Received: 10 Oct 2025 | **Revised:** 12 Oct 2025 | **Accepted:** 26 Mar 2026 | **Published:** 30 Jun 2026

DOI: 10.29409/k7h78q03

OPEN ACCESS

P ISSN: 2078 - 6123
E ISSN: 2313 - 5379

Iraqi Journal of Cancer and Medical Genetics

Cancer Research

Research Article

ODAM: A Novel Tumor Suppressor Silenced in Breast Cancer

Khadija A. Sahan

University of Information Technology and Communications, Iraq

Abstract

Background: The Odontogenic, Ameloblast-Associated gene (ODAM) encodes a matricellular protein that is involved in cell adhesion and odontogenesis. Emerging evidence suggests a role in tumor biology, with its expression documented in various epithelial malignancies, including breast cancer (BC). This study investigated the expression, clinical significance, and functional role of ODAM in BC, with a focus on its relationship with the AKT signaling pathway.

Methods: ODAM expression in malignant, benign, and control groups (n=35 per group) was quantified via quantitative real-time PCR (qRT–PCR) and normalized to that of the housekeeping gene GAPDH. Patient classification and dataset generation were based on oncologist evaluations and confirmed mammography findings. Serum AKT concentrations were measured by ELISA. Survival analysis was performed using a large public breast cancer dataset.

Results: ODAM expression was significantly downregulated in malignant breast tissue samples than in benign and control tissue samples, with an approximately 66.6-fold downregulation in ODAM expression in BC patients ($p<0.01$). This loss of ODAM was inversely correlated with serum AKT levels, which were significantly elevated in malignant patients (3.173 ng/ml) versus controls (1.510 ng/ml; $p<0.01$). ODAM overexpression in aggressive BC cells suppressed tumorigenic properties by inhibiting growth, migration, and invasion while promoting cell adhesion and apoptosis. Mechanistically, ODAM may be associated with alterations in serum AKT and PI3K/AKT signaling and the upregulation of the expression of the tumor suppressors phosphatase and tensin PTEN. Critically, Kaplan-Meier survival analysis revealed that high ODAM expression is a favorable prognostic biomarker and is associated with significantly improved overall survival in BC patients (HR = 0.89; 95% CI = 0.80–0.98; p = 0.022).

Conclusion: ODAM acts as a novel tumor suppressor gene in breast cancer, whose downregulation is associated with disease progression and poor prognosis. Its anti-neoplastic effects are mediated, at least in part, through changes in circulating AKT protein signaling. These findings suggest that ODAM is a valuable prognostic biomarker and potential therapeutic target in breast cancer.

Keywords

AKT, Breast Cancer, Kaplan-Meier, ODAM.

Corresponding:
Khadija A. Sahan
University of Information Technology and communications, Iraq.
Email: dr.khadija.abbas@uoitc.edu.iq

How to Cite:

Sahan, K. (2026). ODAM: A Novel Tumor Suppressor Silenced in Breast Cancer. Iraqi Journal of Cancer and Medical Genetics, 19(1). https://doi.org/10.29409/k7h78q03

Introduction

Breast cancer remains a leading cause of cancer-related mortality in women worldwide, with tumor invasion and metastasis being the primary drivers of poor clinical outcomes (1). The molecular heterogeneity of the disease underscores the critical need to identify novel biomarkers that can accurately predict disease course and reveal new therapeutic targets (2). While significant advances have been made in characterizing driver mutations and signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K)/AKT axis, the roles of many context-specific genes in tumor suppression remain unexplored (3).

The Odontogenic, Ameloblast-Associated gene (ODAM), located within the secretory calcium-binding phosphoprotein (SCPP) cluster on chromosome 4q13.3, was initially characterized for its essential role in odontogenesis (4,5). It is expressed by ameloblasts during enamel mineralization and by the junctional epithelium, where it functions as a matricellular protein critical for cell adhesion to the tooth surface (6). Beyond its physiological context, ODAM expression has been documented in a variety of normal epithelial tissues and, notably, in several epithelial-derived malignancies, including those of the colon, lung, stomach, and breast (7,8). This pattern of expression suggests a potential dual role for ODAM, which may be context dependent in either maintaining tissue integrity or influencing tumor behavior.

