In Vivo Modelling of ACTN4 in Oral Cancer: Tracking Tumor Progression and Metastasis

Dr Pravin Badhe

Swalife Biotech Ltd North Point House, North Point Business Park, New Mallow Road, Cork (Republic of Ireland)*

Corresponding author: drpravinbadhe@swalifebiotech.com
Doi: https://doi.org/10.5281/zenodo.17898256
Received: 10 October 2025
Accepted: 18 November 2025

Abstract

Oral squamous cell carcinoma (OSCC) is marked by its aggressive clinical behavior and high propensity for metastasis, necessitating deeper insights into the molecular drivers of tumor progression. ACTN4, an actin-binding protein, has been independently linked to increased cell motility, invasion, and metastatic potential in OSCC, with gene amplification and overexpression serving as prognostic indicators of poor patient outcomes. Recent investigations highlight ACTN4 as a critical modulator in epithelial–mesenchymal transition, extracellular matrix remodeling, and cytoskeletal reorganization, thereby directly promoting both local invasion and distant dissemination of cancer cells. To unravel the mechanistic underpinnings and functional impact of ACTN4 in OSCC, advanced in vivo models—utilizing gene-editing, orthotopic implantation, and metastatic tracking protocols—are now employed to dynamically monitor tumor growth and metastatic events. Integration of artificial intelligence-assisted histopathology, leveraging machine learning algorithms on digitized tissue sections, enables robust quantitative assessments of cellular architecture, invasion patterns, and molecular marker distribution, thus enhancing the precision of tissue phenotyping and metastatic burden analysis. Collectively, these approaches provide a transformative framework for elucidating ACTN4's pro-metastatic role and identifying actionable therapeutic targets in OSCC.

Keywords: ACTN4, Oral Cancer, Metastasis, In Vivo Models, AI Histopathology, Tumor Microenvironment, Angiogenesis

1.Introduction

Oral cancer remains a major public health challenge globally, with oral squamous cell carcinoma (OSCC) as the most prevalent subtype, accounting for significant morbidity and mortality across varied geographic regions. Recent Global Burden of Disease (GBD) studies report a marked upward trend in incidence, mortality, and disability-adjusted life years (DALYs) for oral cancer between 1990 and 2021, with rates rising from 3.26 to 5.34 per 100,000 for incidence and 1.83 to 2.64 per 100,000 for mortality. The disease burden is exacerbated in regions with high prevalence of risk factors such as tobacco usage, betel quid chewing, and alcohol consumption, particularly in parts of Asia and the Pacific. Despite advances in diagnostic modalities and therapeutic interventions, predicting metastatic behavior accurately—and intervening before dissemination—remains hindered by complex tumor biology and unreliable biomarkers. Late-stage diagnosis and frequent cervical lymph node metastasis contribute to poor prognosis and high recurrence rates among OSCC patients.

In this context, the cytoskeletal protein ACTN4 (α-actinin-4) has emerged as a key driver in cancer cell motility and metastatic progression. As a member of the actin-binding protein family, ACTN4 orchestrates cellular adhesion, migration, and invasion by crosslinking actin filaments and modulating focal adhesion dynamics. Functional studies reveal that ACTN4 not only regulates protrusive forces for cell movement, but is also essential for nuclear translocation and efficient migration through tissue matrices, primarily via transcriptional regulation of non-muscle myosin IIB and association with myosin IIA. Genetic alterations and dysregulation of ACTN4 activate several oncogenic pathways including epithelial–mesenchymal transition and extracellular matrix remodeling, directly fueling local invasion and metastatic dissemination. Knockdown or pharmacological inhibition of ACTN4 attenuates tumor cell motility, blocks malignant transformation, and impairs metastatic potential, suggesting its pivotal role in aggressive disease phenotypes.

Extensive clinical evidence supports the association of ACTN4 gene amplification and protein overexpression with adverse outcomes in oral cancer. Retrospective analyses and cohort studies demonstrate that increased ACTN4 copy number in tumor tissues correlates with higher rates of cervical lymph node metastasis, shorter overall survival, and elevated risk of recurrence, independent of traditional histopathological parameters. Kaplan–Meier survival curves consistently show poorer outcomes for patients with ACTN4-positive tumors compared to those lacking amplification or overexpression, underscoring its importance as a prognostic biomarker for OSCC. Moreover, these clinical correlations have been validated across multiple cancer types—including breast, lung, and gastric cancers—reinforcing ACTN4's broad oncogenic role.

However, while associations abound, establishing direct causal relationships between ACTN4 activity, tumor progression, and metastasis requires rigorous functional validation using in vivo models. Preclinical studies employing gene knockout, overexpression, orthotopic xenograft implantation, and metastatic tracking protocols serve to dissect ACTN4's mechanistic role in OSCC biology. Such models enable real-time observation of tumor growth kinetics, invasive patterns, and metastatic dissemination, providing empirical evidence to support clinical correlations. Integrating animal models with advanced imaging and molecular profiling allows researchers to delineate ACTN4-driven cellular programs and identify potential therapeutic vulnerabilities.

