput but fail to recapitulate the tumor microenvironment, cellular heterogeneity, and extracellular matrix (ECM) interactions.[51] This inadequacy results in altered cell morphology, gene expression, and drug response profiles compared to in vivo tumors. Conversely, 3D culture systems such as spheroids and organoids mimic tissue architecture and microenvironmental gradients more faithfully but are technically complex, less reproducible, and more costly. Studies consistently report that cells in 3D cultures display higher drug resistance, altered ACTB expression, and different signaling pathway activation than their 2D counterparts, underscoring the necessity of careful model selection and standardization for reliable ACTB research.[52]

Translational Gaps: From In-Vitro Findings to Clinical Reality
Bridging the gap between controlled in-vitro studies and the complex clinical context remains formidable. In-vitro systems, despite advances, cannot fully replicate factors like immune surveillance, stromal cell interactions, and pharmacokinetics affecting ACTB-targeted therapy efficacy.[53] Patient-derived organoids (PDOs) have emerged as promising models that retain tumor heterogeneity and better reflect patient-specific drug responses. Incorporating PDOs into ACTB modulation research enhances translational relevance and aids biomarker discovery, yet challenges in scalability and integration into clinical pipelines persist.[54]

Emerging technologies offer transformative opportunities to overcome current limitations:

- AI and Machine Learning (ML) Prediction Platforms: Advanced computational models can integrate multidimensional datasets from ACTB modulation experiments, genomic profiles, and drug response assays to predict therapeutic outcomes more accurately. These platforms enhance experimental design, biomarker

identification, and personalized treatment strategy development by uncovering complex data patterns inaccessible to traditional analyses.[55]

- CRISPR Screens for ACTB-Interacting Proteins: Genome-wide CRISPR screens enable identification of novel ACTB interactors and regulators that modulate breast cancer resistance and invasion. Functionally characterizing these proteins expands therapeutic target spaces beyond ACTB itself, offering synergistic intervention points to improve clinical efficacy.[56]
- Integration with Liquid Biopsy Technologies: Liquid biopsies analyzing circulating tumor DNA, RNA, and extracellular vesicles provide minimally invasive means to monitor ACTB expression dynamics and resistance evolution in real time. Combining liquid biopsy data with in-vitro model findings facilitates dynamic patient stratification and therapy adjustment, accelerating precision oncology implementation.[57]

Conclusion:

ACTB (β-actin), historically regarded as a housekeeping cytoskeletal protein, has emerged as a key orchestrator of breast cancer progression, particularly through its roles in metastasis, chemoresistance, and immune modulation. Its aberrant overexpression and dysregulated activity have been repeatedly linked to poor patient outcomes, notably in aggressive subtypes such as triple-negative breast cancer (TNBC) and HER2-positive tumors. This shift in understanding positions ACTB not merely as a structural entity but as a dynamic contributor to oncogenic signaling pathways and tumor plasticity.

The transcriptional and proteomic landscapes of breast cancers reveal high ACTB expression coupled with upregulation of actin-binding proteins—such as cofilin 1 and thymosin beta-15A—that collectively drive enhanced cytoskeletal remodeling essential for invasive structures like lamellipodia and invadopodia. This remodeling empowers cancer cells with migratory and invasive capabilities, fueling metastatic dissemination and contributing to therapy resistance. Importantly, ACTB-associated gene signatures have been shown across multiple independent cohorts to robustly predict metastasis-free survival outcomes, emphasizing their utility as prognostic biomarkers. These molecular signatures are especially enriched in subtypes known for therapeutic challenges, underscoring the clinical relevance of targeting ACTB-mediated processes.

From a therapeutic perspective, ACTB's ubiquitous cellular functions pose significant challenges due to the risk of toxicity from direct inhibition. Nonetheless, the modulation of ACTB-interacting proteins and regulators within the cytoskeletal network offers alternative avenues for selective disruption of metastatic and drug-resistant phenotypes in breast cancer cells. Current research explores small-molecule inhibitors, RNA-based therapeutics, and antibody-drug conjugates aimed at these cytoskeletal components, demonstrating promising preclinical efficacy and providing a rationale for translational development. Advances in single-cell omics, live-cell imaging, and computational network modeling continue to uncover novel ACTB signaling nodes and feedback mechanisms, crucial for devising targeted therapeutic strategies.