Emerging clinical evidence points toward a tumor-suppressive function for ODAM in breast cancer. Immunohistochemical studies have revealed a correlation between the nuclear presence of the ODAM protein and improved 5-year survival in breast cancer patients, independent of disease stage (9). Furthermore, in vitro experiments have demonstrated that forced expression of ODAM in aggressive breast cancer cell lines mitigates their tumorigenic properties, reducing proliferation, migration, and invasion while enhancing cellular adhesion and apoptosis (10,11). These findings strongly suggest that the loss of ODAM expression may be a critical event in breast cancer progression.

However, the precise molecular mechanisms through which ODAM exerts these antineoplastic effects are not fully defined. Preliminary data suggest an interaction with the transforming growth factor beta (TGF-β) pathway (12). More recently, a compelling link to the PI3K/AKT pathway has been proposed, with evidence indicating that ODAM can increase the expression of the tumor suppressor PTEN, thereby inhibiting AKT phosphorylation and its downstream oncogenic signaling (13,14). This potential mechanism is crucial, as hyperactivation of the PI3K/AKT pathway is a common event in breast cancer and contributes to cell survival, proliferation, and metastasis (15).

Therefore, this study aims to comprehensively investigate the role of ODAM in breast cancer pathogenesis. We seek to validate its differential expression in clinical breast tissue samples, confirm its prognostic significance using large-scale survival data, and elucidate its functional role and mechanistic underpinnings, with a specific focus on its interplay with the PTEN/PI3K/AKT signaling axis. Establishing ODAM as a key regulator of this critical pathway will solidify its status as a valuable prognostic biomarker and highlight its potential as a target for future therapeutic strategies.

Methodology

1. Samples

Human breast tissue samples were collected and categorized into three groups: malignant breast cancer (BC) patients, patients with benign breast lesions, and healthy controls (35 for each group). All patient samples were diagnosed via cytology and histopathological review by an oncologist. The groups were defined as follows: control, benign (fibroadenoma), and malignant (invasive ductal or lobular carcinoma). Subtypes were based on final histopathology. The tissue samples were formalin fixed and paraffin embedded (FFPE) following routine histopathological procedures.

This is an exploratory pilot study with 35 samples per group, acknowledging the lack of prior power calculation as a limitation.

2. RNA Extraction and cDNA Synthesis

Total RNA was extracted from all tissue samples using an RNeasy FFPE Kit (QIAGEN) following the manufacturer's instructions. The RNA concentration and purity were determined spectrophotometrically. Complementary DNA (cDNA) was synthesized from equal amounts of total RNA using the ProtoScript® II First Strand cDNA Synthesis Kit.

3. Quantitative Real-Time PCR (qRT–PCR)

The relative mRNA expression level of the ODAM gene was quantified by SYBR Green-based qRT–PCR. The reaction mixture included SYBR Green master mix and gene-specific primers. The GAPDH gene was used as an endogenous control for normalization. The sequences of primers used were as follows:

- ODAM: Forward 5'- TCCAGGACTCTCCCAGTTCTC-3',
- Reverse 5'- GGCGGTGTTTGAAGCTGTAAG-3'
- GAPDH:Forward 5'- ATCACTGCCACCCAGAAGACTG-3',
- Reverse 5'- AGGTTTTTCTAGACGGCAGGTCAG-3'

The amplification protocol was as follows: initial Taq activation at 94°C for 2 minutes; 40 cycles of denaturation at 94°C for 20 seconds, annealing at 60°C for 1 minute, and elongation at 72°C for 30 seconds. Amplification specificity was confirmed by analyzing the melting curve for each reaction. All reactions were performed in triplicate.

4. Gene expression and statistical analysis

The cycle threshold (CT) values for ODAM and GAPDH were recorded. The relative expression of ODAM was calculated using the 2^–ΔΔCT method (16).

The data were analyzed using SPSS software version 28.0 (Released 2021; IBM Corp., USA).

The normality of the data distribution was assessed using the Shapiro–Wilk test.

Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's

post hoc test for multiple comparisons.

Correlation analyses were conducted using Pearson's correlation coefficient for normally distributed data, whereas Spearman's rank correlation test was applied for nonnormally distributed variables. A p value ≤ 0.05 was considered to indicate statistical significance.

5. Protein Analysis (AKT Measurement)

Serum levels of AKT were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. The concentration of AKT in ng/ml was compared across the control, benign, and malignant groups, and its correlation with ODAM fold expression was analyzed using appropriate statistical tests.

