ORIGINAL RESEARCH

Unraveling Calcification in Keratin Pearls: Insights into OSCC Pathogenesis

Suganya Panneer Selvam[1], Nitya Krishnasamy[2], Niranjan KC[3], Ramya Ramadoss[4]

Abstract

Calcification in oral squamous cell carcinoma (OSCC), particularly within keratin pearls, is a rare yet intriguing phenomenon with uncertain clinical implications. While keratin pearls are characteristic of well-differentiated OSCC and are often associated with a favorable prognosis, calcification may signal chronic tumor progression or underlying necrosis. This study aims to explore the etiopathogenesis of calcified keratin pearls through histopathological observations supported by bioinformatics-based gene expression analysis. Histologically, apoptotic debris, chronic inflammation, epithelial-mesenchymal transition (EMT), tumor necrosis, and desmoplasia contribute to a hypoxic tumor microenvironment that favors dystrophic calcification. Bioinformatics analysis revealed that genes such as BMP2, BMP4, RUNX2, SPP1, ALPL, and SPARC, typically involved in bone mineralization and ossification, are aberrantly expressed in OSCC epithelial cells. These genes are interconnected via signaling pathways like BMP4–TGF-β–NFκB, which regulate osteogenic differentiation, extracellular matrix remodeling, and chronic inflammation—key factors contributing to calcium deposition. The expression of cytokeratins and osteogenic markers suggests that keratin pearl calcification could be initiated intracellularly, reflecting a unique pathological adaptation of epithelial tumor cells. The KEGG "Pathways in Cancer" enrichment further supports the involvement of these pathways in OSCC mineralization. While calcification alone does not directly predict poor prognosis, its presence may indicate a tumor microenvironment with aggressive features and treatment resistance. Our findings highlight the need for further investigation into the molecular basis of calcification in OSCC, which could provide novel diagnostic markers and therapeutic targets to improve patient outcomes.

Keywords: Cancer, calcification, keratin pearls, gene, quality of life

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignancy in the oral cavity, ranking 16th among the global incidence and it remains a significant challenge in oncology due to its complex etiology and often late diagnosis.[1] Although several histopathological features are associated with prognosis, the presence of keratin pearls is a hallmark of well-differentiated oral squamous cell carcinoma (OSCC) and is considered an indicator of the tumor's biological behavior.[2] However, calcification within keratin pearls is a rare occurrence. While keratin pearls are generally linked to a favorable prognosis, the presence of calcification does not share this positive implication.[3] These calcifications, often considered mere byproducts of tumor pathology, are increasingly recognized for their potential to uncover hidden genetic clues that could enhance our understanding of tumor behavior and progression. Our work explores the intriguing relationship between calcifications and genetic alterations in OSCC, highlighting how these seemingly incidental findings may reveal previously obscured genetic landscapes and contribute to more effective diagnostic and therapeutic strategies.

Department and Institution Affiliation: [1,2,4]Department of Oral Biology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, [3]Department of Oral & Maxillofacial Pathology & Oral Microbiology, SDM College of Dental Sciences & Hospital, Shri Dharmasthala Manjunatheshwara University, Dharwad- 580 009, Karnataka, India.

Corresponding Author: Suganya Panneer Selvam, Associate Professor, Department of Oral Biology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India Email: suganresearch29@gmail.com

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Conflict of Interest: None

Methodology:

Study Design and Ethics

A retrospective histopathological and bioinformatics study was performed on anonymised archival FFPE tissues of well-differentiated OSCC containing keratin pearls with suspected calcification, conducted under institutional ethical approval with a waiver of informed consent.

Case Selection and Histopathology

Only well-preserved tissues without artefacts, autolysis, or prior decalcification were included. H&E-stained sections (3–4 μm) were examined for keratin pearl morphology, calcification pattern, necrosis, atypia, mitoses, stromal fibrosis, and inflammation. Representative microscopic fields were photographed at standard magnifications using a calibrated microscope-mounted digital camera and processed uniformly for clarity.

