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REVIEW

From diagnostic marker-to-therapeutic modality: emerging paradigms in tumor biomineralization

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ABSTRACT Biomineralization is a highly regulated, multi-scale biological process that is well-characterized in physiologic contexts but remains poorly understood in pathologic settings. Tumor-associated calcification frequently occurs in clinical practice but is often regarded as an imaging feature rather than an active biological phenomenon. Existing evidence has indicated that tumor calcification is closely linked to cancer progression, prognosis, and underlying biological behavior. This review synthesizes current knowledge on tumor-associated biomineralization from a multidisciplinary perspective, integrating insights from oncology, cell biology, pathology, materials science, and systems biology. The major types and microstructural features of calcium deposition that occur in tumors are summarized and the clinical correlations are discussed. The proposed molecular and cellular mechanisms, including organelle-mediated mineral nucleation, metabolic regulation, differentiation-driven processes, and cell fate-dependent pathways, were further examined. Advances in multi-omics technologies, biomimetic models, and analytical characterization methods are highlighted as critical enablers for mechanistic investigation across biological scales. Finally, emerging strategies that exploit induced tumor calcification using engineered materials as a potential drug free therapeutic and diagnostic approach are discussed. By framing tumor calcification as an active, regulated phenotype, this review aims to provide a unified conceptual framework, identify current knowledge gaps, and encourage future research toward translational applications in cancer diagnosis and treatment.

KEYWORDS Cancer-associated calcification; tumor biomineralization; diagnostic and prognosis; microstructure and microenvironment; material-based therapy; artificial induced tumor calcification

Introduction

Biomineralization is a fundamental biological process that is tightly regulated across multiple scales, from molecular and cellular levels-to-tissues and organs. While physiologic biomineralization, exemplified by bone and tooth formation, has been extensively characterized, pathologic biomineralization is comparatively underexplored[1,2]. Among pathologic forms, tumor-associated calcification frequently occurs in clinical practice, yet tumor-associated calcification is often regarded as an imaging feature rather than a biologically meaningful phenomenon. Although the clinical significance of tumor-associated calcification is not fully appreciated, existing evidence has demonstrated a correlation with disease progression, prognosis, and intrinsic tumor biology[3-13].

Tumor calcification represents a complex, multidisciplinary subject at the intersection of oncology, cell biology, pathology, materials science, and systems biology. Although calcified deposits within tumors have long been recognized in radiology

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and histopathology, the biological origins, regulatory mechanisms, and functional significance are poorly understood. Emerging evidence suggests that tumor calcification involves coordinated interactions across multiple biological scales. At the molecular and cellular levels, calcifications are driven by a network of genes, proteins, structural domains, organelles, and metabolic pathways[1,7,14,15]. Cellular processes, including differentiation, stress responses, and regulated cell death, appear to critically influence mineral nucleation, growth, and spatial organization[5,15,16]. Nevertheless, the mechanistic logic linking these cellular and molecular events to the formation and distribution of calcified structures is largely unresolved.

Current research involving tumor calcifications can be broadly categorized into four principal areas: (1) correlating calcification patterns with prognosis and distinguishing benign from malignant lesions; (2) elucidating underlying biological mechanisms, particularly at the cellular and subcellular levels, including differentiation- and metabolism-driven pathways; (3) using multi-omics approaches to map regulatory networks; and (4) developing *in vitro* and *in vivo* models to experimentally investigate calcifications. While the morphology and composition of calcified deposits are well-documented, integrative studies connecting mineral phenotype to multi-scale biological regulation are limited. Tumor calcification is predominantly dystrophic and serves as a marker of metabolic stress and cell death, while some cancers exhibit active osteo-mimicry, which enables tumor cells to mimic bone formation and facilitate bone metastasis. In addition, the mineral deposits from tumor calcifications alter the biomechanics of the tumor microenvironment (TME) to enhance aggressiveness and impede drug delivery. Moreover, the deposits modulate immunity *via* inflammasome activation, driving chronic inflammation and tumor progression. Tumor calcification represents a distinctive cancer phenotype, a mineralized state regulated by cellular metabolism, signaling pathways, and microenvironmental cues[4]. Elucidating the principles of tumor biomineralization thus provides a unique opportunity to link mineral formation with tumor behavior within a unified biological framework.

This review provides an overview of calcium deposition that occurs in tumors and examines the relationship with tumor progression, including a particular emphasis on microstructural characteristics and microenvironmental interactions. Tumor biomineralization is used in the title and final section for a broader scope and calcification is included in the clinical examples for specificity. The proposed mechanisms underlying tumor-associated calcification, including differentiation processes, are discussed. Methodologic considerations are explicitly addressed, recognizing the importance of cross-disciplinary convergence in understanding this complex phenomenon. Finally, emerging strategies inspired by biomineralization are discussed, including the use of designer materials to induce tumor calcification as a "drug free" therapeutic approach[17-19]. By integrating findings across diverse fields, this review aims to provide a new perspective on tumor-associated biomineralization, stimulate mechanistic investigation, and contribute to improved diagnosis and treatment strategies for cancer.

Fundamentals of biomineralization in the human body

Physiologic bone biomineralization model

Bone is a complex, hierarchical biomineralized tissue that is primarily composed of nanocrystalline apatite (≈60 wt%), type I collagen (≈22 wt%), water (≈15 wt%), and non-collagenous organics (≈3 wt%)[20,21]. These components are organized into >10 levels of hierarchy. The mineral phase is generally identified as a carbonated apatite containing various ionic substitutions, such as sodium, magnesium, and acid phosphate, and molecular water[22,23]. While the exact formula is debated, common representations include $Ca_{10-x}[(PO_4)_{6-x}(CO_3)_x](OH)_{2-x}{\cdot}nH_2O$[1]. These anisotropic nanocrystals are structurally supported by type I collagen to form mineralized fibrils, where the crystallographic c-axes of the apatite align with the long axes of the collagen. This composition evolves over time. Indeed, older bone tissue typically features larger crystals with fewer defects compared to newly deposited tissue[1].

The biomineralization process may follow a coordinated cellular sequence, as follows: ion sequestration, osteoblasts collect calcium and orthophosphate species from the bloodstream; matrix deposition, osteoblasts secrete the osteoid, an extracellular matrix (ECM) primarily composed of oriented type I collagen fibers and regulatory non-collagenous proteins (NCPs)[24,25]; precursor transport, mineral building blocks or amorphous calcium phosphate (ACP) precursors are transported through the cell in membrane-enclosed structures; mineral release, these precursors are released into the osteoid, potentially *via* matrix vesicles containing mineralization proteins, like alkaline phosphatase (ALP); and crystallization/phase transformation, the precursors transform from a poorly ordered phase into final crystalline apatite within the collagen fibrils.

Bone remodeling is orchestrated by the following three primary cell types: osteoclasts, multinucleated cells that resorb bone by acidifying a sealed environment to dissolve mineral and degrade the matrix; osteoblasts, the primary cells responsible for bone formation deposit the osteoid, regulate pH and ion flux, and manage polyphosphate reserves; and osteocytes, differentiated osteoblasts embedded within the mineral that form a sensory and communications network to mediate global bone homeostasis.[26-28]

Well-known bone matrix NCPs include glycoproteins, such as ALP, osteonectin, fetuin-A, osteopontin (OPN), and bone

sialoprotein (BSP). The latter two belong to the SIBLING protein family, in addition to Gla proteins (primarily osteocalcin) and proteoglycans (e.g., decorin). These NCPs have been identified in mineralized bone and regulate mineralization, organize the organic matrix, define nucleation sites, and control mineral growth. Most NCPs are expressed by osteoblasts, whereas other NCPs, such as fetuin-A, are serum-derived. Many NCPs also function as signaling molecules. The mineralization roles are spatially and temporally coordinated and influenced by multiple factors. In addition to NCPs, there are small molecules in which pyrophosphate acts as a mineralization inhibitor and ALP substrate, while citrate may serve as an apatite stabilizer[29].

Physiologic mineralization is a highly regulated, multi-stage process wherein specialized cells orchestrate a localized microenvironment to facilitate targeted mineral deposition with precise chemical compositions and crystalline structures. This biomineralization framework typically integrates four fundamental components: regulated ion sources; specialized mineralizing cells; an organic matrix of structural and regulatory proteins; and the transport of mineral precursors *via* extracellular vesicles. These elements culminate in a functional, hierarchically organized architecture of mineralized collagen fibers in healthy bone tissue. Conversely, pathologic mineralization involves a diversion from these homeostatic pathways. While pathologic mineralization may utilize similar cellular and molecular machinery, pathologic mineralization is often characterized by the recruitment of additional aberrant components that lead to disorganized, ectopic mineral deposition.

Pathologic and tumor calcification

Pathologic biomineralization is a dysregulated biological process in which biominerals are abnormally deposited in soft tissues, disrupting normal structure and function. Unlike physiologic biomineralization, such as bone and tooth formation, this ectopic mineralization arises from imbalances between mineralization promoters and inhibitors, which are often driven by metabolic disturbances, inflammation, and cellular dysfunction. Major clinical manifestations of pathologic biomineralization include kidney stone disease, gout, and vascular calcification[30-41]. Pathologic calcifications are mainly addressed here (**Table 1**).

Nephrolithiasis primarily involves the formation of calcium oxalate stones, especially calcium oxalate monohydrate (COM). Stone development begins with urinary supersaturation, followed by nucleation, crystal growth, aggregation, and retention in the kidney. COM crystals adhere strongly to renal epithelial cells *via* surface interactions, whereas calcium oxalate dihydrate exhibits lower adhesion and pathogenicity. Renal epithelial responses, such as upregulation of adhesion molecules, apoptosis, and vesicle release, further promote crystal retention. Subepithelial calcium phosphate deposits, which are known as Randall's plaques, provide nucleation sites that link microscopic calcification to macroscopic stone formation[42].