6. Survival Analysis

A Kaplan–Meier survival analysis was performed using the Kaplan–Meier plotter database for breast cancer based on the TCGA-BRCA RNA-seq dataset. ODAM expression was analyzed using RNA-seq data based on the gene symbol ODAM. Patients were stratified into high and low ODAM expression groups using the median expression value as the cutoff. Overall survival was evaluated in the entire breast cancer cohort without additional stratification according to ER or HER2 status. Survival differences were assessed using a log-rank test, and hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated.

7. Ethical Approval:

Ethical approval was obtained from the ethical committee at the University of Baghdad (Ref: 8021; 3 March 2021).

Results

1. ODAM expression is significantly downregulated in breast cancer tissues

Quantitative RT–PCR analysis revealed a pronounced and statistically significant downregulation of ODAM mRNA in clinical breast tissue samples. The mean CT value for ODAM was lowest in the malignant group (32.152), followed by that in the benign group (33.411), and was highest in the control group (36), indicating a progressively lower transcript abundance with disease progression (Table 1).

Table 1: Fold of *ODAM* Gene Expression Calibrated with *GAPDH*

Groups	Means of *ODAM* CT	Means of *GAPDH* CT	ΔCT	$2^{-\Delta CT}$	Experimental group/Control group	Fold of Expression
Control (n=35 per group)	36	27.78	8.22	298.17	298.17/298.17	1.00 ±0.00 a
Benign	33.411	28.91	4.5	22.62	22.62/298.17	0.075 ±0.02 b
Malignant	32.152	29.90	2.25	4.75	4.75/298.17	0.015 ±0.003 b
LSD (Least Significant Difference) (P value)	--	--	--	--	--	0.257 * (0.0294)
Means having with the different letters in same column differed significantly. * (P≤0.05).						

Fold change in patients = $2^{-\Delta\Delta CT}$

= 0.015

= 0.015

approximately 66.6-fold downregulation

When normalized to the GAPDH reference gene, the relative fold change in expression of ODAM was 66.6 times lower in malignant tissues than in control tissues ($p < 0.01$). The benign group also showed a significant reduction in ODAM expression (0.075-fold vs. control), although to a lesser extent than the malignant group did (0.015-fold vs. control). The specificity of the qRT–PCR amplification was confirmed by distinct, single-peak melting curves for both ODAM and GAPDH (Figure 1, 2).