Bioinformatics Data Sources and Gene Selection

Mineralization-related and regulatory genes (BMP2, BMP4, RUNX2, SPP1, SPARC, ALPL, COL1A1, MGP and pathway-associated mediators) were identified from literature and analysed using transcriptomic data from the TCGA-HNSC cohort accessed via GEPIA2 and UCSC Xena.

Analytical Tools and Workflow

Differential expression analysis (TPM, log2FC, FDR) was performed using GEPIA2/Xena. Protein–protein interaction networks were constructed using STRING v12.0 to assess node degree, network enrichment, and GO/KEGG functional pathways. ShinyGO v0.77 provided independent GO Biological Process and KEGG enrichment analysis for validation.

Integrative Interpretation

Histopathological observations were synthesised with transcriptomic, network, and pathway data to infer the molecular mechanisms contributing to calcification within keratin pearls, with emphasis on osteogenic, inflammatory, and ECM-related signalling pathways.

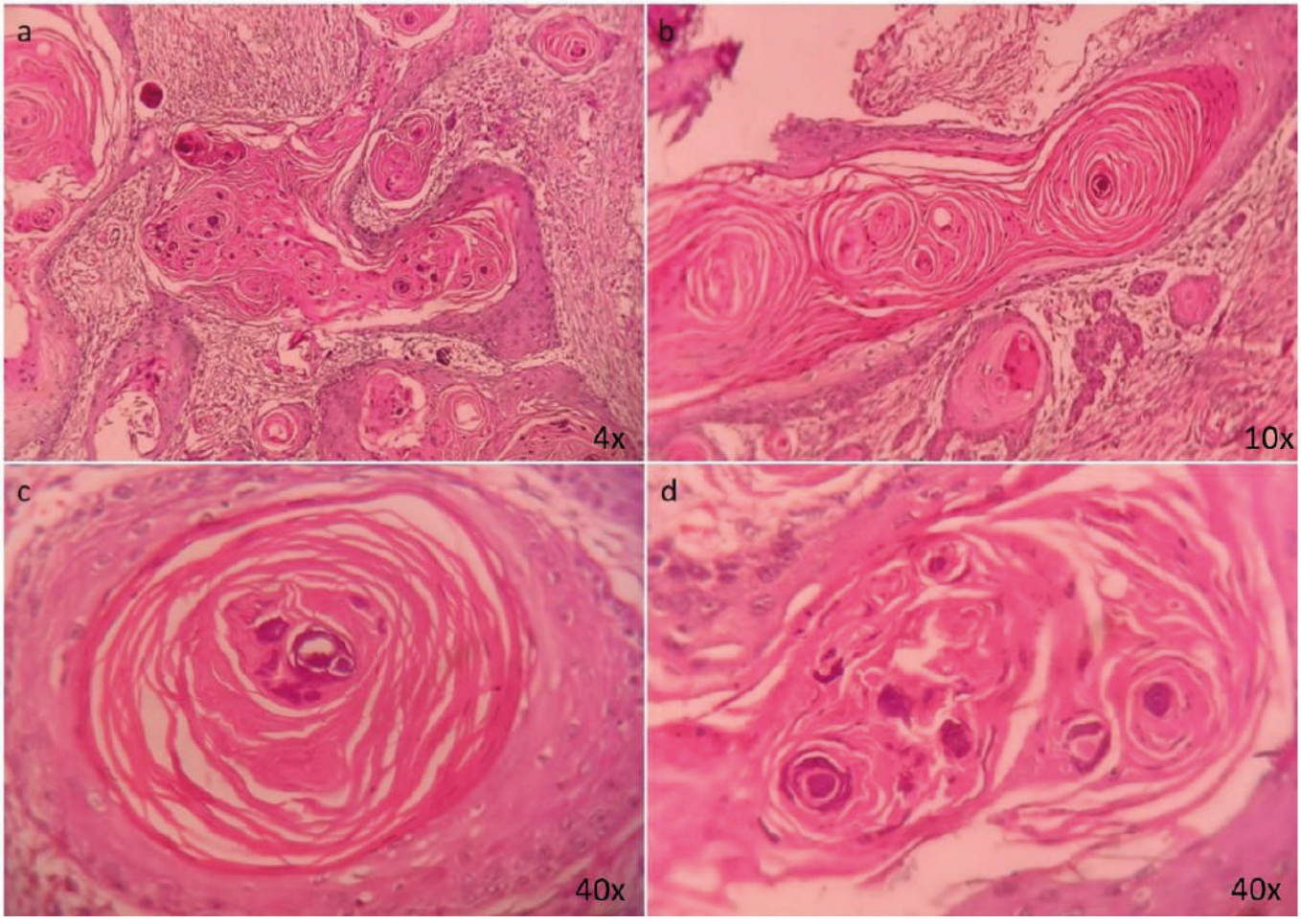
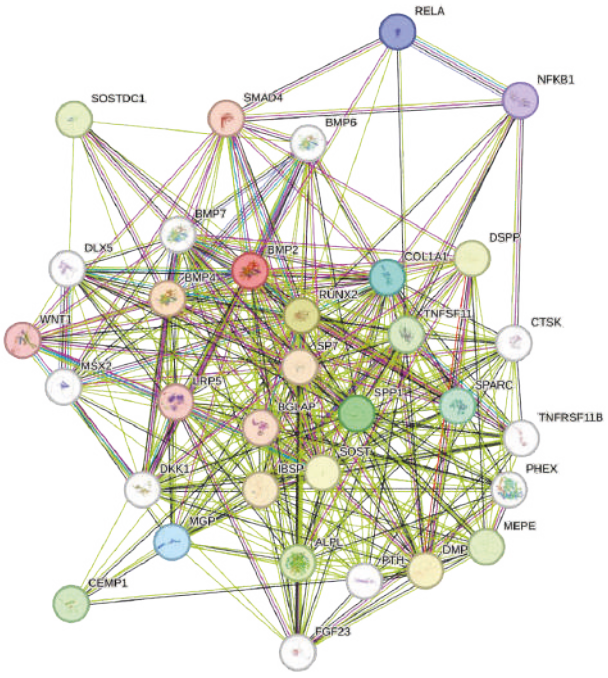