Vascular calcification, which is common in aging, diabetes, and chronic kidney disease, involve hydroxyapatite deposition in arterial walls or atherosclerotic plaques. Vascular smooth muscle cells (VSMCs) undergo osteogenic trans-differentiation in response to elevated phosphate, oxidative stress, and inflammation, and express bone-related proteins, such as Runx2 and alkaline phosphatase. These cells release matrix vesicles that nucleate hydroxyapatite, paralleling skeletal mineralization processes but occurring inappropriately within the vasculature[43].

Both conditions are regulated by overlapping molecular pathways. Elevated phosphate, degradation of the mineralization inhibitor (pyrophosphate) by tissue non-specific alkaline phosphatase (TNAP), and deficiencies in inhibitors, such as fetuin-A, matrix Gla protein, and FGF-23, promote ectopic calcification. Organic matrix proteins, like osteopontin and Tamm-Horsfall protein, act as inhibitors or promoters of crystallization depending on local conditions. Emerging evidence has also implicated regulated cell death pathways, including ferroptosis, in releasing calcifying vesicles[44].

Overall, pathologic biomineralization reflects shared physicochemical principles with normal biomineralization but lacks proper spatial and temporal control. Understanding the cellular and molecular drivers of ectopic mineral deposition is essential for developing therapies that inhibit pathologic calcification while preserving physiologic mineralization.

Tumor calcification, the pathologic deposition of inorganic minerals (i.e., calcium) within neoplastic tissues, has emerged as a critical phenomenon with profound implications for cancer diagnosis, prognosis, and therapeutic intervention. Unlike physiologic biomineralization that occurs in bone and teeth formation, tumor-associated calcification often reflects dysregulated cellular processes, microenvironmental alterations, and systemic metabolic imbalances. This process primarily manifests as deposition of calcium phosphate, which is increasingly recognized as a biomarker for tumor behavior and patient outcomes[17]. The clinical detection of such mineral deposits, particularly in breast, ovarian, colorectal, and thyroid cancers, provides non-invasive imaging signatures that can guide diagnostic workflows and risk stratification.

Microcalcifications (**Figure 1**) detected *via* mammography serve as one of the earliest radiologic indicators of malignancy in breast cancer, especially ductal carcinoma *in situ* (DCIS). These calcifications arise from necrotic cell debris, apoptotic bodies, and matrix vesicles that act as nucleation sites for hydroxyapatite crystal growth[1,3]. Importantly, the morphology, distribution, and density of these calcifications correlate with tumor aggressiveness and recurrence potential. Similarly, psammoma bodies, which are concentrically

Table 1 Examples of pathologic calcifications in the human body

	Mineral type	Medical name of calcification	Location
Calcium phosphat	Apatite $[Ca_{10}(PO_4)_6(OH)_2]$ common known ionic substituents: Ca: Na, Mg; PO_4: CO_3, HPO_4; OH: Cl, F, H_2O	Microcalcifications	Breast[45,46]
		Psammoma bodies	Thyroid[47]
			Ovaries[48]
			Brain (meningioma)[49]
			Brain (pineal gland)[49]
		Microliths	Testicles[50]
		Kidney stones (urinary calculi)	Kidney, urinary system[51]
		Calcifications (macular degeneration)	Eye (nodules, Bruch's membrane plaques)[52]
		Calcifications	Vascular tissue (not including heart valves)[53]
		Calcifications	Vascular tissue (heart valves)[53]
		Calcifications	Bioprosthetic heart valves[54]
	β-tricalcium phosphate $[Ca_3(PO_4)_2]$ Whitlockite $(Ca,Mg)_3(PO_4)_2$	Microcalcifications	Breast[55]
		Calcifications	Testicles[56]
		Calcifications	Vascular tissue (not including heart valves)[1]
		Calcifications (macular degeneration)	Eye (retinal drusen)[57]
	Octacalcium phosphate $[Ca_8H_2(PO_4)_6{\cdot}5H_2O]$	Calcifications	Vascular tissue (not including heart valves)[58]
	Amorphous calcium phosphate	Microcalcifications	Breast[59]
Calcium oxalate	Weddellite (dihydrate) $(CaC_2O_4{\cdot}2H_2O)$	Microcalcifications	Breast[60]
		Kidney stones (urinary calculi)	Kidney, urinary system[61]
	Whewellite (monohydrate) $(CaC_2O_4{\cdot}H_2O)$	Kidney stones (urinary calculi) (major component)	Kidney, urinary system[61]

laminated calcified structures that are hallmark features of papillary thyroid carcinoma and some subtypes of ovarian cancer, are linked to chronic inflammation, oxidative stress, and aberrant expression of mineralization-regulating proteins, such as osteopontin and ALP. For example, the identification of lymph node calcification in thyroid cancer significantly enhances diagnostic specificity and serves as an adverse prognostic indicator due to the association with advanced disease and metastatic spread.

The prognostic value of tumor calcification varies across cancer types. Spontaneous intratumoral calcification is paradoxically associated with improved survival in glioblastoma, colorectal, and lung cancers, possibly reflecting lower proliferative activity, enhanced immune surveillance, or reduced angiogenesis. Conversely, calcifications may indicate more aggressive phenotypes and resistance to therapy in pancreatic and renal cell carcinomas. These divergent outcomes underscore the microenvironment-specific nature of biomineralization, necessitating tumor-specific interpretation frameworks.

Researchers have begun to exploit biomineralization therapeutically not merely as a passive marker but as an active target (**Table 2**). "Blockade therapy" strategies induce artificial mineralization within tumors to occlude blood vessels and nutrient supply, which effectively starves malignant cells[18]. One innovative approach involves liposomal delivery of black phosphorus and calcium peroxide to mitochondria, which

triggers *in situ* calcification that selectively disrupts cancer cell metabolism without affecting healthy tissue, a promising drug-free modality for brain tumors[62]. Furthermore, macromolecular agents, like folate-polySia, selectively induce extracellular calcification on tumor surfaces by hijacking systemic calcium homeostasis, offering a targeted chemotherapy alternative with minimal off-target toxicity[63].

Despite these advances, challenges remain in standardizing the assessment of tumor mineralization across imaging modalities and histopathologic practices. Variability in detection sensitivity, interobserver interpretation, and biological heterogeneity limits widespread clinical adoption. Future directions include integrating artificial intelligence for automated calcification pattern recognition, developing dynamic probes for real-time monitoring of mineralization kinetics, and exploring epigenetic regulators of ectopic mineralization.

Tumor calcification – clinical implications and new study paradigm

Spontaneous calcification in various tumor types

Breast

Microcalcifications are among the most important radiologic features in breast cancer detection and have been recognized as a marker of malignancy since the early 1950s. Microcalcifications are present in 30%–50% of non-palpable screen-detected breast cancers and in the majority of DCIS cases (**Figure 3**)[64,65]. Mammographic screening programs have contributed substantially to the early diagnosis of breast

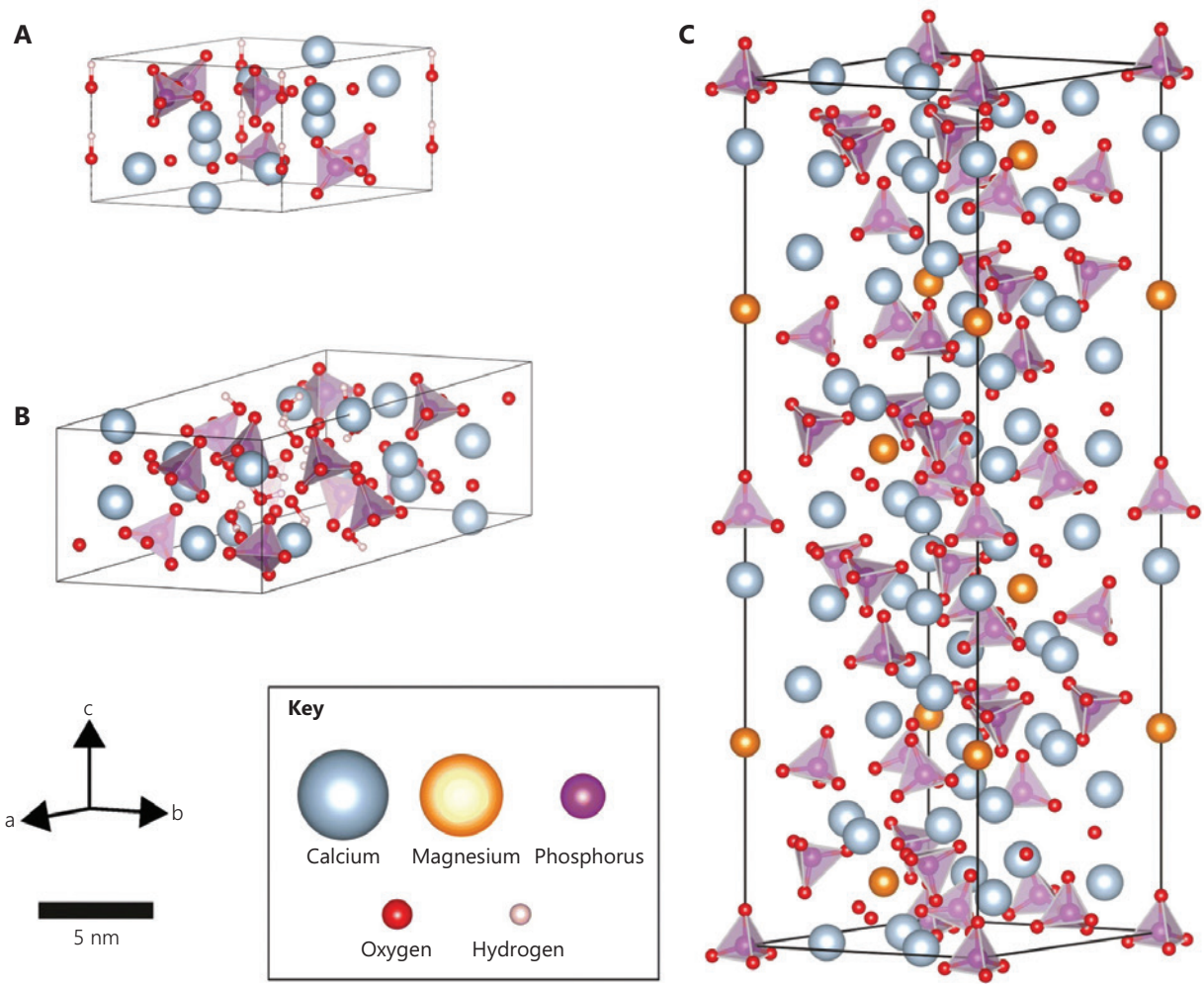


Figure 1 The structure of unit cells (single repeating units) of breast microcalcification phases. (A) a unit cell of hydroxyapatite (HAp), (B) a unit cell of octacalcium phosphate (OCP), (C) a unit cell of magnesium whitlockite. The arrows indicate the three directions of the lattice parameters ('a,' 'b,' and 'c')[4]. Reproduced under the terms of the CC-BY license[4]. Copyright 2019 Gosling et al., published by Springer Nature.