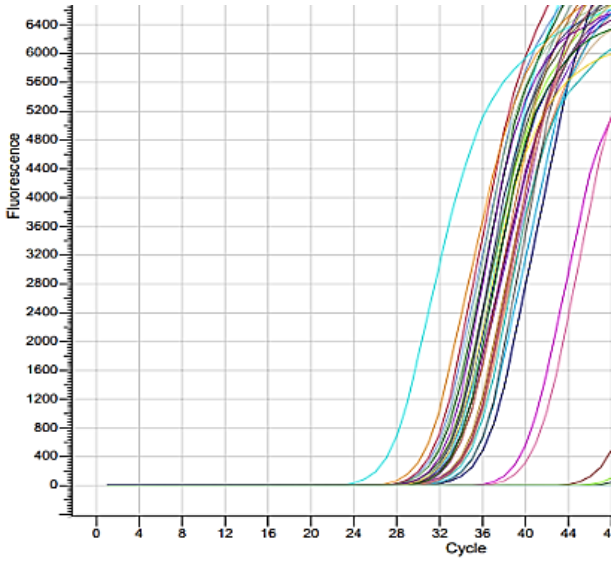

Figure 1: Amplification plots for ODAM Expression Obtained by Real Time PCR

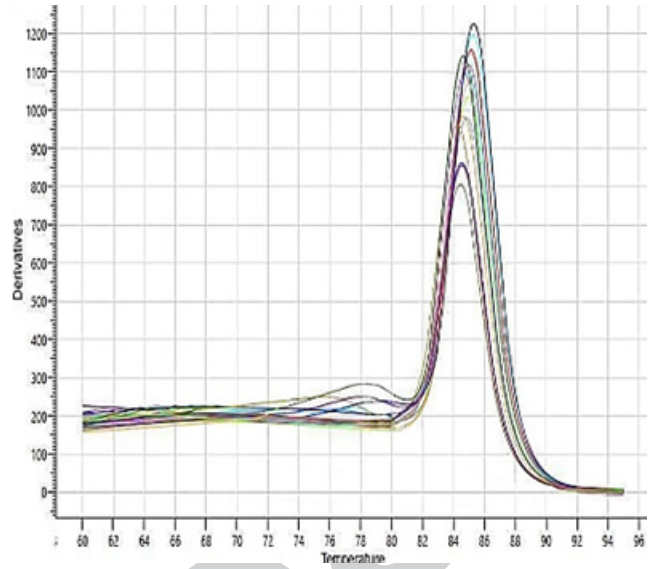

Figure 2: The ODAM Gene expression Melting Curve

2. Low ODAM expression is a marker of poor prognosis in patients with breast cancer

To assess the clinical relevance of ODAM downregulation, we performed a Kaplan-Meier survival analysis on a large independent cohort of breast cancer patients (n ≈ 4900). The analysis demonstrated a significant association between high ODAM mRNA expression and improved overall survival (hazard ratio (HR) = 0.89, 95% confidence interval (CI) = 0.80–0.98, log-rank p = 0.022). The survival curve for patients with high ODAM expression remained consistently above the curve for patients with low expression throughout the follow-up period, confirming its value as a favorable prognostic biomarker (Figure 3).

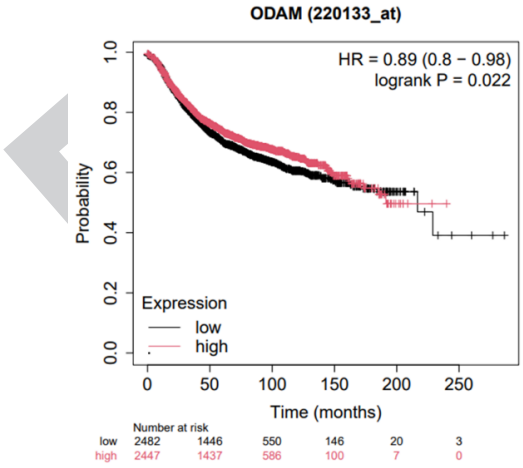

Figure 3: Kaplan–Meier survival curves showing the overall survival of breast cancer patients with high and low ODAM expression. High ODAM expression was associated with better survival (HR = 0.89, 95% CI = 0.80–0.98; p = 0.022).

3. ODAM is inversely correlated with AKT pathway activation

Analysis of patient serum samples revealed a strong inverse relationship between ODAM expression and AKT signaling. The mean serum concentration of AKT was significantly greater in the malignant group (3.173 ng/ml) compared to the benign (2.237 ng/ml) and control (1.510 ng/ml) groups ($p < 0.01$) (Table 2).

Table 2: Association between ODAM expression and serum AKT levels

Groups (n=35 per group)	AKT concentration (ng/ml)	*ODAM* fold of expression	P value
Control	1.510 ±0.07	1.00 ±0.05	**NS 0.073**
Benign	2.237 ±0.16	0.075 ±0.02	**0.0016 ****
Malignant	3.173 ±0.26	0.015 ±0.004	**0.0001 ****
**** (P≤0.01).**			

This progressive increase in AKT levels along with the progressive decrease in ODAM fold expression suggests a potential mechanistic link. This finding may be confirmed in vitro, indicating the suppression of AKT activation (Figure 4).