Fig. 1 (a–d): Histopathological features of calcification within keratin pearls in OSCC (H&E stain). Several concentric keratin pearls containing coarse basophilic calcific foci (a&b); lamellated psammoma-like calcification within the central keratin core (c); Dense basophilic calcium with necrotic keratin (d)



Results

Microscopic examination of the retrieved OSCC specimens revealed well-formed nests of malignant squamous epithelial cells containing classic concentric keratin pearls, several of which exhibited distinct basophilic mineral deposits. These deposits ranged from fine granular foci to coarse irregular aggregates and lamellated psammoma-like rings, representing progressive stages of calcification, and were closely associated with necrotic keratin, apoptotic bodies, and compacted lamellae, suggesting a dystrophic process initiated around degenerated cellular fragments (Figure 1). The surrounding stroma showed marked desmoplasia with densely collagenised connective tissue, activated fibroblasts, and chronic inflammatory infiltrates predominantly composed of lymphocytes and plasma cells. Tumour islands adjacent to calcified areas demonstrated reduced vascularity, nuclear shrinkage, cytoplasmic eosinophilia, and micro-necrosis, indicative of local hypoxia, while peripheral tumour cells occasionally displayed spindle morphology and decreased intercellular cohesion, consistent with early epithelial–mesenchymal transition (EMT).