In conclusion, given the mounting global burden of OSCC and persistent challenges in predicting and intercepting metastatic spread, investigating ACTN4 is both timely and essential. In vivo modeling not only bridges the gap between clinical observations and causality but also paves the way toward targeted therapies aimed at mitigating tumor progression and metastasis. This review consolidates current evidence on ACTN4's role in oral cancer, highlights clinical and epidemiological context, and advocates for translational, mechanistic studies to unravel its oncogenic functions in vivo.

2. ACTN4 as a Molecular Driver of Tumor Invasion and Metastasis

ACTN4 (α-actinin-4) is a cytoskeletal actin-binding protein that plays a pivotal role in organizing actin filaments, thereby orchestrating cell motility, adhesion, and morphological changes critical for tumor invasion and metastasis. Structurally, ACTN4 forms anti-parallel dimers with two calponin homology domains at the N-terminus to bind actin and a calmodulin-like domain at the C-terminus facilitating protein-protein interactions. This organization enables ACTN4 to crosslink actin filaments into bundles within dynamic cellular protrusions such as filopodia and lamellipodia, essential for cellular migration.

Functionally, ACTN4 regulates focal adhesion dynamics by interacting with integrins, vinculin, and catenins, bridging the actin cytoskeleton to the plasma membrane. This modulates adhesion turnover facilitating cell movement through extracellular matrices. Moreover, ACTN4 is implicated in matrix remodeling by localizing to invadopodia, specialized structures that secrete matrix metalloproteinases (MMPs) to degrade the extracellular matrix and enable invasion. Additionally, ACTN4 influences angiogenesis by modulating cytoskeletal arrangements in endothelial cells, contributing to new blood vessel formation that supports tumor growth and metastasis.

Clinical studies across various epithelial cancers, including oral squamous cell carcinoma (OSCC), reveal consistent overexpression and gene amplification of ACTN4 in aggressive tumor phenotypes. In OSCC, immunohistochemical analysis shows robust ACTN4 expression at invasive fronts correlating with enhanced migratory and invasive capabilities. Increased ACTN4 copy number detected by fluorescence in situ hybridization (FISH) has been associated with poor prognosis, advanced stage, and higher metastatic rates in oral, pancreatic, ovarian, lung, and colorectal cancers. These clinical correlations underscore ACTN4 not only as a biomarker but also as an active molecular driver contributing to malignant progression and metastatic dissemination.

Overall, mechanistic and clinical evidence converges to position ACTN4 as a key regulator of cytoskeletal reorganization, focal adhesion dynamics, extracellular matrix degradation, and angiogenesis, collectively facilitating tumor invasion and metastasis. Targeting ACTN4-mediated pathways could thus offer promising therapeutic strategies against aggressive epithelial malignancies including oral cancer.

3.In Vivo Models for ACTN4 Functional Studies

In vivo models are indispensable for dissecting the functional contributions of ACTN4 to oral squamous cell carcinoma (OSCC) progression and metastasis, providing a dynamic physiological context absent in cell culture systems. These models recapitulate tumor-host interactions, including stromal remodeling, immune surveillance, and vascularization, allowing precise evaluation of ACTN4's causal role through genetic manipulations and longitudinal monitoring. Rodent-based approaches, from chemical induction to advanced xenografts, enable mechanistic insights into ACTN4-driven cytoskeletal dynamics, invasion, and dissemination, while emerging humanized platforms enhance translational relevance. This section explores key models, highlighting their application to ACTN4 studies,

advantages, limitations, and potential for therapeutic validation. By integrating multi-modal readouts such as imaging, histopathology, and omics profiling, these models bridge preclinical findings to clinical outcomes in oral cancer.

DMBA-Induced Oral Carcinogenesis Models

The 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced model represents a cornerstone for studying chemical carcinogenesis in oral tissues, mimicking tobacco-related OSCC etiology prevalent in high-risk populations. In this paradigm, DMBA, a polycyclic aromatic hydrocarbon, is topically applied to the buccal pouch, tongue, or lip of rodents such as Syrian hamsters or NMRI mice, initiating DNA adducts that trigger mutational events akin to those in human smokers. The process unfolds over 10-20 weeks, with repeated applications (e.g., thrice weekly) leading to premalignant lesions and invasive tumors, providing a timeline to track ACTN4 expression dynamically.

Temporal progression in DMBA models mirrors human OSCC histogenesis, starting with epithelial hyperplasia and dysplasia within 4-8 weeks, progressing to papillomas and invasive squamous cell carcinoma by 12-18 weeks. ACTN4 expression correlates with advancing stages, showing baseline levels in normal mucosa but upregulation in dysplastic foci, peaking in invasive fronts where it colocalizes with actin bundles and MMPs. Immunohistochemical analyses in DMBA-treated hamster buccal pouches reveal intensified ACTN4 staining in hyperkeratotic lesions, associating with enhanced cell motility and early invasion, while qRT-PCR confirms transcriptional activation via oncogenic pathways like PI3K/Akt. In NMRI mice, ACTN4 immunoreactivity intensifies by week 12, paralleling Ki-67 proliferation and p53 dysregulation, underscoring its role in mitotic instability during multistep carcinogenesis.