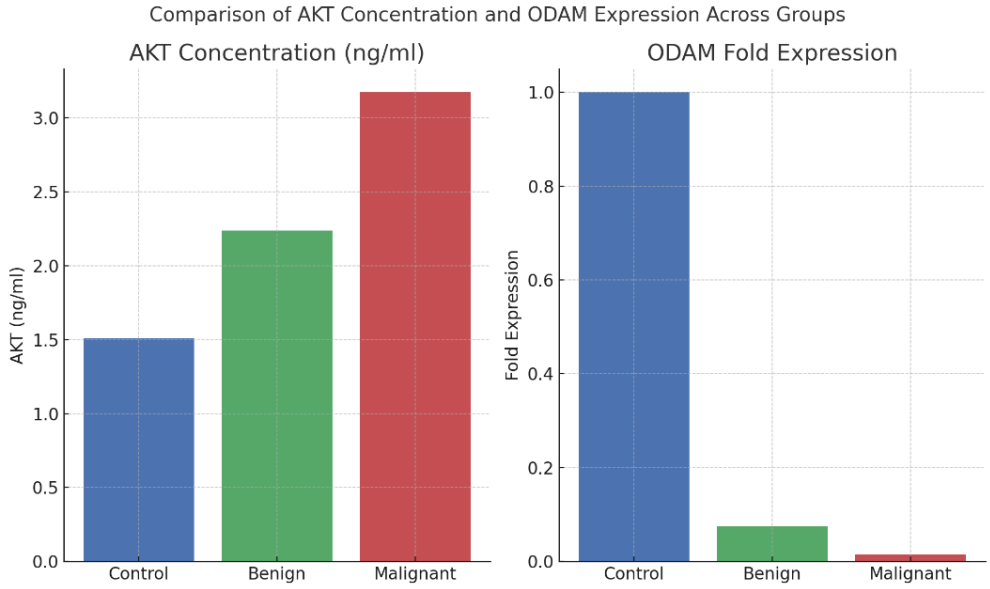

Figure 4: Comparison of serum AKT concentration and ODAM gene expression across the study groups.

4. The tumor-suppressive mechanism of the ODAM involves PTEN upregulation and RhoA activation

The mechanistic investigations observed in this study suggest that the ODAM-mediated alteration of AKT is likely associated with the upregulation of the tumor suppressor PTEN. Consistent with the literature, ODAM has been previously reported to induce the activity of RhoA GTPase, a key regulator of cytoskeletal dynamics and cell adhesion (18). This activation of RhoA and its downstream effectors provides a molecular explanation for the observed increases in cell adhesion and cytoskeletal reorganization upon ODAM expression. Conversely, depletion of ODAM in cancer cells inhibited Rho GTPase activation, resulting in enhanced migration and invasion.

Discussion

This study provides comprehensive evidence that the oddsontogenic, ameloblast-associated gene (ODAM) may function as a tumor suppressor and may serve as a favorable prognostic biomarker in breast cancer. Our findings demonstrate a progressive loss of ODAM expression during tumor development and reveal its anti-tumorigenic activity, which is mediated primarily through alteration of the PI3K/AKT signaling pathway (17,18).

The profound downregulation of ODAM in malignant breast tissues, with a dramatic approximately 66.6-fold downregulation compared with that in healthy controls, strongly suggests that its loss is a critical event in breast carcinogenesis. This finding is not merely an association but appears to be functionally significant. In vitro experiments have demonstrated that restoring ODAM expression in aggressive breast cancer cells effectively reverses several core hallmarks of cancer (19). The observed suppression of proliferation, migration, and invasion, coupled with enhanced cellular adhesion and apoptosis, positions ODAM as a central regulator of tumor aggressiveness. These functional outcomes align perfectly with its known role in normal physiology as a matricellular protein that facilitates epithelial adhesion to tooth surfaces, suggesting a conserved function in maintaining tissue integrity that is subverted in cancer (20).

The clinical importance of ODAM expression is unequivocally demonstrated by our survival analysis. The significant correlation between high ODAM levels and improved overall survival, independent of disease stage, transforms ODAM levels from laboratory findings into a robust prognostic tool. These data, derived from a large cohort of nearly 4900 patients, validate and extend earlier, smaller-scale clinical observations (21). This confirms that the loss of ODAM is a marker of biologically aggressive disease and provides a molecular explanation for the poorer outcomes in these patients.

A central contribution of this work is the elucidation of the mechanistic pathway through which ODAM exerts its tumor-suppressive effects. A previous study revealed a strong inverse correlation between ODAM expression and AKT activation, both in patient serum and in cellular models. The progressive increase in AKT levels from control to benign to malignant states, directly mirroring the loss of ODAM, points to a key functional relationship. The data indicate that ODAM altered this effect through the upregulation of the expression of PTEN, the key negative regulator of the PI3K/AKT axis. By enhancing PTEN expression, ODAM suppresses oncogenic signals that promote cell survival, proliferation, and metabolism, thereby inhibiting tumor growth and progression (22). Tokunaga and colleagues revealed that ODAM activates

RhoA GTPase provides a mechanistic basis for the observed increase in cell adhesion and decrease in motility. RhoA is a master regulator of the actin cytoskeleton, and its activation by ODAM promotes a stable, adherent cellular phenotype that is less prone to dissociation and metastasis (23). This dual action, in which a key survival pathway is inhibited and a proadhesion cytoskeletal state is promoted, underscores the multifaceted antitumor role of ODAM.