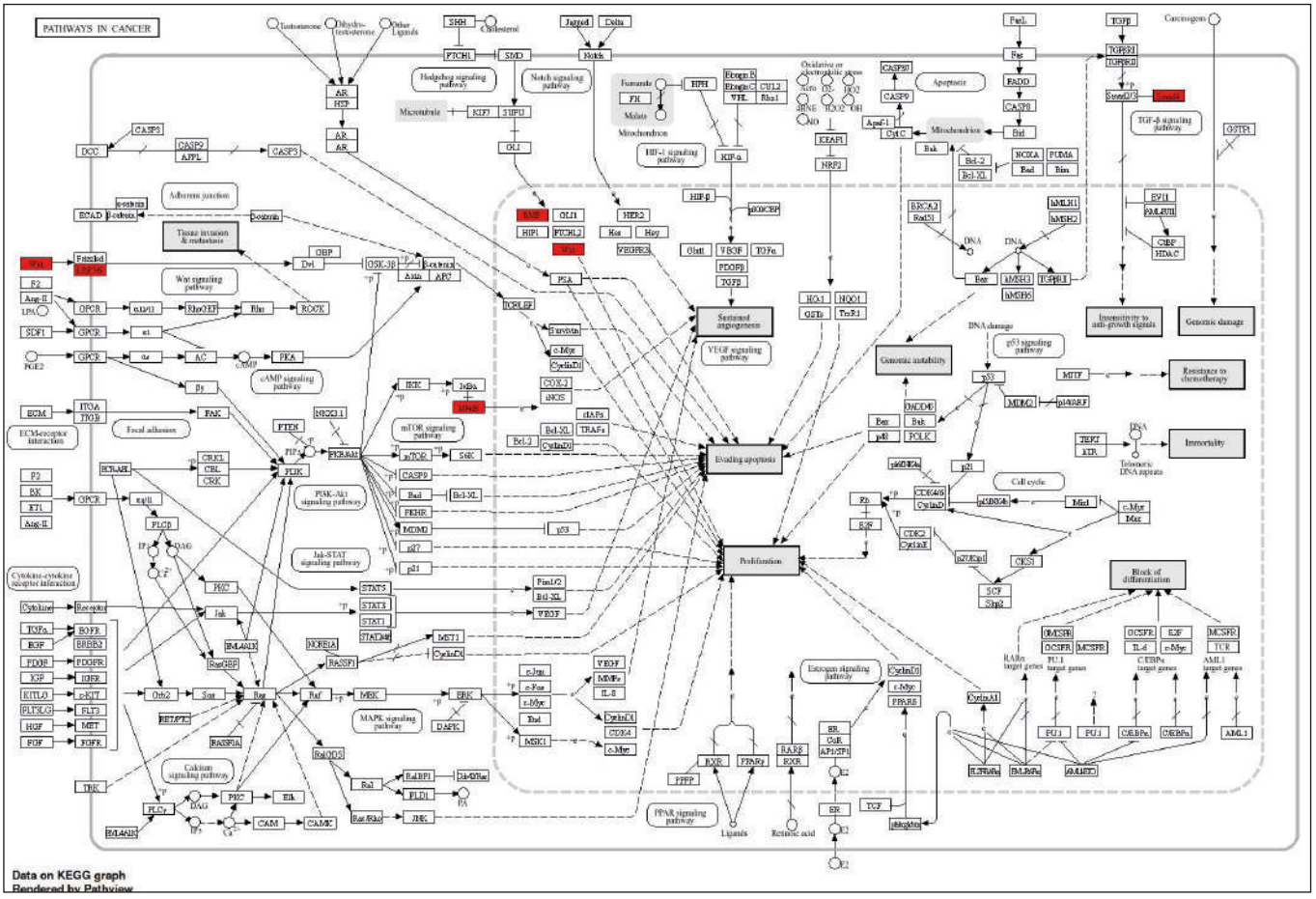


Fig. 2A: STRING network analysis showing the correlation and interaction of genes involved in calcification in OSCC & **Fig. 2B:** KEGG "Pathways in Cancer" map highlighting key molecular pathways related to ECM remodeling, mineralization, inflammation, and tumor progression in calcified OSCC.