Table 2 Summary of induced tumor biomineralization

Category	Core mechanism	Main inducers/strategies	Primary therapeutic effect	Representative model/study
Pericellular/extracellular mineralization (tumor blockade)	Forms a dense mineral shell around tumor cells/tissues; blocks nutrient exchange, invasion, and signaling	- Folate-polySia (folate receptor targeting)[63] - Bisphosphonate-conjugated polymers (DSPE-PEG-ALN)[91] - Acid-triggered self-assembling peptides (BINP)[19]	Physical blockade, inhibition of proliferation/migration, reduced metastasis	Breast cancer, cervical cancer, osteosarcoma (mouse models)
Intracellular/mitochondrial mineralization	Induces mineral deposition inside tumor cells (especially mitochondria); disrupts organelle function and induces apoptosis	- Mitochondria-targeted liposomes (BCLT: BP + CaO_2)[62] - Silica precursors (trialkoxysilane)[95] - Iron-based mineral inducers (HC)[94]	Direct cell killing *via* organelle damage; drug-free therapy	SCC7 tumor, lung cancer (A549), osteosarcoma
Stimuli-responsive mineralization	Activated by tumor-specific stimuli (acidic pH, NIR light, enzymes); spatiotemporally controlled mineralization	- pH-responsive peptide nanoparticles (BINP)[19] - NIR-triggered BP/CaO_2 liposomes (BCLT)[62] - Enzyme-activatable mineral precursors[96]	On-demand mineralization; reduced side effects	Various solid tumors (pH/NIR responsive)
Biomineralization for tumor diagnosis	Generates mineral contrast agents for imaging; enables non-invasive tumor detection	- Iron-based mineral inducers (CT contrast)[94] - Calcium mineralization (Micro-CT imaging)[87]	Tumor visualization, quantification, and treatment monitoring	Lung cancer, osteosarcoma (Micro-CT/PET-CT)

cancer with large meta-analyses reporting up to a 20% reduction in breast cancer mortality. Despite ongoing debate regarding over-diagnosis and false-positive rates, mammography remains a cornerstone of breast cancer screening, particularly when combined with clinical examination and biopsy as part of the triple assessment approach.

The diagnostic value of microcalcifications depends on morphology and distribution. Benign calcifications, such as popcorn-like, eggshell, or dystrophic forms, are typically associated with involutional or post-traumatic changes. In contrast, suspicious morphologies, including amorphous, coarse heterogeneous, fine pleomorphic, and fine linear (casting) calcifications, are strongly associated with malignancy[66,67]. Meta-analyses demonstrate increasing malignancy risk across these patterns, with fine linear calcifications carrying the highest predictive value. The distribution of microcalcifications further refines risk assessment. Specifically, linear and segmental patterns suggest ductal spread and are highly suspicious, while clustered calcifications have an intermediate risk and diffuse patterns are usually benign. Accurate interpretation requires combined assessment of morphology and distribution to avoid diagnostic misclassification.

Breast microcalcifications are classified into two main chemical types: calcium oxalate (Type I), which is exclusive to benign lesions; and hydroxyapatite (Type II), which is present in both benign and malignant tissues and is universal in invasive and *in situ* carcinomas. Conventional mammography cannot distinguish between these types, prompting the development of advanced spectroscopic techniques. Raman and Fourier transform infrared (FTIR) spectroscopy enable molecular characterization of calcifications, revealing features associated with malignancy, such as reduced carbonate substitution in hydroxyapatite and increased protein-to-mineral ratios[68,69]. Emerging evidence also implicates magnesium-containing minerals, such as whitlockite, as potential markers of malignant calcifications.

In addition to diagnosis, microcalcifications, particularly the casting-type, have significant prognostic implications. Multiple studies have associated the presence of microcalcifications with higher tumor grade, lymph node involvement, HER2 overexpression, increased recurrence risk, and reduced long-term survival. Patients with casting calcifications exhibit markedly worse outcomes compared to patients without calcifications, including higher mortality hazard ratios and recurrence rates. Genomic assays, such as Oncotype DX, frequently classify tumors with calcifications as high risk. Although some studies have reported conflicting findings, large-scale analyses consistently support an association between microcalcifications and aggressive tumor biology, particularly HER2 positivity[70-72].

Hydroxyapatite, the predominant mineral in malignant microcalcifications, actively contributes to tumor progression rather than representing a passive byproduct. *In vitro* studies

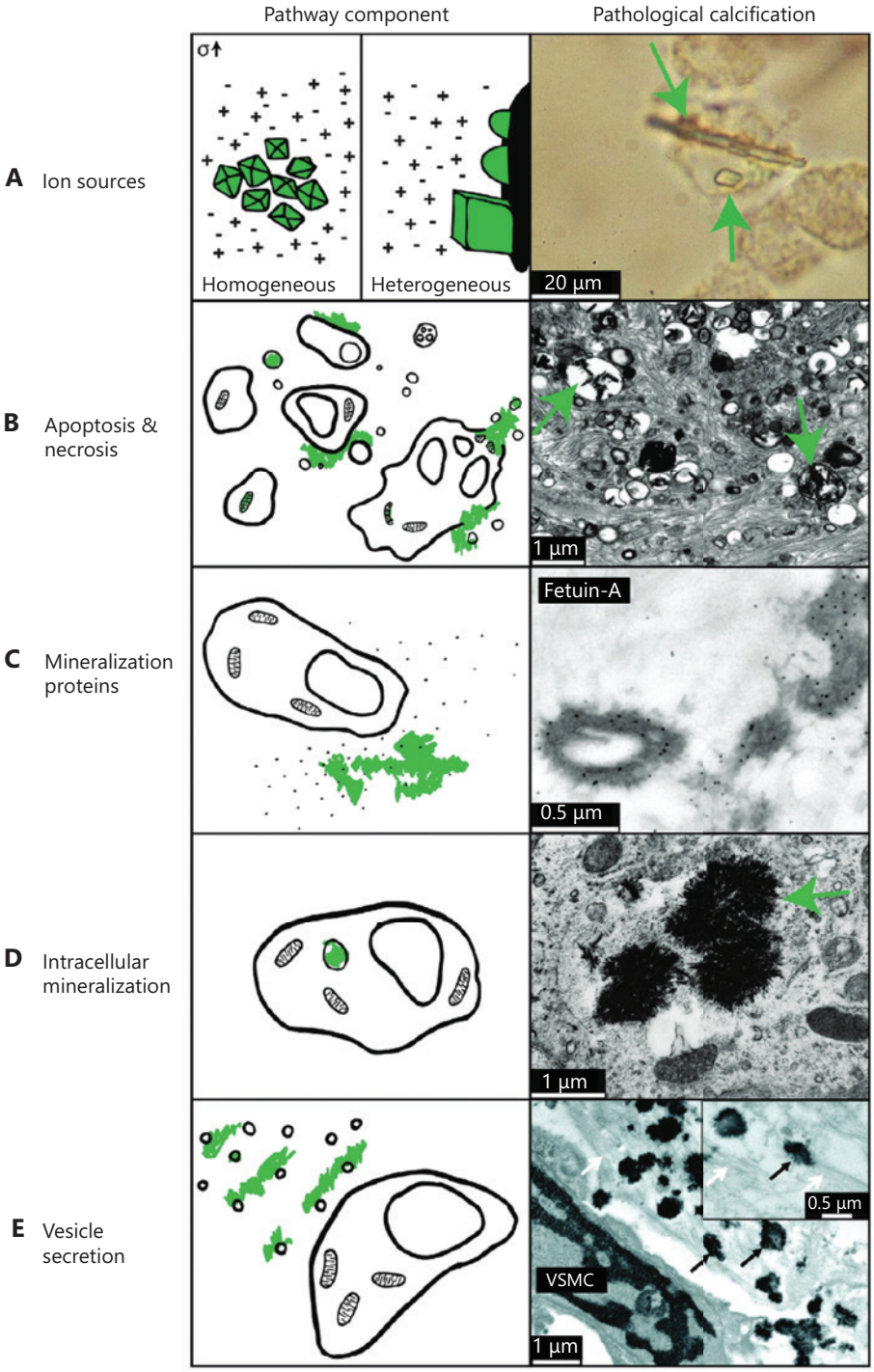


Figure 2 Pathway components identified in pathologic mineralization; see text for more details about each component. Left: Schematic representations (green: mineral). Right: Examples of pathologic calcifications where the corresponding pathway component is observed. Although a single cell is shown schematically (left), most pathologic mineralization occurs in a multicellular environment: (A) Ion sources in solution, i.e., serum and other biological fluids. Homogeneous nucleation can occur in bulk solution when the local supersaturation increases and heterogeneous nucleation can occur on cellular components, debris, or on foreign bodies at lower supersaturations due to a reduction in the energetic barrier to nucleation. Light microscope image of CPPD crystals extracted from the synovial fluid of a patient with joint disease. Rhomboidal and needle-shaped crystals are observed (arrows). (B) Apoptosis/necrosis. TEM image shows vascular calcification. Calcium deposits are observed in cell debris areas, some within vesicles (arrows). (C) Mineralization proteins. High-resolution TEM image of

immunogold labeling of calcifications in human arteries shows fetuin-A, a known mineralization inhibitor, associated with calcifications. (D) Intracellular mineralization. TEM image of human breast carcinoma. Clusters of needle-shaped crystals are observed (arrow) in an intracytoplasmic lumen. (E) Vesicle secretion. TEM of human arteries showing a vascular smooth muscle cell (VSMC) surrounded by vesicles containing calcifications (black arrows) in the ECM. White arrows indicate collagen fibrils. Inset: higher magnification of the calcification and collagen[1]. Reproduced with permission[1]. Copyright 2020 Wiley-VCH GmbH.

have demonstrated that hydroxyapatite promotes breast cancer cell proliferation and migration through calcium-dependent signaling pathways. Hydroxyapatite also induces inflammatory responses by upregulating cyclooxygenase-2 (COX2) and prostaglandin E2 (PGE2), facilitating epithelial–mesenchymal transition and invasion[73]. In addition, hydroxyapatite stimulates matrix metalloproteinase expression, promoting ECM degradation, and may activate inflammasome pathways, further linking calcification to tumor-promoting inflammation.