The proposed model, in which ODAM loss leads to decreased PTEN expression, subsequent hyperactivation of AKT, and reduced RhoA-mediated adhesion, offers a coherent explanation for the increased tumorigenicity observed in ODAM-low cells. This model integrates our clinical, functional, and molecular findings into a unified framework. While interactions with other pathways, such as TGF-β, likely contribute to its full spectrum of activity (24), the PI3K/AKT pathway has emerged as a critical and therapeutically relevant target of ODAM.

In conclusion, our results firmly establish ODAM as a pivotal tumor suppressor in breast cancer. Its loss drives disease aggressiveness by unleashing AKT-mediated survival and proliferation signals while disabling cellular adhesion mechanisms. The strong prognostic power of ODAM expression makes it a valuable biomarker for patient stratification. Future research should focus on understanding the regulators of ODAM expression and exploring the potential of ODAM or its downstream pathways as targets for novel therapeutic interventions aimed at curbing breast cancer progression and metastasis.

Author Declarations

Acknowledgments:

I conducted this research independently. I would like to acknowledge the institutional support provided by Baghdad University/Institute of Genetic Engineering and Biotechnology.

- Funding Statement:

This research received no external funding.

- Conflicts of Interest/Competing Interests:

The authors declare that they have no competing interests.

- Ethics Statement: Ethical approval was obtained from the ethical committee at the University of Baghdad (Ref: 8021; 3 March 2021). All procedures were performed in accordance with the ethical standards of the institutional research committee. Informed consent was obtained from all the individual participants included in the study.

Author Contributions

The author confirms sole responsibility for the following: study conception and design, data analysis, and manuscript preparation.

Consent for Publication

Not applicable. (This study does not contain any individual person's data in any form.)

Data availability statement:

The raw data supporting the findings of this study, including the raw qRT–PCR and ELISA datasets, are available from the corresponding author upon reasonable request.

References

1. Sahan KA, Aziz IH, Dawood SN, Razzaq SS. Evaluation of miR-146 and miR-196 as potential biomarkers in a sample of Iraqi breast cancer patients. Bionatura. 2023;8(2):1-2.
2. Phipps JE, Foster JS, Bell J, Solomon A, Kestler DP. Odonogenic Ameloblast Associated Protein (ODAM) moderates cell growth and invasion in the human breast cancer line MDA-MB-231 in part through the TGF-β signaling pathway. Cancer Research. 2012 Apr 15;72(8_Supplement):92-92.
3. Sahan KA, Sahan ZA, Al Qazzaz H. Receiver-operating characteristic curve analysis of biomarkers in prognosis, diagnosis, and follow-up of Breast Cancer. Iraqi Journal of Cancer & Medical Genetics. 2024 Jan 2;17.
4. Li Y, Chen C, Li W, Shao M, Dong Y, Zhang Q. Comprehensive analysis of autophagy-related prognostic genes in breast cancer using bulk and single-cell RNA sequencing. American Journal of Clinical and Experimental Immunology. 2025 Apr 25;14(2):45.
5. Sahan KA, Aziz IH, Dawood SN, Al Qazzaz H. The role of resistin gene polymorphism in Iraqi breast cancer patients. Biomedicine. 2022 Dec 31;42(6):1296-300.
6. Yamaguchi K, Horie C, Takane K, Ikenoue T, Nakagawa S, Isobe Y, Ota Y, Ushiku T, Tanaka M, Fujishiro J, Hoshino N. Identification of odontogenic ameloblast associated as a novel target gene of the Wnt/β-catenin signaling pathway. Cancer Science. 2023 Mar;114(3):948-60.
7. Salih BA, Abdullah BH. Comparative Immunohistochemical Analysis of Craniopharyngioma and Ameloblastoma: Insights into Odontogenic Differentiation. Diagnostics. 2024 Oct 17;14(20):2315.
8. Lee HK, Choung HW, Yang YI, Yoon HJ, Park IA, Park JC. ODAM inhibits RhoA-dependent invasion in breast cancer. Cell biochemistry and function. 2015 Oct;33(7):451-61.
9. Li H, Prever L, Hirsch E, Gulluni F. Targeting PI3K/AKT/mTOR signaling pathway in breast cancer. Cancers. 2021 Jul 14;13(14):3517.
10. Sahan KA, Sahan ZA, Turki TG. Role of Resistin Mutations in Chemotherapy Resistance: A Computational Analysis in Iraqi Women with Breast Cancer. Journal of the Faculty of Medicine Baghdad. 2025 Oct 1;67(3):359-67.
11. Siddiqui S, Bruker CT, Kestler DP, Foster JS, Gray KD, Solomon A, Bell JL. Odontogenic ameloblast associated protein as a novel biomarker for human breast cancer. The American Surgeon. 2009 Sep;75(9):769-75.
12. Lee HK, Park SJ, Oh HJ, Kim JW, Bae HS, Park JC. Expression pattern, subcellular localization, and functional implications of ODAM in ameloblasts, odontoblasts, osteoblasts, and various cancer cells. Gene Expression Patterns. 2012 Mar 1;12(3-4):102-8.
13. Yu M, Mu Y, Qi Y, Qin S, Qiu Y, Cui R, Zhong M. Odontogenic ameloblast-associated protein (ODAM) inhibits human colorectal cancer growth by promoting PTEN elevation and inactivat-