Mechanisms and Hypotheses behind Calcification in OSCC:

Calcium ions (Ca^{2+}) are critical in regulating various cellular functions, including apoptosis. Nevertheless, they play a multifaceted role in regulating apoptosis in cancer cells via mitochondrial dysfunction, caspase activation, and endoplasmic reticulum stress.[4,5] Disruption of calcium pumps in cancer cells elevates intracellular calcium, driving proliferation, apoptosis resistance, invasion, and angiogenesis, thereby enhancing tumor progression and aggressiveness.[6]

The four reasons given for the calcifications are higher calcium concentration in the malignant epithelial cells, a higher concentration of apoptotic cells in well-differentiated OSCC, hypercalcemia in the tumor environment, and keratin calcification initiated at the intra-cellular level was considered as the probable etiology by the authors.[7] However, tumors in other organs such as the lung, breast, and ovary postulate that the calcification is due to the tumor cell degeneration, disturbance in the calcium metabolism in the tumor microenvironment, tumor cell secretion, shift in the functioning of cells due to epithelial-mesenchymal interaction, engulfment of calcified scar tissue or granulomatous tissue by tumor cells[8-10] Despite these insights of calcification of keratin pearls in OSCC remains poorly understood, warranting further in-depth investigation.

Genetic Network and Pathway Analysis:

Bioinformatics-based gene expression analysis reveals a complex gene network associated with calcification in oral squamous cell carcinoma (OSCC), highlighting the involvement of multiple genes and signaling pathways. Central to this network is BMP2, a key inducer of bone formation, supported by genes such as ALPL, SPP1, and RUNX2, which are critical for bone mineralization and remodeling. Structural matrix components like COL1A1 and regulators of the tumor microenvironment such as FGF2, SPARC, and MGP further underscore the multifaceted nature of pathological calcification in OSCC. KEGG pathway enrichment also identifies BMP4, TGFβ1, SMAD4, and components of the Wnt/β-catenin pathway (WNT1, LRP5, FZD2) as upregulated, indicating active roles in osteogenic differentiation, matrix deposition, and fibrosis (Figure 2A). The activation of the NF-κB signaling pathway (NFκB1, RELA, CHUK) and overexpression of anti-apoptotic genes BCL2 and BCL2L1 suggest a pro-inflammatory, apoptosis-resistant tumor environment conducive to dystrophic calcification. Additionally, oxidative stress-related genes like MGST1 reflect responses to hypoxic and necrotic conditions that may promote calcium deposition. Collectively, these gene expression patterns point toward a BMP4-TGFβ-NFκB signaling axis that integrates bone-like mineralization, fibrosis, and inflammation, positioning these genes as potential diagnostic markers and therapeutic targets in calcified OSCC (Figure 2B).

Discussion

Calcification in oral squamous cell carcinoma (OSCC) is a rare phenomenon and can be attributed to multiple factors involving the tumor microenvironment and cellular processes. One of the primary reasons is chronic inflammation and irritation, often seen in long-standing or neglected tumors.[11,12] These conditions, exacerbated by risk factors such as smoking, alcohol, or mechanical trauma, alter calcium metabolism and create a pro-inflammatory environment conducive to calcification. Additionally, tumor cell degeneration, often associated with necrosis or apoptosis, releases intracellular calcium, which, in the presence of hypercalcemia or disrupted calcium homeostasis, facilitates abnormal calcium deposition.[13,14] Our genetic analysis also showed the involvement of NFκB1, RELA, CHUK via NF-κB signaling pathway that promotes persistent inflammation, angiogenesis, and tumor progression, and it can also influence osteogenic differentiation and calcification.

Another contributing factor is epithelial-mesenchymal transition (EMT) and the interaction between tumor cells and stromal fibroblasts, which alter the tumor microenvironment. These interactions can stimulate the secretion of pro-calcifying factors like transforming growth factor-beta (TGF-β), which remodels the extracellular matrix and fosters calcification.[15] Also, the tumor cells of OSCC are known to secrete parathyroid hormone-related peptide, which eventually causes a paraneoplastic condition called cancer-related hypercalcemia.[16,17] Also, our genetic analysis showed that SPP1 (Osteopontin) has a major role in linking inflammation to mineralization, especially in dystrophic calcification.