Microcalcification formation in breast cancer resembles physiologic mineralization processes, such as bone formation, and shares mechanisms with pathologic vascular calcification. Central to this process is dysregulation of the phosphate–pyrophosphate balance, which is mediated by ALP activity. Breast cancer cells exhibit osteo-mimicry, expressing bone-associated genes, including *BMP2*, *RUNX2*, osteopontin, and ALP, enabling active mineral deposition. Additional contributors include carbonic anhydrase I and secretory pathway Ca^{2+}-ATPases, underscoring that microcalcifications arise from regulated cellular processes rather than passive calcium precipitation[73,74].

Microcalcifications provide a critical link between early detection and prognostic stratification in breast cancer. The strong association between microcalcifications and aggressive molecular features, particularly HER2 overexpression, highlights the potential role in guiding targeted therapies. Advances in non-invasive chemical characterization may reduce unnecessary biopsies and improve diagnostic specificity. Future research should focus on integrating calcification biology into risk prediction models, refining imaging techniques, and exploring therapeutic strategies targeting calcification-associated pathways, such as COX2 or ALP inhibition.

Microcalcifications are not merely radiologic markers but are biologically active components of breast cancer progression. The imaging characteristics, chemical composition, and molecular effects collectively inform diagnosis, prognosis, and treatment planning. Continued integration of imaging, molecular biology, and computational techniques will enhance the clinical utility of microcalcifications and support more personalized approaches to breast cancer management.

Prostate

Prostate calcification and the association with prostate cancer has also been reported[7,76,77]. A prospective blinded study evaluated the prevalence and classification of prostatic calcifications on transrectal ultrasonography (TRUS) and the correlation with histology in men undergoing prostate biopsy[12]. Using a standardized imaging protocol and a novel classification system, prostate cancer was identified in 58.8% of patients, inflammation in 18.9%, and benign pathology in 22.3%. Interface calcification was common (42.3%) and showed no association with specific pathology, whereas peripheral zone calcification was uncommon (6.8%) but significantly associated with prostate cancer with 78.1% of affected patients demonstrating malignant histology, suggesting potential diagnostic relevance.

Solid tumor growth and metastasis depend on angiogenesis. However, the biological properties of tumor-associated endothelial cells (ECs) have historically been considered largely normal and functionally passive. Dudley et al. has challenged this assumption by identifying a distinct population of multipotent prostate tumor endothelial cells (TECs) capable of undergoing a mesenchymal-like transition[7]. This transition enables ectopic differentiation into bone and cartilage-like tissues, resulting in vascular calcification within the TME. These findings revealed an unexpected level of endothelial plasticity and suggested that the tumor vasculature may have an active role in cancer progression rather than serving solely as a conduit for nutrients and oxygen. TECs have been shown to retain endothelial identity using the TRAMP mouse model of prostate cancer, while exhibiting stem cell and mesenchymal features, which distinguished TECs from normal ECs. Functionally, TECs demonstrated restricted multipotency, differentiating into bone- and cartilage-like tissues and undergoing mineralization, leading to vascular calcification within the TME. These findings were supported by human prostate cancer tissue analyses, which revealed frequent tumor calcification and vascular-associated mineral deposits co-localize with endothelial markers. Together, these results highlight unexpected endothelial plasticity and suggest that the tumor vasculature may actively contribute to prostate cancer progression rather than serving solely as a passive conduit.

Gynecologic

Gynecologic tumors, including ovarian cancer, uterine fibroids, endometrial cancer, and cervical cancer, pose a significant threat to the health of women globally. While these tumors have been extensively studied, calcification in the microenvironments is an overlooked issue, despite being a common occurrence. Calcification exhibits distinct patterns across different gynecologic tumors. Calcification is most

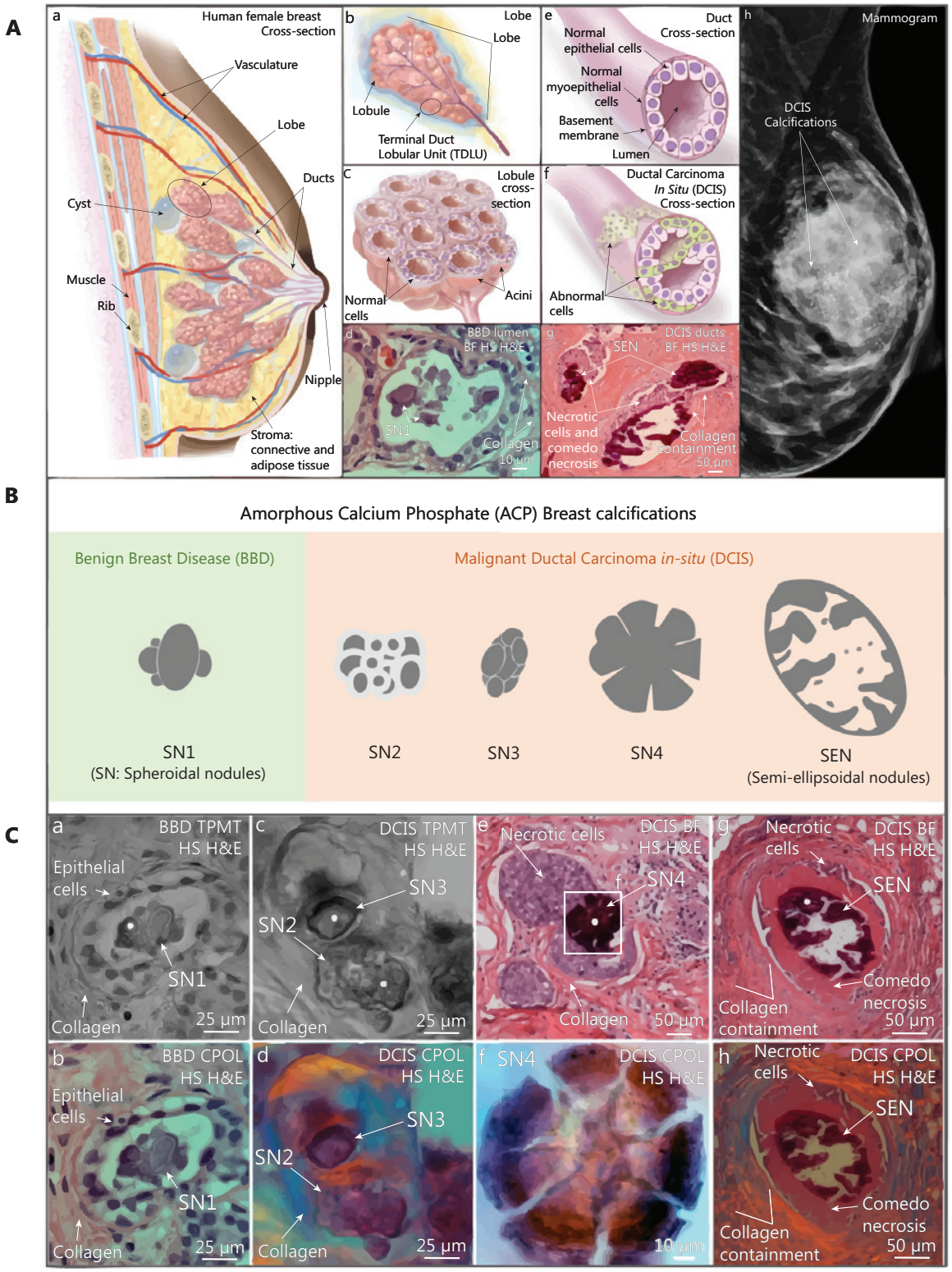


Figure 3 (A) Anatomic illustrations, microscopy imaging, and a mammogram projection showing calcifications associated with the progressive development of BBD and DCIS in the human female breast[75]. (B) Schematic of amorphous calcium phosphate (ACP) breast calcifications. (C) Microscopy and Raman spectroscopy analyses of calcifications associated with BBD and DCIS. Reproduced under the terms of the CC-BY license[75]. Copyright 2025 Sivaguru et al., published by Springer Nature.

prevalent in uterine fibroids and ovarian cancer (**Figure 4**), yet rare in endometrial and cervical cancers. Calcification typically presents as a degenerative change in uterine fibroids, often appearing as a dense shell or arc-shaped deposit in the tumor periphery with an incidence ranging from 4%–13%, according to clinical studies[8]. Ovarian cancer, particularly the serous papillary subtypes, frequently exhibits psammomatous calcification, which are granular psammoma bodies formed by lamellated calcification of necrotic cells. Notably, 8% of ovarian tumors have been shown to have calcifications on a CT scan with serous tumors (13%) being more prone to calcifications than mucinous tumors (4%). In contrast, endometrial and cervical cancers rarely develop calcifications. When present, calcifications often manifest as microcalcifications or psammoma bodies, with most cases associated with benign conditions.