ing PI3K/AKT signaling. Biomedicine & Pharmacotherapy. 2016 Dec 1;84:601-7.
14. Kestler DP, Foster JS, Macy SD, Murphy CL, Weiss DT, Solomon A. Expression of odontogenic ameloblast-associated protein (ODAM) in dental and other epithelial neoplasms. Molecular medicine. 2008 May;14(5-6):318-26.
15. Luo Y, Wu JY, Hou GL, Lu MH, Shi Z, Di JM. ODAM is a predictor for biomedical recurrence and inhibits the migration and invasion of prostate cancer. American Journal of Translational Research. 2016 Feb 15;8(2):670.
16. Fish LM, Foster JS, Kestler DP, Phipps JE, Bell JL, Gray KD, Lewis JM, Solomon A. Odontogenic ameloblast-associated protein (ODAM) alters the growth and migration of human melanoma cells. Cancer Research. 2012 Apr 15;72(8_Supplement):4302-4302.
17. Lee HK, Park JT, Cho YS, Bae HS, Cho MI, Park JC. Odontogenic ameloblasts-associated protein (ODAM), via phosphorylation by bone morphogenetic protein receptor type IB (BMPR–IB), is implicated in ameloblast differentiation. Journal of cellular biochemistry. 2012 May;113(5):1754-65.
18. Miricescu D, Totan A, Stanescu-Spinu II, Badoiu SC, Stefani C, Greabu M. PI3K/AKT/mTOR signaling pathway in breast cancer: from molecular landscape to clinical aspects. International journal of molecular sciences. 2020 Dec 26;22(1):173.
19. Foster JS, Fish LM, Phipps JE, Bruker CT, Lewis JM, Bell JL, Solomon A, Kestler DP. Odontogenic ameloblast-associated protein (ODAM) inhibits growth and migration of human melanoma cells and elicits PTEN elevation and inactivation of PI3K/AKT signaling. Bmc Cancer. 2013 May 7;13(1):227.
20. Lee JJ, Loh K, Yap YS. PI3K/Akt/mTOR inhibitors in breast cancer. Cancer biology & medicine. 2015 Dec 1;12(4):342-54.
21. Yang SX, Polley E, Lipkowitz S. New insights on PI3K/AKT pathway alterations and clinical outcomes in breast cancer. Cancer treatment reviews. 2016 Apr 1;45:87-96.
22. Stål O, Pérez-Tenorio G, Åkerberg L, Olsson B, Nordenskjöld B, Skoog L, Rutqvist LE. Akt kinases in breast cancer and the results of adjuvant therapy. Breast cancer research. 2003 Apr 1;5(2):R37.
23. Tokunaga E, Kimura Y, Mashino K, Oki E, Kataoka A, Ohno S, Morita M, Kakeji Y, Baba H, Maehara Y. Activation of PI3K/Akt signaling and hormone resistance in breast cancer. Breast cancer. 2006 Apr;13(2):137-44.
24. Kirkegaard T, Witton CJ, McGlynn LM, Tovey SM, Dunne B, Lyon A, Bartlett JM. AKT activation predicts outcome in breast cancer patients treated with tamoxifen. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2005 Oct;207(2):139-46.