Keratin dystrophy and the formation of keratin pearls are characteristic features of well-differentiated oral squamous cell carcinoma (OSCC), providing a structural foundation for calcification. The presence of apoptotic or necrotic debris within these pearls can serve as nucleation sites for calcium deposition. Additionally, desmoplastic reactions and fibrosis, commonly observed in OSCC, create a dense tumor stroma that reduces perfusion, induces hypoxia, and traps necrotic cell remnants, further promoting localized calcification. A previous study also proposed that the epithelial malignant cells of oral cancer may possess a genetic memory related to hard-cornified skin modifications, leading to the expression of cytokeratins.[7] Based on this, we propose that calcification in keratin pearls arises due to the differential expression of specific genes within these malignant epithelial cells—namely BMP2/BMP4, RUNX2, SPP1, ALPL, and SPARC. These genes, functioning through the BMP4–TGFβ–NFκB signaling axis, are implicated in osteogenic-like processes and pathological mineralization. Thus, keratin pearl calcification may be an intrinsically regulated intracellular event, orchestrated by the unique molecular profile of OSCC epithelial cells.

The prognosis of calcification in oral squamous cell carcinoma (OSCC) is complex and not directly linked to worse outcomes. While well-differentiated OSCCs generally have a favourable prognosis due to their slower growth and lower metastatic potential, the presence of calcifications may suggest chronic tumor progression or underlying necrosis, which could reflect more aggressive tumor behavior. Calcifications in OSCC, particularly when associated with extensive necrosis or poor vascular perfusion, may indicate a tumor microenvironment that is more resistant to treatment and prone to complications. However, in the absence of severe necrosis

or local complications, calcifications alone do not significantly worsen survival rates. As such, while calcifications serve as important diagnostic markers, their prognostic value depends on the overall tumor stage, degree of differentiation, and the presence of other adverse features, highlighting the need for a comprehensive evaluation when determining the prognosis for OSCC patients.

Limitation

The study is limited by its retrospective design, small sample size, and reliance on archival FFPE tissues, which may affect tissue quality and completeness of clinical data. Bioinformatics findings are based on bulk RNA-seq datasets that do not distinguish tumour from stromal components, and no functional validation experiments were performed to confirm molecular mechanisms.

Future Scope

Larger prospective studies with comprehensive clinical correlation are needed to validate findings. Advanced techniques such as spatial or single-cell transcriptomics and in-vitro/in-vivo models can help clarify cellular sources and mechanisms of calcification. Experimental validation of key signalling pathways may identify potential biomarkers or therapeutic targets.

Conclusion

In conclusion, calcification in oral squamous cell carcinoma (OSCC) is a multifaceted phenomenon influenced by a combination of tumor-specific and microenvironmental factors. Chronic inflammation, keratin dystrophy, necrosis, and disruptions in calcium metabolism are key contributors to this process, with the dense fibrotic stroma and hypoxic conditions further facilitating calcium deposition. While calcifications are not inherently indicative of poor prognosis, their presence may reflect chronic tumor progression or microenvironmental changes that could influence tumor behavior. Understanding the underlying mechanisms driving calcification in OSCC provides valuable insights into tumor biology and highlights the importance of evaluating both cellular and stromal components for better diagnostic, prognostic, and therapeutic strategies.

AUTHOR'S CONTRIBUTION: Suganya Panneer Selvam - study conceptualization, critically reviewed and edited the manuscript, and ensured the integrity and accuracy of the work. **Nitya Krishnaswamy** - study conceptualization, literature review, data interpretation, and manuscript writing. **Niranjan KC** performed the bioinformatics analysis, interpreted the gene expression data, and assisted in figure preparation. **Ramya Ramadoss** contributed to bioinformatics data analysis, and interpretation of gene expression data related to calcification in OSCC.

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