This model's physiological relevance stems from its stepwise progression, recapitulating field cancerization and genetic heterogeneity observed in human OSCC, including TP53 mutations and chromosomal instability. Unlike abrupt xenograft implantation, DMBA induction allows observation of ACTN4's temporal dynamics, from hyperplasia to metastasis, facilitating biomarker validation and chemoprevention studies. For instance, ACTN4 antagonists could be tested during premalignant phases to halt progression, mirroring preventive strategies for at-risk individuals.

However, limitations include prolonged induction times (up to 20 weeks), increasing variability from animal strain differences and environmental factors, which can delay ACTN4-specific mechanistic studies. Genetic heterogeneity arises from DMBA's broad mutagenicity, complicating attribution of phenotypes solely to ACTN4 dysregulation without targeted interventions. Ethical concerns and labor-intensive monitoring further restrict scalability, though refinements like accelerated regimens in NMRI mice (lesions by 5-18 weeks) mitigate these issues.

Overall, DMBA models offer robust platforms for correlating ACTN4 expression with carcinogenesis stages, despite challenges, providing foundational insights into its prognostic and therapeutic implications in oral cancer.

Orthotopic and Xenograft Models

Orthotopic implantation models position ACTN4-manipulated OSCC cells directly into the murine tongue or buccal mucosa, restoring anatomical fidelity and enabling site-specific tumor-host interactions critical for invasion studies. Human or syngeneic OSCC cell lines, engineered for ACTN4 overexpression via lentiviral vectors or knockdown using shRNA/CRISPR-Cas9, are injected submucosally (e.g., 1-5 × 10^6 cells in Matrigel), forming tumors within 2-4 weeks that mimic primary lesion growth. This setup allows real-time tracking of ACTN4's impact on local expansion, with bioluminescent imaging revealing accelerated kinetics in ACTN4-high cells due to enhanced lamellipodia formation and adhesion turnover.

These models yield profound insights into ACTN4-driven local invasion and angiogenesis, as orthotopic tumors exhibit stromal invasion patterns indistinguishable from clinical OSCC, with ACTN4 promoting MMP-9 secretion for basement membrane breach. In tongue-implanted ACTN4-overexpressing SCC-9 cells, tumors show increased vascular density via VEGF upregulation, quantified by CD31 staining, supporting nutrient supply for aggressive growth. Metastatic dissemination to cervical lymph nodes occurs in 40-60% of cases by week 6, with lung micrometastases detected via IVIS imaging, directly linking ACTN4 to EMT induction and circulating tumor cell survival.

Genetic manipulation is pivotal, with CRISPR knockout of ACTN4 in CAL-27 cells reducing tumor volume by 70% and abolishing nodal spread, confirming causality in cytoskeletal-mediated invasion. shRNA silencing attenuates focal adhesion kinase (FAK) signaling, impairing angiogenesis and metastasis, while rescue experiments with ACTN4 mutants restore phenotypes, delineating domain-specific functions. Overexpression models, conversely, accelerate progression, with tumors showing hypermethylated promoters and aneuploidy, mirroring human amplifications.

The relevance to therapeutic targeting is evident, as these models facilitate anti-metastatic drug testing; for example, ACTN4 inhibitors like ellagic acid combined with cisplatin reduce invasion in orthotopic xenografts, extending survival by 50%. Syngeneic models in immunocompetent C57BL/6 mice (e.g., ROC cell lines) incorporate immune evasion, revealing ACTN4's role in PD-L1 upregulation and T-cell exclusion, ideal for immunotherapy combinations. Pharmacodynamic endpoints, including reduced Ki-67 and E-cadherin restoration, validate efficacy against ACTN4-driven phenotypes.

Limitations include immunodeficiency in nude mice, underrepresenting immune dynamics, and implantation artifacts potentially inflating metastasis rates. Standardization of cell doses and monitoring (e.g., weekly calipers/ultrasound) is crucial to minimize variability.

In summary, orthotopic xenografts provide mechanistic depth for ACTN4's causal roles, bridging to clinical translation through targeted interventions.

Emerging Models

Patient-derived xenografts (PDXs) advance ACTN4 studies by engrafting fresh OSCC biopsies into immunodeficient mice (e.g., NSG strains), preserving tumor heterogeneity and stroma for authentic metastasis recapitulation. Passaging up to 10 generations maintains genomic fidelity, including ACTN4 amplifications in 20-30% of high-grade OSCC PDXs, allowing evaluation of patient-specific ACTN4 variants. Humanized mice, engrafted with CD34+ hematopoietic stem cells or PBMCs, restore immune competence, enabling ACTN4's interplay with T-cell infiltration and cytokine milieus in metastatic niches.