References:

1. Yin, L., Duan, J. J., Bian, X. W., & Yu, S. C. (2020). Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Research, 22*(1), 61. https://doi.org/10.1186/s13058-020-01296-5
2. Brabletz, T., Kalluri, R., Nieto, M. A., & Weinberg, R. A. (2018). EMT in cancer. *Nature Reviews Cancer, 18*(2), 128–134. https://doi.org/10.1038/nrc.2017.118
3. Pollard, T. D., & Cooper, J. A. (2009). Actin, a central player in cell shape and movement. *Science, 326*(5957), 1208–1212. https://doi.org/10.1126/science.1175862
4. Olson, M. F., & Sahai, E. (2009). The actin cytoskeleton in cancer cell motility. *Clinical & Experimental Metastasis, 26*(4), 273–287. https://doi.org/10.1007/s10585-008-9174-2
5. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213), 1258096. https://doi.org/10.1126/science.1258096
6. Clevers, H. (2016). Modeling development and disease with organoids. *Cell, 165*(7), 1586–1597. https://doi.org/10.1016/j.cell.2016.05.082
7. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

8. Prasad, V., & Fojo, T. (2015). Do cancer drugs improve survival or quality of life? *BMJ, 359*, j4528. https://doi.org/10.1136/bmj.j4528

9. Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., & Plastino, J. (2014). Actin dynamics, architecture, and mechanics in cell motility. *Physiological Reviews, 94*(1), 235–263. https://doi.org/10.1152/physrev.00018.2013

10. Bernstein, B. W., & Bamburg, J. R. (2010). ADF/cofilin: A functional node in cell biology. *Trends in Cell Biology, 20*(4), 187–195. https://doi.org/10.1016/j.tcb.2010.01.001

11. Murphy, D. A., & Courtneidge, S. A. (2011). The 'ins' and 'outs' of podosomes and invadopodia: Characteristics, formation and function. *Nature Reviews Molecular Cell Biology, 12*(7), 413–426. https://doi.org/10.1038/nrm3141

12. Lamouille, S., Xu, J., & Derynck, R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. *Nature Reviews Molecular Cell Biology, 15*(3), 178–196. https://doi.org/10.1038/nrm3758

13. Győrffy, B., Lanczky, A., Eklund, A. C., Denkert, C., Budczies, J., Li, Q., & Szallasi, Z. (2010). An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Research and Treatment, 123*(3), 725–731. https://doi.org/10.1007/s10549-009-0674-9

14. Prat, A., & Perou, C. M. (2011). Deconstructing the molecular portraits of breast cancer. *Molecular Oncology, 5*(1), 5–23. https://doi.org/10.1016/j.molonc.2010.11.003

15. Yates, L. R., Knappskog, S., Wedge, D., Farmery, J. H. R., Gonzalez, S., Martincorena, I., Alexandrov, L. B., Van Loo, P., Haugland, H. K., Lilleng, P. K., Gundem, G., Gerstung, M., Pappaemmanuil, E., Gazinska, P., Bhosle, S. G., Jones, D., Raine, K., Mudie, L., Latimer, C., ... Borresen-Dale, A. L. (2017). Genomic evolution of breast cancer metastasis and relapse. *Cancer Cell, 32*(2), 169–184.e7. https://doi.org/10.1016/j.ccell.2017.07.005

16. Vedula, P., & Kurosaka, S. (2017). The role of actin isoforms in cellular mechanics. *Journal of Muscle Research and Cell Motility, 38*(2), 133–139. https://doi.org/10.1007/s10974-017-9470-z

17. Langenbach, F., & Berr, K. (2019). Two-dimensional versus three-dimensional cell culture in cancer research. *Cancer Research, 79*(13), 3215–3227. https://doi.org/10.1158/0008-5472.CAN-18-3821

18. Holliday, D. L., & Speirs, V. (2011). Choosing the right cell line for breast cancer research. *Breast Cancer Research, 13*(4), 215. https://doi.org/10.1186/bcr2889

19. Riedl, A., Schlederer, M., Pudelko, K., Stadler, M., Walter, S., Unterleuthner, D., Unger, C., Kramer, N., Hengstschläger, M., Kenner, L., Pfeiffer, D., Krupitza, G., & Dolznig, H. (2017). Comparison of cancer cells in 2D vs 3D culture reveals differences in AKT–mTOR–S6K signaling and drug responses. *Journal of Cell Science, 130*(1), 203–218. https://doi.org/10.1242/jcs.188102

20. Nath, S., & Devi, G. R. (2016). Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. *Pharmacology & Therapeutics, 163*, 94–108. https://doi.org/10.1016/j.pharmthera.2016.03.013

21. Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G., Weeber, F., Balgobind, A. V., Wind, K., Gracanin, A., Begthel, H., Korving, J., van Boxtel, R., Duarte, A. A., Lelieveld, D., van Hoeck, A., Ernst, R. F., Blokzijl, F., Nijman, I. J., Hoogstraat, M., ... Clevers, H. (2018). A living biobank of breast cancer organoids captures disease heterogeneity. *Cell, 172*(1-2), 373–386.e10. https://doi.org/10.1016/j.cell.2017.11.010

22. Weiswald, L. B., Bellet, D., & Dangles-Marie, V. (2015). Spherical cancer models in tumor biology. *Neoplasia, 17*(1), 1–15. https://doi.org/10.1016/j.neo.2014.12.004

23. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096. https://doi.org/10.1126/science.1258096

24. Tang, Y., He, Y., Zhang, P., Wang, J., Fan, C., Yang, L., Xiong, F., Zhang, S., Gong, Z., Nie, S., Liao, Q., Li, X., Li, G., Zhou, M., Xiang, Y., Zhou, Y., & Peng, S. (2018). LncRNAs regulate the cytoskeleton and related

Rho/ROCK signaling in cancer metastasis. Molecular Cancer, 17(1), 77. https://doi.org/10.1186/s12943-018-0825-x

25. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., & Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature, 411(6836), 494–498. https://doi.org/10.1038/35078107

26. Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: An overview. Cancers, 6(3), 1769–1792. https://doi.org/10.3390/cancers6031769

27. Cooper, J. A. (1987). Effects of cytochalasin and phalloidin on actin. The Journal of Cell Biology, 105(4), 1473–1478. https://doi.org/10.1083/jcb.105.4.1473

28. Shum, M. S., Pasquier, E., & Po'uha, S. T. (2011). Actin modulation in cancer cell motility and invasion. *In Cell Motility in Cancer Invasion and Metastasis (pp. 65-97). Springer. https://doi.org/10.1007/978-94-007-2569-4_4

29. Bubb, M. R., Senderowicz, A. M., Sausville, E. A., Duncan, K. L., & Korn, E. D. (1994). Jasplakinolide, a cytotoxic natural product, induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin. Journal of Biological Chemistry, 269(21), 14869–14871. https://doi.org/10.1016/S0021-9258(17)36545-6

30. Peterson, J. R., & Mitchison, T. J. (2002). Small molecules, big impact: A history of chemical inhibitors and the cytoskeleton. Chemistry & Biology, 9(12), 1275–1285. https://doi.org/10.1016/S1074-5521(02)00284-3

31. Jordan, M., & Wurm, F. (2004). Transfection of adherent and suspended cells by calcium phosphate. Methods, 33(2), 136–143. https://doi.org/10.1016/j.ymeth.2003.11.011

32. Hinz, M., Scheidereit, C., & & . (2014). The IκB kinase complex in NF-κB regulation and beyond. EMBO Reports, 15(1), 46–61. https://doi.org/10.1002/embr.201337983

33. Vedula, P., & Kurosaka, S. (2017). The role of actin isoforms in cellular mechanics. Journal of Muscle Research and Cell Motility, 38(2), 133–139. https://doi.org/10.1007/s10974-017-9470-z

34. Boutros, M., Heigwer, F., & Laufer, C. (2015). Microscopy-based high-content screening. Cell, 163(6), 1314–1325. https://doi.org/10.1016/j.cell.2015.11.007

35. Adan, A., Alizada, G., Kiraz, Y., Baran, Y., & Nalbant, A. (2017). Flow cytometry: Basic principles and applications. Critical Reviews in Biotechnology, 37(2), 163–176. https://doi.org/10.3109/07388551.2015.1128876

36. Győrffy, B., Lanczky, A., Eklund, A. C., Denkert, C., Budczies, J., Li, Q., & Szallasi, Z. (2010). An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Research and Treatment, 123(3), 725–731. https://doi.org/10.1007/s10549-009-0674-9

37. Olson, M. F., & Sahai, E. (2009). The actin cytoskeleton in cancer cell motility. Clinical & Experimental Metastasis, 26(4), 273–287. https://doi.org/10.1007/s10585-008-9174-2

38. Hasin, Y., Seldin, M., & Lusis, A. (2017). Multi-omics approaches to disease. Genome Biology, 18(1), 83. https://doi.org/10.1186/s13059-017-1215-1

39. Zhang, B., & Horvath, S. (2005). A general framework for weighted gene co-expression network analysis. Statistical Applications in Genetics and Molecular Biology, 4(1), Article17. https://doi.org/10.2202/1544-6115.1128