The association between calcification and histologic stage of gynecologic tumors is most evident in ovarian cancer. A meta-analysis of 3 studies, including 175 calcified lesions, revealed that 74% of calcified ovarian tumors are low grade, while only 50% of high-grade serous cancers exhibit calcification. This finding suggested that calcification is predominantly linked to lower histologic grades. Most microcalcifications in endometrial tumors correlate with benign factors, such as postmenopausal status or endometrial polyps, with only 3% of cases associated with malignancy. Due to the rarity of calcification in cervical cancer, no clear correlation with histologic stage has been established.

Calcification also has a pivotal role in predicting prognosis, although the impact varies by tumor type. Calcification indicates benign degeneration and tumor necrosis in uterine fibroids, serving as a marker of good prognosis. Conversely, ovarian cancer patients with calcification face poorer outcomes. A study conducted by Burkill et al. reported a median survival of 61 months for calcified cases compared to 132 months for non-calcified cases[78]. However, conflicting findings exist and some studies have noted no significant difference in survival based on psammoma body presence, highlighting the complexity of this association[8]. Limited data preclude definitive conclusions for endometrial and cervical cancers but the rarity of calcification in malignant cases suggests potential prognostic value.

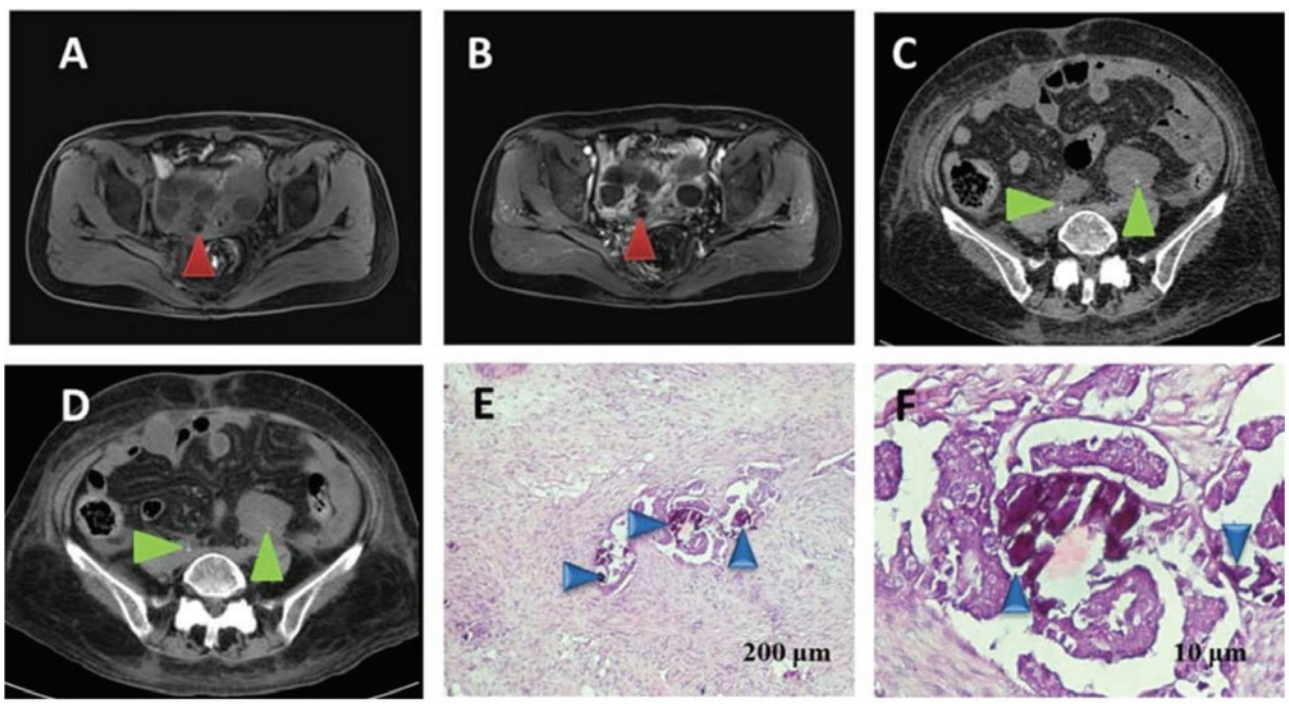


Figure 4 Morphology of calcification in ovarian cancer. (A) Pelvic magnetic resonance imaging scan showing nodular calcifications (arrowhead) in the left ovary. (B) Pelvic magnetic resonance imaging scan showing nodular calcification (arrowhead) in the left ovary. (C) Pelvic CT showing punctate calcifications (arrowhead) in the left ovary. (D) Pelvic thin section CT scans showing punctate calcification (arrowhead) in the left ovary. (E) Sporadic psammoma body in a biopsy specimen. The background contains ovarian tumor cells with rich, bright cytoplasm and vesicular nuclear chromatin (hematoxylin and eosin stain, original magnification ×100). (F) Concentric, laminated calcifications (psammoma body) in a biopsy specimen. The background contains ovarian tumor cells with rich, bright cytoplasm and vesicular nuclear chromatin (hematoxylin and eosin stain, original magnification ×400)[8]. Reproduced with permission[8]. Copyright 2018 Elsevier.

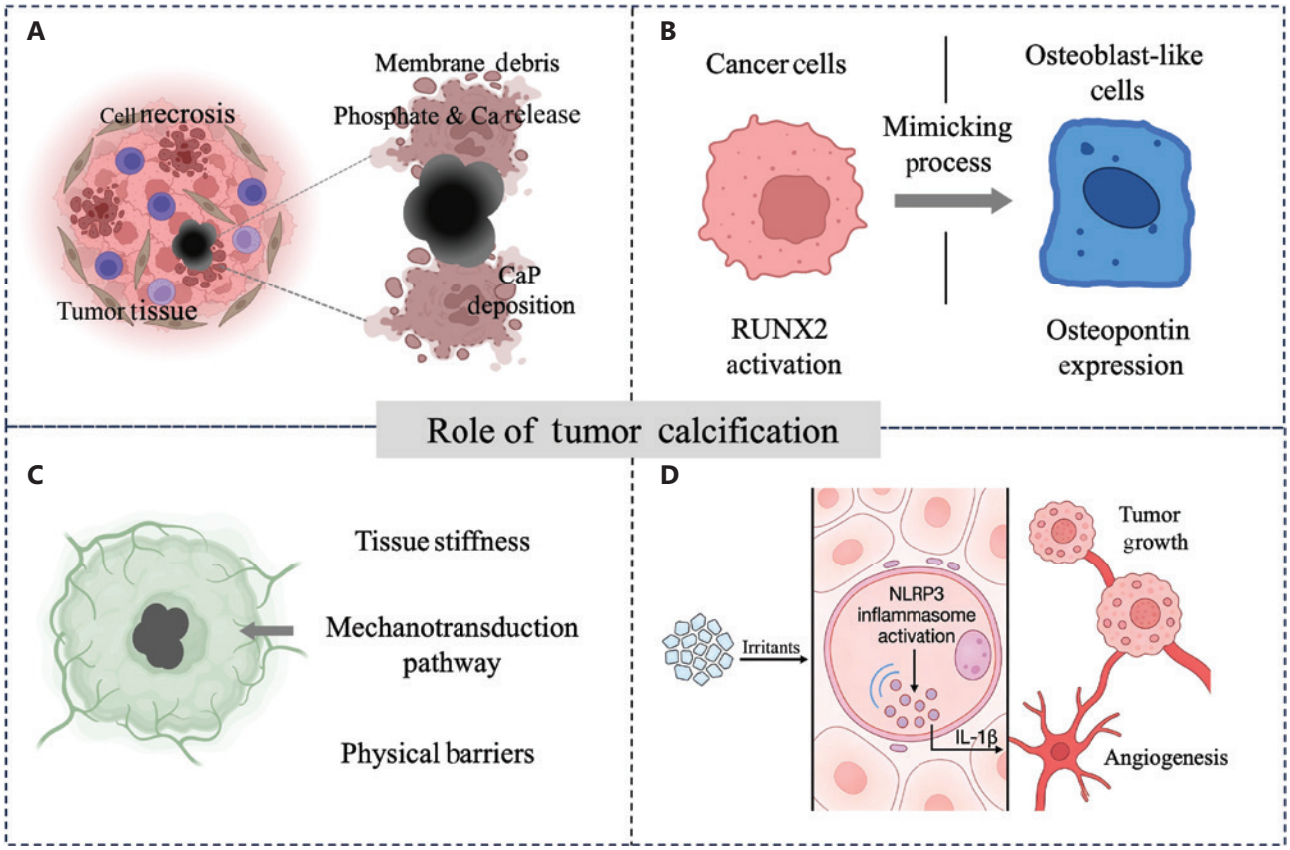


Figure 5 Schematic diagram of tumor-associated calcification as a core biological event in tumors. (A) Metabolic stress and cell death (dystrophic deposition with membrane phosphate and calcium from dying cells). (B) Osteo-mimicry (gene co-option). (C) Biomechanical alteration (matrix hardening). (D) Inflammation and immune modulation (inflammasome activation). Created with BioRenders.

The mechanisms driving calcification in gynecologic TMEs remain unclear with two primary theories proposed. One theory attributes calcification to tumor cell degeneration. Factors like ischemia, chemotherapy, or radiotherapy induce necrosis, leading to dystrophic calcification as calcium salts deposit in dying tissue. The other theory emphasizes disturbed calcium metabolism in the local microenvironment, driven by secretions from tumor cells, stromal cells, or osteoblast-like cells derived from the epithelial-mesenchymal transition. Studies have suggested that molecules, such as bone morphogenetic protein 2 and type IV collagen, may promote psammoma body formation in ovarian cancer, while hormonal influences contribute to calcification in uterine fibroids.