PDXs from metastatic OSCC reveal ACTN4 overexpression correlating with lymph node engraftment rates >50%, with stromal fibroblasts enhancing invasion via TGF-β paracrine loops. In humanized setups, ACTN4-high PDXs exhibit immune-cold phenotypes, with reduced CD8+ T-cells and increased MDSCs, highlighting immunotherapy resistance mechanisms. These models support co-clinical trials, where ACTN4-targeted siRNAs delivered via nanoparticles suppress progression in patient-matched PDXs.

3D organoid implantation models integrate tumor-stroma crosstalk by deriving OSCC organoids from biopsies, co-cultured with CAFs or endothelial cells, then implanted subcutaneously or orthotopically. Organoids retain ACTN4 expression profiles, forming vascularized structures that metastasize to lungs in 30% of cases, driven by invadopodia maturation. PDOX (patient-derived organoid xenografts) combine advantages, showing 80% engraftment and stable ACTN4-driven mutations over passages, ideal for stroma-dependent studies.

These models enable multi-omics readouts of ACTN4 behavior, with single-cell RNA-seq unveiling transcriptional programs in invasive clusters and proteomics identifying phosphorylated substrates like myosin IIB. Spatial transcriptomics maps ACTN4 gradients at tumor margins, correlating with metastatic propensity, while CRISPR screens in organoids pinpoint synthetic lethals. Integration with AI-driven image analysis quantifies invasion metrics, accelerating discovery of ACTN4 inhibitors.

Challenges include low engraftment (20-40% for early-stage tumors) and high costs, but advancements like air-liquid interface cultures enhance viability. Ethical sourcing from consented patients ensures diversity, representing global OSCC subtypes.

Emerging models thus refine ACTN4 interrogation, fostering precision oncology through human-centric, multi-faceted platforms.

4.ACTN4 in Angiogenesis, Stromal Remodeling, and Metastatic Spread

ACTN4 (α-actinin-4) plays a multifaceted role in promoting tumor progression by orchestrating angiogenesis, stromal remodeling, and metastatic dissemination through key molecular pathways. Central to its pro-angiogenic function is the regulation of vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and integrin signaling pathways that collectively facilitate endothelial migration, extracellular matrix (ECM) degradation, and neovascularization within the tumor microenvironment.

Mechanistically, ACTN4 enhances VEGF expression and secretion, thereby stimulating endothelial cells to migrate and form new blood vessels necessary for tumor nourishment and growth. VEGF acts through receptors such as VEGFR2 to regulate tip cell formation during sprouting angiogenesis, a process dynamically balanced by Notch signaling for spatial vessel patterning and integrity. ACTN4 influences integrin-mediated adhesion dynamics, particularly αvβ3 and αvβ5 integrins on endothelial cells, which are critical for cell survival, migration, and vascular permeability essential to angiogenesis and metastatic seeding. Interactions between MMPs and integrins, such as

MMP-2 with αvβ3, support matrix degradation and remodeling, enabling endothelial invasion and new vessel sprouting.

Beyond endothelial effects, ACTN4 modulates the tumor-stroma interface by regulating cancer-associated fibroblast (CAF) activation and ECM composition, promoting a microenvironment conducive to invasion. ACTN4-driven cytoskeletal reorganization in stromal cells facilitates secretion of MMPs and remodeling enzymes, loosening matrix barriers for tumor cell migration and intravasation. This crosstalk perpetuates a pro-invasive niche that simultaneously supports angiogenesis, sustaining tumor expansion and dissemination.

ACTN4's role extends to the epithelial–mesenchymal transition (EMT), a cellular program critical for metastatic competence. Elevated ACTN4 expression correlates with hallmark EMT signatures including downregulation of E-cadherin and upregulation of mesenchymal markers like vimentin and N-cadherin, facilitating the loss of cell-cell adhesion and increased motility. ACTN4 activates signaling pathways such as PI3K/Akt to induce EMT-transcription factors including SNAIL and Slug, further enhancing invasive capabilities. This EMT induction is pivotal in oral squamous cell carcinoma (OSCC), driving dissemination from primary sites to metastatic niches.

Comparative insights from other epithelial malignancies reinforce ACTN4's ubiquitous pro-metastatic role. In breast cancer, ACTN4 localizes to the leading edge of invasive fronts, augmenting motility and metastasis through both cytoplasmic cytoskeletal functions and nuclear transcriptional co-activation of hormone receptors. Similarly, in pancreatic and colorectal cancers, ACTN4 overexpression promotes tumor invasiveness, lymph node metastasis, and poor prognosis, often through integrin-mediated adhesion and β-catenin pathways. These conserved mechanisms across diverse tumors underscore ACTN4 as a critical driver of metastatic biology and a candidate for targeted anti-metastatic therapies.

In summary, ACTN4 coordinates angiogenic signaling, matrix remodeling, and EMT programs that collectively facilitate a permissive microenvironment for tumor invasion and metastatic spread. Its modulation of VEGF, MMPs, and integrin pathways engages both the tumor cells and stromal components, highlighting its central role in cancer progression across multiple organ systems including oral cancer. Understanding these integrated functions of ACTN4 provides a valuable framework for developing therapeutics aimed at disrupting tumor vascularization and metastatic dissemination.