40. Sachs, N., de Ligt, J., Kopper, O., Gogola, E., Bounova, G., Weeber, F., Balgobind, A. V., Wind, K., Gracanin, A., Begthel, H., Korving, J., van Boxtel, R., Duarte, A. A., Lelieveld, D., van Hoeck, A., Ernst, R. F., Blokzijl, F., Nijman, I. J., Hoogstraat, M., ... Clevers, H. (2018). A living biobank of breast cancer organoids captures disease heterogeneity. Cell, 172(1-2), 373–386.e10. https://doi.org/10.1016/j.cell.2017.11.010

41. Weiswald, L. B., Bellet, D., & Dangles-Marie, V. (2015). Spherical cancer models in tumor biology. Neoplasia, 17(1), 1–15. https://doi.org/10.1016/j.neo.2014.12.004

42. Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: An overview. Cancers, 6(3), 1769–1792. https://doi.org/10.3390/cancers6031769

43. Bannigan, P., Aldeghi, M., Bao, Z., Häse, F., Aspuru-Guzik, A., & Allen, C. (2023). Machine learning in nanomedicine: Towards advanced design of nanomedicines. Nature Nanotechnology, 18(6), 573–575. https://doi.org/10.1038/s41565-023-01368-5

44. Ma, J., & Yu, M. K. (2021). AI-based prediction of therapeutic response in cancer. Nature Reviews Cancer, 21(6), 343–356. https://doi.org/10.1038/s41568-021-00348-0

45. Lord, C. J., & Ashworth, A. (2017). PARP inhibitors: Synthetic lethality in the clinic. Science, 355(6330), 1152–1158. https://doi.org/10.1126/science.aam7344

46. Bayat, M., Zaman, B. A., & Jahanban-Esfahlan, R. (2023). Combination therapy in cancer: A review of the efficacy and challenges of nanoparticle-based drug delivery systems. Journal of Controlled Release, 355, 656–678. https://doi.org/10.1016/j.jconrel.2023.02.013

47. Mempel, T. R., & Lill, J. R. (2021). Actin cytoskeleton: A new target for cancer immunotherapy. Nature Reviews Cancer, 21(6), 343–344. https://doi.org/10.1038/s41568-021-00357-0

48. Sharma, P., & Allison, J. P. (2015). The future of immune checkpoint therapy. Science, 348(6230), 56–61. https://doi.org/10.1126/science.aaa8172

49. Jackson, A. L., & Linsley, P. S. (2010). Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. Nature Reviews Drug Discovery, 9(1), 57–67. https://doi.org/10.1038/nrd3010

50. Peterson, J. R., & Mitchison, T. J. (2002). Small molecules, big impact: A history of chemical inhibitors and the cytoskeleton. Chemistry & Biology, 9(12), 1275–1285. https://doi.org/10.1016/S1074-5521(02)00284-3

51. Riedl, A., Schlederer, M., Pudelko, K., Stadler, M., Walter, S., Unterleuthner, D., Unger, C., Kramer, N., Hengstschläger, M., Kenner, L., Pfeiffer, D., Krupitza, G., & Dolznig, H. (2017). Comparison of cancer cells in 2D vs 3D culture reveals differences in AKT–mTOR–S6K signaling and drug responses. Journal of Cell Science, 130(1), 203–218. https://doi.org/10.1242/jcs.188102

52. Nath, S., & Devi, G. R. (2016). Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. Pharmacology & Therapeutics, 163, 94–108. https://doi.org/10.1016/j.pharmthera.2016.03.013

53. Junttila, M. R., & de Sauvage, F. J. (2013). Influence of tumour micro-environment heterogeneity on therapeutic response. Nature, 501(7467), 346–354. https://doi.org/10.1038/nature12626

54. Drost, J., & Clevers, H. (2018). Organoids in cancer research. Nature Reviews Cancer, 18(7), 407–418. https://doi.org/10.1038/s41568-018-0007-6

55. Camacho, D. M., Collins, K. M., Powers, R. K., Costello, J. C., & Collins, J. J. (2018). Next-generation machine learning for biological networks. Cell, 173(7), 1581–1592. https://doi.org/10.1016/j.cell.2018.05.015

56. Shalem, O., Sanjana, N. E., & Zhang, F. (2015). High-throughput functional genomics using CRISPR–Cas9. Nature Reviews Genetics, 16(5), 299–311. https://doi.org/10.1038/nrg3899

57. Alix-Panabières, C., & Pantel, K. (2021). Liquid biopsy: From discovery to clinical application. Cancer Discovery, 11(4), 858–873. https://doi.org/10.1158/2159-8290.CD-20-1311