Brain

Oligodendroglioma (ODG), defined by IDH mutation and 1p/19q co-deletion, is well-known for characteristic intratumoral calcification, a feature commonly used for radiologic diagnosis[79]. Despite this characteristic, the prognostic significance of calcification is unclear. A large retrospective study involving 305 patients with molecularly confirmed ODG addressed this gap by systematically examining the clinical, radiologic, molecular, and survival implications of tumor calcification[11]. Patients were stratified into calcified and non-calcified groups with outcomes assessed using univariate and multivariate survival analyses.

Calcification was present in approximately one-third of cases and was associated with more aggressive tumor features, including larger size, higher histologic grade, elevated Ki-67 index, lower extent of resection, and adverse radiologic characteristics, such as midline crossing, contrast enhancement, and cystic components. Molecularly, calcified tumors exhibited higher MGMT methylation levels and increased rates of 1q/19p co-polysomy. Importantly, calcification correlated with significantly worse progression-free and overall survival and remained an independent negative prognostic factor on multivariate analysis. Specific imaging patterns, particularly T2-hypointense calcification and calcification accompanied by cyst formation, identified a subset of ODGs with especially aggressive behavior and poorer outcomes. Together, these findings established intratumoral calcification as a diagnostic hallmark and a clinically meaningful prognostic marker in ODG.

Calcified brain metastases (CBMs) have traditionally been considered rare and most often associated with prior radiotherapy, leading to frequent misclassification of intraparenchymal calcifications as benign findings. However, recent evidence suggests that CBM are more common than previously recognized[9]. In a retrospective analysis of 1,002 brain CT scans from oncologic patients, calcified metastases were identified in 17.9% of patients with brain metastases and 9.5% presenting with calcifications *ab initio* without prior radiotherapy. Lung adenocarcinoma was the most frequent primary tumor associated with CBM, followed by breast ductal carcinoma and small cell lung cancer. Calcifications were more commonly punctate and often present in multiple lesions with no clear correlation between calcification morphology and primary tumor histology. These findings underscore the importance of considering metastatic disease in the differential diagnosis of intraparenchymal brain calcifications, particularly in oncologic patients, to avoid delays in diagnosis and treatment.

Others

Thyroid nodule calcification is regarded as one of the most important features in ultrasound (US) diagnosis of thyroid cancer [papillary thyroid carcinoma (PTC)]. Recent advances in machine learning have enabled more objective and reproducible assessment of US features associated with thyroid cancer, particularly calcifications[80]. A DeepLabv3+-based deep learning framework was developed to automatically detect thyroid nodules and to identify and quantify intra-nodular calcifications on US images[81]. Using a large multicenter dataset, the model demonstrated high agreement (>90%) with experienced radiologists in detecting calcifications, supporting its reliability and potential utility in routine clinical practice.

Beyond detection, the study introduced novel quantitative parameters to characterize US calcifications and explored the prognostic significance. These parameters differed significantly between PTC patients with and without cervical lymph node metastasis (LNM) and were shown to contribute meaningfully to LNM risk prediction. A multivariable logistic regression model incorporating calcification metrics, patient age, and additional US features achieved higher specificity and accuracy than calcification parameters alone. These findings suggested that automated, quantitative assessment of calcifications may not only enhance diagnostic consistency but also aid in identifying more aggressive PTC phenotypes, thereby supporting risk stratification and personalized management strategies.

Mesenteric carcinoid tumors commonly exhibit calcification and fibrosis on CT, features that can aid diagnosis. In a retrospective series of 29 cases, calcification was present in approximately 70% of mesenteric masses, most often appearing as stippled, coarse, or diffuse patterns and corresponding histologically to areas of mature fibrous tissue. The degree of radiating strands on CT correlated with the extent of fibrosis, supporting the classic imaging triad of a calcified mesenteric mass, radiating strands, and adjacent bowel-wall thickening as highly suggestive of carcinoid tumor[82].

Calcification within primary lung cancer is an uncommon but clinically important imaging finding that may lead to diagnostic uncertainty. In a review of CT scans from 353 patients undergoing initial evaluation for lung cancer, intratumoral calcification was identified in approximately 6% of cases[83]. Calcified tumors spanned a range of sizes and histologic subtypes, including small-cell carcinoma, squamous cell carcinoma, adenocarcinoma, and undifferentiated carcinoma, with no clear association between calcification pattern, tumor size, or cell type. Calcifications appeared amorphous, punctate, or reticular and were variably distributed within the lesions. These findings indicated that the presence of intratumoral calcification on chest radiographs or CT does not exclude the diagnosis of bronchogenic carcinoma and should be interpreted cautiously in the evaluation of pulmonary masses.

Role of tumor calcification as a core biological event

Tumor calcification, particularly in the form of dystrophic calcification, serves as a critical indicator of metabolic stress and cell death within the TME. Unlike metastatic calcification driven by systemic hypercalcemia, dystrophic calcification arises from localized changes in tumor physiology, predominantly associated with cellular necrosis. As tumors rapidly proliferate beyond the vascular supply, cells in the central regions undergo necrosis due to insufficient nutrient and oxygen delivery. These dying cells release membrane-derived phosphates and accumulate calcium ions, creating a favorable ionic environment for mineral formation. Furthermore, the acidic milieu of metabolically stressed tumors, coupled with the release of alkaline phosphatase from compromised cells, triggers the precipitation of calcium phosphate crystals. In this regard, tumor calcification acts as a "biological record" that reflects the internal struggle of the tumor for nutrient acquisition and metabolic homeostasis.

Beyond being a passive marker of cellular stress, tumor calcification can also occur through active "osteo-mimicry" (bone mimicry), a phenomenon most frequently occurring in breast and prostate cancers. Cancer cells undergo phenotypic reprogramming during osteo-mimicry to resemble bone-building osteoblasts, a process driven by the aberrant activation of genes normally restricted to bone formation, including RUNX2 and osteopontin. This gene co-option is not merely a coincidental phenotypic change but a critical adaptive mechanism. Tumor cells are primed for metastasis to the skeletal system by acquiring the ability to create and manipulate calcium-based minerals. This process aligns with the "seed and soil" hypothesis, in which osteo-mimetic tumor cells (the "seed") develop the biological tools necessary to survive and proliferate in the bone

microenvironment (the "soil"), highlighting the functional relevance of biomineralization in tumor progression.

The deposition of solid mineral aggregates also induces profound biomechanical alterations in the TME, which in turn modulate tumor behavior. Calcification significantly increases the mechanical stiffness of tumor tissue and this elevated stiffness activates mechano-transduction pathways in cancer cells. Through these pathways, tumor cells sense the rigid microenvironmental cues and respond with enhanced aggressiveness, increased mobility, and reduced sensitivity to chemotherapeutic agents, which are key hallmarks of disease progression. In addition, large mineral deposits can disrupt interstitial fluid pressure within the tumor, creating physical barriers that hinder the uniform distribution of therapeutic drugs to the tumor core. This biomechanical dysregulation not only promotes tumor progression but also contributes to treatment resistance, underscoring the multifaceted impact of biomineralization on tumor pathophysiology.

Notably, calcium crystals formed during tumor calcification are not biologically inert. Instead, calcium crystals act as immunostimulatory "irritants" that modulate local inflammatory and immune responses. Hydroxyapatite, one of the most common calcium phosphate crystals in tumor calcification, can specifically activate the NLRP3 inflammasome in resident macrophages within the TME. Activation of this inflammasome triggers the release of pro-inflammatory cytokines (most prominently of IL-1β), which paradoxically fosters tumor growth and angiogenesis. These mineral-induced immune responses create a pro-tumorigenic microenvironment by sustaining a state of chronic, low-grade inflammation that supports tumor cell survival, proliferation, and metastasis, further integrating biomineralization into the complex network of tumor progression mechanisms.

Therapy-induced tumor calcification

Tumor calcification following chemotherapy or radiotherapy represents a significant radiologic and pathologic phenomenon that reflects complex biological responses to cytotoxic cancer treatments. This process, characterized by the deposition of calcium salts within tumor tissue after therapeutic intervention, is increasingly recognized not only as a marker of treatment-induced cell death but also as a potential prognostic indicator across multiple malignancies. The underlying mechanisms involve a cascade of events initiated by therapy-induced tumor necrosis, apoptosis, and microenvironmental alterations that collectively favor mineralization.

Chemotherapy and radiotherapy induce direct and indirect damage to tumor cells, leading to widespread cellular demise. In response to this injury, dying tumor cells release intracellular contents, such as phosphatidylserine and nucleic acids, which act as nucleation sites for calcium phosphate crystal formation[84]. In addition, therapy-induced vascular damage disrupts perfusion, resulting in local hypoxia and acidosis, conditions conducive to calcium salt precipitation. These changes are particularly evident in tumors with high baseline metabolic activity and dense cellularity, where extensive necrosis post-treatment creates an ideal milieu for dystrophic calcification. Notably, tumor calcification has been observed across diverse cancer types, including colorectal carcinoma, hepatocellular carcinoma (HCC), germ cell tumors, and sarcomas following multimodal therapy regimens[85,86].

The presence of tumor calcification in metastatic colorectal cancer after cetuximab-combined chemotherapy correlates significantly with improved survival outcomes. A retrospective study of 111 patients demonstrated that patients exhibiting calcified metastases had markedly better progression-free and overall survival rates compared to the non-calcifying counterparts[86]. Similarly, another cohort analysis involving 159 patients receiving bevacizumab plus chemotherapy as first-line treatment confirmed these findings, showing that calcification was independently associated with favorable prognosis[13]. These clinical observations suggested that calcification may serve as a non-invasive imaging biomarker reflecting effective tumor response to systemic therapy.

Calcification is uncommon in high grade glioma (HGG) and often overlooked on MRI. CT imaging suggests calcification may reflect tumor chronicity, treatment effects, or have prognostic significance. Unexpected tumor calcification was noted in a retrospective review of HGG patients treated with bevacizumab (BVZ), particularly in patients with longer treatment duration. Calcification appeared more frequent with anti-angiogenic therapy and longer survival compared to glioblastoma patients not exposed to BVZ, suggesting that calcification may be a marker of treatment response[6].