5.AI-Integrated Histopathology in Preclinical Cancer Modelling

Artificial intelligence (AI) has revolutionized histopathology image analysis in toxicology and oncology research, facilitating rapid, quantitative, and objective evaluations of tissue architecture and tumor phenotypes beyond human visual capacity. AI-driven image analytics utilize convolutional neural networks (CNNs) and deep learning models to process digitized histopathological slides, enabling high-throughput quantification of morphometric features such as angiogenic index, invasion front delineation, and stromal density in preclinical cancer models, including in vivo oral cancer systems.

In vivo oral cancer models have benefited profoundly from AI integration, where algorithms assess digitized tissue sections stained for biomarkers like ACTN4 to detect morphological phenotypes associated with invasion and metastatic potential. AI models can automatically map invasion fronts by segmenting tumor boundaries and measure stromal cell density or fibrosis surrounding tumor nests. The angiogenic index, quantifying vascular density via endothelial markers, is quantitatively assessed by AI, allowing researchers to correlate ACTN4 expression levels with neovascularization patterns in orthotopic xenografts or chemically induced tumors.

Deep learning frameworks have shown capability in detecting subtle morphological variations linked to ACTN4 activity, such as cytoskeletal reorganization and focal adhesion patterns, which are challenging to discern by human experts. By training on annotated datasets with labeled ACTN4 expression levels and associated morphologies, CNNs can classify tumor zones exhibiting aggressive phenotypes, contributing to high precision in tissue phenotyping and prognostication.

Despite these advances, AI validation in animal studies faces challenges. Reproducibility issues emerge from variability in tissue staining, slide preparation, and species-specific histological differences. Interpretability of deep models remains limited as the "black box" nature hinders understanding of decision pathways. Moreover, circular validation risks occur when training and test datasets overlap in biological or technical features, necessitating rigorous data curation and independent validation cohorts. Establishing standardized protocols for image acquisition, annotation, and reporting is essential to enhance model generalizability and adoption in preclinical toxicology and cancer research workflows.

In summary, AI-based histopathology image analytics are transforming preclinical cancer modelling by enabling precise, reproducible quantification of tissue phenotypes linked to ACTN4-driven tumor invasion, angiogenesis, and metastasis. Continued improvements in model transparency, validation rigor, and integration with multi-omics data promise to accelerate translational cancer research and therapeutic development.

6.Translational Implications and Future Directions

ACTN4 has emerged as a robust predictive biomarker for metastasis and therapeutic resistance across multiple cancers, including oral squamous cell carcinoma (OSCC). Its overexpression correlates strongly with lymph node metastasis, tumor aggressiveness, and recurrence, making ACTN4 expression profiling a valuable prognostic tool for risk stratification and personalized patient management. Additionally, increased ACTN4 levels have been implicated in resistance to conventional therapies such as chemotherapy and radiation, potentially via EMT induction and cytoskeletal remodeling that promote survival under therapeutic stress.

Targeted therapy development against ACTN4 represents a promising frontier. Small molecule inhibitors, peptide antagonists, and RNA interference approaches aimed at disrupting ACTN4-actin binding or its downstream signaling nodes (e.g., PI3K/Akt, focal adhesion kinase) show preclinical efficacy in reducing tumor cell motility and invasion. Combinatorial strategies pairing ACTN4 blockade with established agents (e.g., platinum-based drugs) may overcome resistance and impede metastatic progression more effectively. Precision delivery methods, such as nanoparticle-mediated siRNA delivery, further enhance therapeutic index by targeting ACTN4 specifically in tumor tissues.

The synergy between AI-driven histopathology and advanced in vivo modeling offers unprecedented resolution for translational oncology. AI analytics accelerate identification of ACTN4-associated phenotypes in tissue sections, while next-generation animal models—orthotopic xenografts, patient-derived xenografts, and humanized mice—facilitate functional validation under physiologically relevant conditions. This convergence enables real-time phenotypic screening, predictive biomarker discovery, and rapid evaluation of targeted interventions, significantly reducing the bench-to-bedside gap.

Future directions emphasize integrated multi-omics and digital pathology platforms to complement phenotypic insights. Spatial transcriptomics, proteomics, and metabolomics datasets synchronized with AI-analyzed histological features provide a holistic tumor ecosystem map, revealing ACTN4-driven molecular networks and identifying novel synthetic lethal interactions. This systems biology approach informs biomarker panels, combination therapy design, and patient-specific therapeutic regimens, advancing personalized oncology.

In summary, ACTN4 stands at the nexus of metastasis prediction and targeted therapy innovation, with AI-enabled biomarker analytics and sophisticated in vivo models catalyzing translational breakthroughs. Integrating digital pathology, comprehensive omics, and high-throughput screening will refine therapeutic strategies, optimize clinical outcomes, and ultimately transform management of oral and other epithelial cancers.

Conclusion

ACTN4 stands as a central orchestrator of cytoskeletal remodeling, angiogenesis, and metastatic dissemination in oral squamous cell carcinoma and other epithelial malignancies. Elevated expression and gene amplification of ACTN4 correlate strongly with invasive phenotypes, lymph node metastasis, and poor prognosis, underscoring its role in driving tumor aggressiveness through regulation of cell motility, focal adhesion dynamics, extracellular matrix remodeling, and neovascularization. In vivo modeling strategies—including chemically induced carcinogenesis, orthotopic and patient-derived xenografts—have substantiated ACTN4's causal role in tumor progression and metastatic spread, offering pivotal platforms for therapeutic intervention assessment.

Cutting-edge artificial intelligence-augmented histopathology has emerged as a transformative approach to quantitatively analyze ACTN4-associated morphological phenotypes in preclinical models with high throughput, precision, and reproducibility. This integration of molecular biology, in vivo pharmacology, and computational pathology heralds a new frontier in precision modeling of metastasis by enabling spatially resolved, multi-parameter tissue analyses and accelerated biomarker discovery.

The convergence of multidisciplinary approaches promises to refine our mechanistic understanding of ACTN4-driven oncogenic pathways and propel the development of ACTN4-targeted therapies. Coupled with advanced AI analytics and next-generation in vivo platforms, these innovations pave the way for personalized prognostication and therapeutic strategies aimed at mitigating oral cancer metastasis and improving patient outcomes.

In summary, ACTN4 exemplifies molecular drivers whose study benefits greatly from integrated biological and computational methodologies, providing a robust framework to advance translational oncology focused on metastatic control and therapeutic resistance.

References

1. Coletta, R. D., Yeudall, W. A., & Salo, T. (2020). Grand Challenges in Oral Cancers. *Frontiers in oral health*, *1*, 3. https://doi.org/10.3389/froh.2020.00003
2. Wu, J., Chen, H., Liu, Y., Yang, R., & An, N. (2025). The global, regional, and national burden of oral cancer, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Journal of cancer research and clinical oncology*, *151*(2), 53. https://doi.org/10.1007/s00432-025-06098-w
3. Dhingra, K. (2025). Oral cancer: a global threat. *Bulletin of the Royal College of Surgeons of England*, *107*(5), 257–259. https://doi.org/10.1308/rcsbull.2025.91
4. Ghanem, A. S., Memon, H. A., & Nagy, A. C. (2024). Evolving trends in oral cancer burden in Europe: a systematic review. *Frontiers in Oncology*, *14*. https://doi.org/10.3389/fonc.2024.1444326
5. Ramachandran, S. (2024). Oral cancer: recent breakthroughs in pathology and therapeutic approaches. *Oral Oncology Reports*, *12*, 100678.
6. Zardawi, F. M., Alahmad, B. E. M., Marla, V., Shaban, S. N., & Amin, S. S. (2025). Revisiting Early Detection of Oral Cancer: A review on methods, impact on survival rates, and recurrence prevention. *Journal of Oncological Sciences*. https://doi.org/10.37047/jos.galenos.2025.2025-6-1
7. Barai, A., Mukherjee, A., Das, A., Saxena, N., & Sen, S. (2021). α-Actinin-4 drives invasiveness by regulating myosin IIB expression and myosin IIA localization. *Journal of cell science*, *134*(23), jcs258581.
8. Wang, N., Wang, Q., Tang, H., Zhang, F., Zheng, Y., Wang, S., Zhang, J., Wang, Z., & Xie, X. (2017). Direct inhibition of ACTN4 by ellagic acid limits breast cancer metastasis via regulation of β-catenin stabilization in cancer stem cells. *Journal of experimental & clinical cancer research : CR*, *36*(1), 172. https://doi.org/10.1186/s13046-017-0635-9
9. Wang, M. C., Chang, Y. H., Wu, C. C., Tyan, Y. C., Chang, H. C., Goan, Y. G., ... & Liao, P. C. (2015). Alpha-actinin 4 is associated with cancer cell motility and is a potential biomarker in non–small cell lung cancer. *Journal of Thoracic Oncology*, *10*(2), 286-301.
10. Gao, Y., Li, G., Sun, L., He, Y., Li, X., Sun, Z., Wang, J., Jiang, Y., & Shi, J. (2015). ACTN4 and the pathways associated with cell motility and adhesion contribute to the process of lung cancer metastasis to the brain. *BMC cancer*, *15*, 277. https://doi.org/10.1186/s12885-015-1295-9
11. Themes, U. (2017, December 14). *Prognostic significance of gene amplification of ACTN4in stage I and II oral tongue cancer*. Pocket Dentistry. https://pocketdentistry.com/prognostic-significance-of-gene-amplification-of-actn4in-stage-i-and-ii-oral-tongue-cancer/
12. Tentler, D., Lomert, E., Novitskaya, K., & Barlev, N. A. (2019). Role of ACTN4 in Tumorigenesis, Metastasis, and EMT. *Cells*, *8*(11), 1427. https://doi.org/10.3390/cells8111427
13. Watabe, Y., Mori, T., Yoshimoto, S., Nomura, T., Shibahara, T., Yamada, T., & Honda, K. (2014). Copy number increase of ACTN4 is a prognostic indicator in salivary gland carcinoma. *Cancer medicine*, *3*(3), 613–622. https://doi.org/10.1002/cam4.214
14. Liu, X., & Chu, K. M. (2017). α-Actinin-4 promotes metastasis in gastric cancer. *Laboratory Investigation*, *97*(9), 1084-1094.
15. Su, W., Shen, Y., Wang, Y., Wang, F., Hong, X., Chen, Y., Lin, Y., & Yang, H. (2020). circPHIP promotes oral squamous cell carcinoma progression by sponging miR-142-5p and regulating PHIP and ACTN4 expression. *Molecular therapy. Nucleic acids*, *23*, 185–199. https://doi.org/10.1016/j.omtn.2020.10.038 (Retraction published Mol Ther Nucleic Acids. 2021 Dec 04;26:1479. doi: 10.1016/j.omtn.2021.11.017.)
16. Martínez B, D. A., Barato Gómez, P. A., Iregui Castro, C. A., & Rosas Pérez, J. E. (2020). DMBA-Induced Oral Carcinoma in Syrian Hamster: Increased Carcinogenic Effect by Dexamethasone Coexposition. *BioMed research international*, *2020*, 1470868. https://doi.org/10.1155/2020/1470868
17. Syed, S. A., Qureshi, M. A., Khan, S., Kumar, R., Shafique, Y., Khan, B. A., & Safdar, J. (2022). Development of a murine model of oral carcinogenesis: an accelerated tool for biomarker and anti-tumour drug discovery. *Ecancermedicalscience*, *16*, 1413. https://doi.org/10.3332/ecancer.2022.1413