Biomineralization signature: advances in material science integration

Breast microcalcifications are routinely used as radiologic indicators for the detection of breast cancer, yet the biological and clinical significance has traditionally been viewed as largely passive. Lara Estroff et al. challenged this paradigm by investigating the "biomineralogic signatures" of breast microcalcifications, demonstrating that these mineral deposits contain complex chemical information linked to local malignancy and patient prognosis[2]. The authors analyzed 93 microcalcifications obtained from 21 patients using high-resolution materials characterization, revealing that calcifications reflect both the TME and broader disease behavior (**Figure 6**).

The study adopted an omics-inspired, multimodal analytical approach to move beyond the limited spatial resolution of mammography. Raman microscopy was used to map mineral phases and organic matrix components at sub-micrometer resolution, while energy-dispersive spectroscopy identified trace elements incorporated within calcium phosphate minerals.

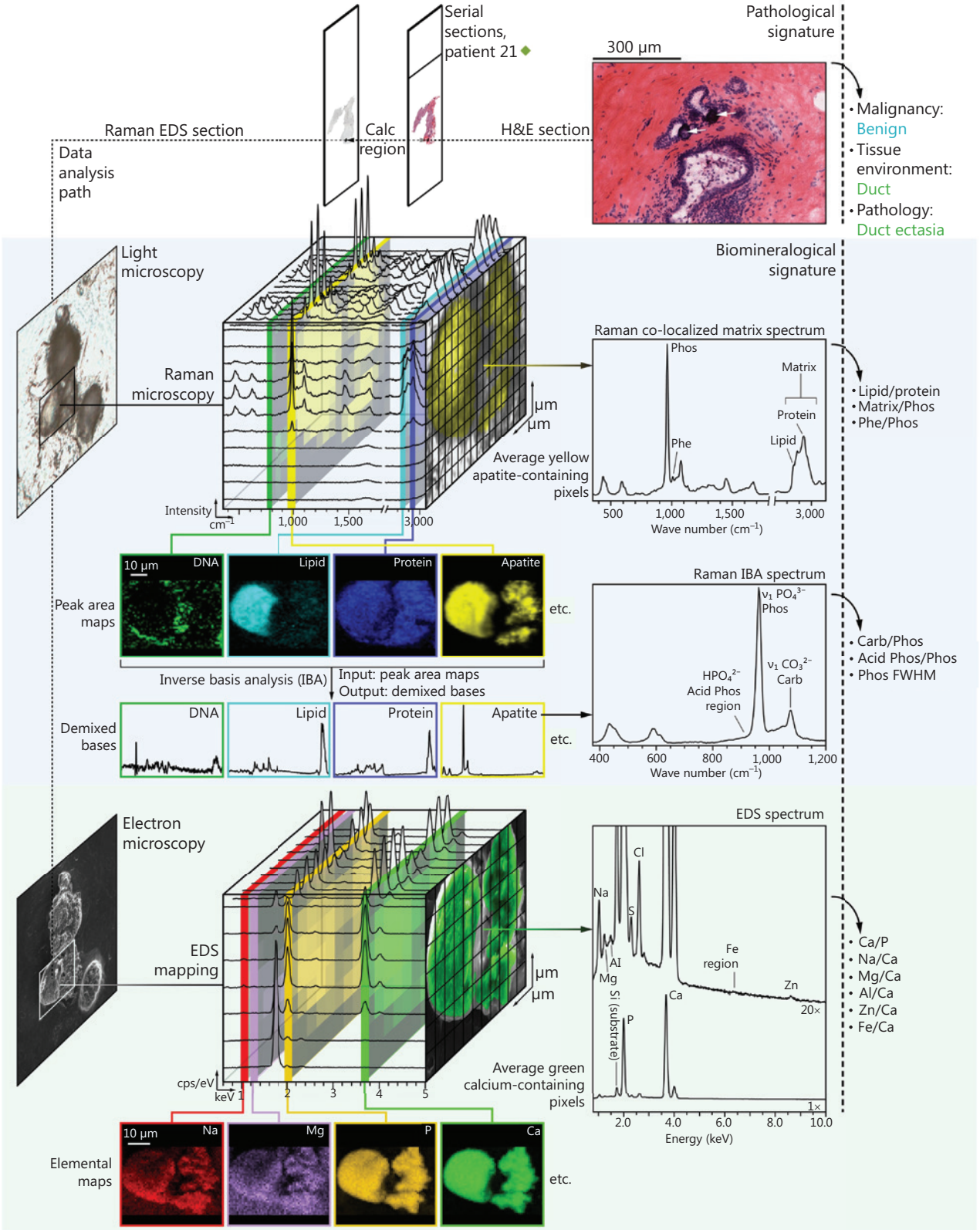


Figure 6 Schematic depicting data analysis flow of "biomineralogic signatures". The schematic shows how the pathologic and biomineralogic signatures are generated for a benign (micro)calcification (Calc) region within a sectioned tissue from a patient. The calcification region is

classified by conventional histopathologic evaluation using the H&E-stained section (right), as indicated. The corresponding unstained section undergoes light optical microscopy followed by rehydration and buffer immersion for non-destructive Raman microscopy (blue-shaded area) of the calcification region (black rectangle). Electron microscopy and EDS mapping (green-shaded area) are performed on the region (white rectangle) after sample dehydration. Both Raman and EDS produce hyperspectral datasets, spatial maps where each xy point (represented by overlaid grids) has a corresponding spectrum. Datasets are represented as cubes, which are shown as spatially binned and spectrally cropped for clarity. The spatial distribution of mineral and organic matrix components is visualized by integrating over-isolated peaks associated with specific signatures. An example of full-resolution false-color peak area heatmaps for Raman and elemental maps for EDS is shown. Averaging the apatite-containing spectra (yellow shading on the gridded image) produces the "Raman co-localized matrix spectrum" for the Raman dataset, from which all organic matrix-containing compositional parameters are calculated (right). Because organic components spectrally overlap with mineral components, an additional demixed mineral spectrum is generated (the "Raman IBA spectrum," from which all Raman mineral-specific compositional parameters are calculated). Averaging the calcium-containing pixels (green shading on the gridded image) produces the "EDS spectrum" for the EDS map, from which all elemental ratios are calculated for the biomineralogic signature[2]. Reproduced under the terms of the CC-BY license[2]. Copyright 2023 Kunitake et al., published by American Association for the Advancement of Science.

These data were integrated with serial histopathology, including H&E and von Kossa staining, to classify surrounding tissue pathology. Together, these techniques enabled the definition of distinct biomineralogic signatures based on mineral crystallinity, carbonate substitution, trace metal content, and entrapped organic matrix.

Analysis revealed that microcalcifications clustered according to tissue type and malignancy status based solely on the biomineralogic features. Benign lobular calcifications grouped together across patients, whereas calcifications associated with invasive lobular and invasive ductal carcinomas showed overlapping signatures, suggesting that the local tissue microenvironment strongly influences mineral composition. Substantial intratumoral heterogeneity was observed, particularly in carbonate content, with lower carbonate levels frequently associated with malignant regions, potentially reflecting the acidic conditions characteristic of TMEs.

The study also identified selective enrichment of trace metals in malignant-associated calcifications. Zinc, iron, aluminum, and sodium were significantly elevated in calcifications located within malignant tissue, whereas magnesium was more commonly associated with benign lesions. These inorganic signatures further support the concept that calcifications actively record biochemical features of the surrounding environment rather than forming as inert byproducts of disease.

Notably, the organic matrix entrapped within the mineral phase demonstrated strong prognostic relevance. A lower lipid-to-protein ratio within microcalcifications correlated with poorer composite patient outcomes, including higher tumor grade, advanced stage, and metastatic recurrence. Strikingly, this organic signature was consistent across calcifications within individual patients, whether a given calcification was or was not adjacent to benign or malignant tissue, suggesting the presence of a patient-level biomineralization "fingerprint."

Collectively, these findings redefined breast microcalcifications as biologically informative structures with diagnostic and prognostic potential. Given that most biopsies prompted by suspicious calcifications are ultimately benign, incorporation of biomineralogic signatures, encompassing trace metals and mineral-entrapped organic components could improve diagnostic precision and risk stratification. This work highlights the value of expanding clinical assessment beyond calcification presence alone to include the detailed chemical and structural properties, with important implications for personalized breast cancer management.

Calcification cell model: a systematic approach

Cell culture models have long served as indispensable tools for investigating physiologic and pathologic calcification, enabling mechanistic studies of tissue formation and therapeutic strategies to either promote or inhibit mineral deposition (**Figure 7**). Although 2024 marked the 50th anniversary of the first true *in vitro* calcification model, the field remains fragmented with diverse experimental approaches and limited methodologic standardization[16]. The development of calcification models began in the 1960s when early bone cell cultures failed to mineralize under standard conditions. A major advance occurred in 1974 with the demonstration that primary rat bone cells produce calcium phosphate mineral when cultured in phosphate-enriched media, establishing that specific chemical cues are required for *in vitro* mineral formation. Subsequent work identified β-glycerophosphate (BGP) as a widely used phosphate source, which must be enzymatically cleaved by tissue TNAP to release free phosphate. Additional supplements, such as ascorbic acid to stimulate collagen synthesis and dexamethasone to promote osteogenic differentiation, became standard components of osteogenic culture systems.