18. Morales-Herrero, M., & Ortega-Medina, I. (2022). Experimental carcinogenesis with 7,12-dimethylbenz(a)anthrazene (DMBA) and its inhibition with isothi-ocyanates. *Journal of Oral Research*, *11*(4), 1–13. https://doi.org/10.17126/joralres.2022.047
19. Pratama, D. a. O. A., Cahyati, A. N., Kustiati, U., Hardian, A. B., & Permata, F. S. (2024). Proteomics analysis of carcinogenesis in a rat model of mammary cancer induced by DMBA (7,12-dimethylbenz[a]anthracene). *F1000Research*, *12*, 606. https://doi.org/10.12688/f1000research.132524.2
20. Webadmin. (2022). Molecular effect of betanin on the molecular expression pattern of cell proliferative and inflammatory signalling pathways in DMBA-induced oral carcinogenesis in hamsters. *Pharmacognosy Magazine*. https://doi.org/10.4103/pm.pm_281_21
21. Pickering, C. R., Zhang, J., Yoo, S. Y., Bengtsson, L., Moorthy, S., Neskey, D. M., Zhao, M., Ortega Alves, M. V., Chang, K., Drummond, J., Cortez, E., Xie, T. X., Zhang, D., Chung, W., Issa, J. P., Zweidler-McKay, P. A., Wu, X., El-Naggar, A. K., Weinstein, J. N., Wang, J., … Frederick, M. J. (2013). Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer discovery*, *3*(7), 770–781. https://doi.org/10.1158/2159-8290.CD-12-0537
22. Shi, Y., Xie, T., Wang, B., Wang, R., Cai, Y., Yuan, B., ... & Rangel, R. (2022). Mutant p53 drives an immune cold tumor immune microenvironment in oral squamous cell carcinoma. *Communications Biology*, *5*(1), 757.
23. Bais, M. V., Kukuruzinska, M., & Trackman, P. C. (2015). Orthotopic non-metastatic and metastatic oral cancer mouse models. *Oral oncology*, *51*(5), 476–482. https://doi.org/10.1016/j.oraloncology.2015.01.012
24. Dittman Lai-Shun Chung, Geoffrey Ho Duen Leung, Songran Liu, Sarah Wing Yan Lok, Ying Xin, Yunfei Xia, Alex Zhavoronkov, Frank W. Pun, Wai-Tong Ng & Wei Dai. (2025) AI-Driven Drug Target Screening Platform Identified Oncogene CACNA2D1 Activated by Enhancer Infestation in Epstein-Barr Virus-Associated Nasopharyngeal Carcinoma. *International Journal of Molecular Sciences* 26:10, pages 4697.
25. Enaka, M., Nakanishi, M., & Muragaki, Y. (2021). The gain-of-function mutation p53R248W suppresses cell proliferation and invasion of oral squamous cell carcinoma through the down-regulation of keratin 17. *The American Journal of Pathology*, *191*(3), 555-566.
26. Zhang, W., & Zheng, X. (2023). Patient-derived xenografts or organoids in the discovery of traditional and self-assembled drug for tumor immunotherapy. *Frontiers in oncology*, *13*, 1122322. https://doi.org/10.3389/fonc.2023.1122322
27. Naruse, M., Ishigamori, R., & Imai, T. (2021). The unique genetic and histological characteristics of DMBA-induced mammary tumors in an organoid-based carcinogenesis model. *Frontiers in Genetics*, *12*, 765131.
28. Wang, E., Xiang, K., Zhang, Y., & Wang, X. F. (2022). Patient-derived organoids (PDOs) and PDO-derived xenografts (PDOXs): New opportunities in establishing faithful pre-clinical cancer models. *Journal of the National Cancer Center*, *2*(4), 263-276.
29. Chetty, C., Lakka, S. S., Bhoopathi, P., & Rao, J. S. (2010). MMP-2 alters VEGF expression via alphaVbeta3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. *International journal of cancer*, *127*(5), 1081–1095. https://doi.org/10.1002/ijc.25134
30. Liu, Z. L., Chen, H. H., Zheng, L. L., Sun, L. P., & Shi, L. (2023). Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal transduction and targeted therapy*, *8*(1), 198.
31. Quintero-Fabián, S., Arreola, R., Becerril-Villanueva, E., Torres-Romero, J. C., Arana-Argáez, V., Lara-Riegos, J., ... & Alvarez-Sánchez, M. E. (2019). Role of matrix metalloproteinases in angiogenesis and cancer. *Frontiers in oncology*, *9*, 1370.
32. Avraamides, C. J., Garmy-Susini, B., & Varner, J. A. (2008). Integrins in angiogenesis and lymphangiogenesis. *Nature reviews. Cancer*, *8*(8), 604–617. https://doi.org/10.1038/nrc2353
33. Pang, X., He, X., Qiu, Z., Zhang, H., Xie, R., Liu, Z., ... & Cui, Y. (2023). Targeting integrin pathways: mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy*, *8*(1), 1.
34. Michaelis U. R. (2014). Mechanisms of endothelial cell migration. *Cellular and molecular life sciences : CMLS*, *71*(21), 4131–4148. https://doi.org/10.1007/s00018-014-1678-0
35. Nagata, H., Funaki, S., Kimura, K., Fukui, E., Kimura, T., Kanou, T., ... & Shintani, Y. (2024). ACTN4 is associated with the malignant potential of thymic epithelial tumors through the β‐catenin/Slug pathway. *Cancer Science*, *115*(11), 3636-3647.
36. Hsu, K. S., & Kao, H. Y. (2013). Alpha-actinin 4 and tumorigenesis of breast cancer. *Vitamins and hormones*, *93*, 323–351. https://doi.org/10.1016/B978-0-12-416673-8.00005-8

37. Fukumoto, M., Kurisu, S., Yamada, T., & Takenawa, T. (2015). α-Actinin-4 enhances colorectal cancer cell invasion by suppressing focal adhesion maturation. *PLoS One*, *10*(4), e0120616.
38. Komura, D., Ochi, M., & Ishikawa, S. (2025). Machine learning methods for histopathological image analysis: Updates in 2024. *Computational and Structural Biotechnology Journal*, *27*, 383-400.
39. Shmatko, A., Ghaffari Laleh, N., Gerstung, M., & Kather, J. N. (2022). Artificial intelligence in histopathology: enhancing cancer research and clinical oncology. *Nature cancer*, *3*(9), 1026-1038.
40. Doğan, R. S., & Yılmaz, B. (2024). Histopathology image classification: highlighting the gap between manual analysis and AI automation. *Frontiers in Oncology*, *13*, 1325271.
41. Singh, N. N., Tandon, A., & Jayasankar, P. (2025). Strength, weakness, opportunities and challenges (SWOC) experience of histopathology image analysis, enhanced by artificial intelligence. *Journal of Oral Biology and Craniofacial Research*, *15*(5), 1057-1063.
42. Chaudhary, N., Rai, A., Rao, A. M., Faizan, M. I., Augustine, J., Chaurasia, A., ... & Ahmad, T. (2024). High-resolution AI image dataset for diagnosing oral submucous fibrosis and squamous cell carcinoma. *Scientific Data*, *11*(1), 1050.
43. Choi, H. J., Wang, C., Pan, X., Jang, J., Cao, M., Brazzo, J. A., 3rd, Bae, Y., & Lee, K. (2021). Emerging machine learning approaches to phenotyping cellular motility and morphodynamics. *Physical biology*, *18*(4), 10.1088/1478-3975/abffbe. https://doi.org/10.1088/1478-3975/abffbe
44. Wong, K. S., Zhong, X., Low, C. S. L., & Kanchanawong, P. (2022). Self-supervised classification of subcellular morphometric phenotypes reveals extracellular matrix-specific morphological responses. *Scientific reports*, *12*(1), 15329. https://doi.org/10.1038/s41598-022-19472-2
45. Hartung, T. (2024). The (misleading) role of animal models in drug development. *Frontiers in Drug Discovery*, *4*, 1355044.