Modern calcification models have expanded beyond static two-dimensional cultures to incorporate more physiologically relevant platforms. Lab-on-a-chip and microfluidic systems enable dynamic perfusion and mechanical stimulation,

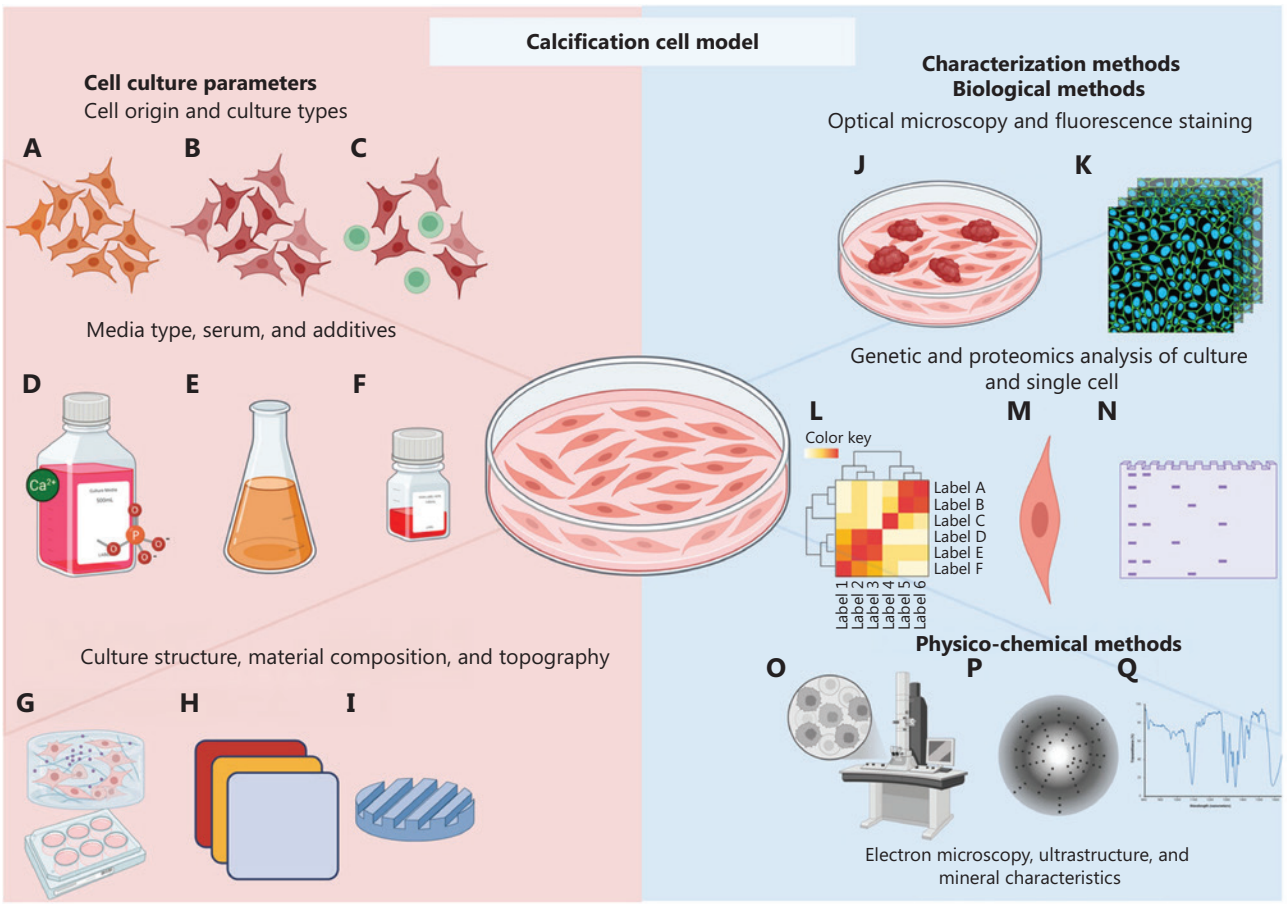


Figure 7 Calcification cell models typically utilize (A) homogeneous cell lines or (B) heterogeneous cultures of primary cells. (C) Deliberate co-cultures of different cell lines or types can be used to study the influence of one cell type on another. (D) Culture media selection is crucial, involving choices about the base medium, (E) mineral supersaturation, source of mineral (inorganic or organic), amount, and quality of serum, (F) antibiotics for contamination prevention, particulate matter as calcification promoters, and sometimes an inhibitor. (G) 3D substrates, like collagen gels, are traditionally spread on flat plastic surfaces, for cells to infiltrate and better mimic the ECM. (H) The design and material of the substrate also have significant roles as the combination of (I) topography and (H) material both determine substrate mechanical and chemical properties, such as elasticity. (J, K) Mineral and cell locations, and morphologies are typically analyzed post-culture, using optical microscopy, with techniques, like Alizarin Red or von Kossa staining, to highlight calcified features. Detailed investigations into the calcification process involve live microscopy for longitudinal studies, fluorescent antibody staining, and confocal microscopy for specific cell structures. (L, M) Cellular responses to calcification, such as differential expression and the analysis of gene regulatory networks, are best examined using unbiased techniques, like single-cell sequencing, including the latest variants, spatial, and time information. (N) Protein expression is best studied by (space-resolved) mass spectrometry and confirmed by immunoblotting. The composition and structure of the minerals formed may be analyzed using (O) electron microscopy, (P) diffraction, and (Q) spectroscopy[16]. Reproduced with Permission[16]. Copyright 2020 Wiley-VCH GmbH.

recapitulating aspects of bone remodeling and osteocyte mechano-transduction. Three-dimensional organoids and scaffold-based cultures using biomimetic materials, such as collagen or silk, better reproduce tissue architecture and matrix interactions. Notably, recent bone-on-a-chip models have successfully generated calcified trabeculae-like structures through osteoblast–osteoclast co-culture. Implant and biomaterial models have further demonstrated that some inorganic substrates, particularly calcium phosphate bioceramics, can induce mineralization in the absence of soluble additives, highlighting the osteoinductive influence of material chemistry and surface microtopography.

In addition to skeletal tissues, calcification models have been developed to study pathologic mineralization in

non-osteogenic sites, where so-called "non-professional mineralizers" undergo aberrant calcification. Cardiovascular models focus on VSMCs and valve interstitial cells to elucidate mechanisms of arterial and valvular calcification. In ocular disease, particularly age-related macular degeneration, retinal pigment epithelium models have replicated drusen formation without classical osteogenic additives, demonstrating that calcification can arise through pathways distinct from canonical bone-forming mechanisms.

Existing calcification models face several important limitations, despite the utility. Many calcification models rely on artificially supersaturated media that may not accurately reflect *in vivo* biochemical conditions, raising concerns about non-specific mineral precipitation. Cellular heterogeneity presents an additional challenge because primary cultures often contain mesenchymal stem cells that readily respond to osteogenic cues, complicating interpretation of cell-type-specific mineralization. Genetic and phenotypic drift in long-term cell cultures further contributes to variability with key regulators, such as TNAP activity, declining even at early passages. Moreover, inconsistent reporting of media composition, serum quality, and culture conditions limits reproducibility across studies.

Several best-practice recommendations have emerged to address these issues, including detailed reporting of base media and culture parameters, careful control or replacement of animal serum, rigorous mineral characterization using techniques, such as electron diffraction, and quantitative assessment of mesenchymal stem cell contamination. Context-specific modeling strategies have also been proposed, such as using calcium and phosphate salts instead of BGP when mimicking disease states, like hyperphosphatemia. Collectively, these measures aim to improve reproducibility and biological relevance across calcification studies.

Tumor calcification models represent a specialized extension of this field that are designed to investigate the biological and chemical mechanisms underlying mineral formation within cancerous tissues. Such models have been used to explore how different mineral phases influence tumor behavior, especially in breast cancer, with hydroxyapatite promoting proliferative and invasive signaling pathways, whereas calcium oxalate may exert suppressive effects. Contemporary approaches increasingly favor three-dimensional organoid systems that better capture the TME, including ECM composition and mechanical stiffness. However, these models frequently rely on osteogenic additive cocktails to induce mineralization, raising concerns about artificial mineral formation and the difficulty in distinguishing tumor cell-driven calcification from contributions by contaminating mesenchymal progenitors. As with other calcification models, continued refinement and rigorous validation are necessary to ensure that tumor calcification systems yield biologically meaningful insights.

Artificially induced tumor biomineralization for anti-tumor treatment

Tumor biomineralization-inducing approaches

Artificially induced tumor biomineralization is an emerging paradigm in "drug-free" cancer therapy[18,19,62,63,87-96]. Unlike traditional chemotherapy, which relies on cytotoxic molecules to kill cells, this approach leverages the biological and chemical environment of the tumor to trigger the *in situ* formation of inorganic minerals, such as calcium phosphate, by some calcium binding materials. These mineral structures act as physical barriers or metabolic disruptors, effectively "petrifying" the tumor from the inside out or sealing it off from the host.

The therapeutic efficacy of induced biomineralization is reported to result from several distinct mechanisms, as follows: 1) Physical blockade (tumor encapsulation). By creating a mineralized layer around the tumor surface, researchers can obstruct the exchange of nutrients, oxygen, and metabolic waste. This "blockade therapy" starves the tumor and prevents the migration of metastatic cells. 2) Intracellular metabolism disruption. Targeting biomineralization to organelles, specifically the mitochondria, leads to "intramitochondrial silicification." The formation of nanostructures within the mitochondrial matrix physically ruptures the organelle, leading to energy failure and rapid apoptosis. 3) Ion homeostasis imbalance. Inducing calcification often involves manipulating calcium or iron concentrations. Overloading cells with these ions not only promotes mineral growth but also triggers secondary cell death pathways, like ferroptosis or calcium-induced oxidative stress. Targeting ability of the biomineralization must be considered and current research focuses on two primary methods. Surface targeting involves engineering precursor molecules with ligands, such as folate, that binds to receptors overexpressed on tumor cell membranes (e.g., folate-conjugated polysialic acid). Subcellular targeting uses molecules with organelle-specific moieties, like triphenylphosphonium (TPP) for mitochondria, which accumulate in specific compartments before undergoing mineralization.

Tang et al. introduced the concept of cancer-cell-targeting calcification (CCTC), leveraging the interaction between folate receptors on tumor cells and folic acid to present carboxyl groups on cell membranes, which chelated Ca^{2+} to form a calcified layer that inhibited tumor activity and metastasis[63]. To overcome the limitations of local folate delivery and exogenous Ca^{2+} supplementation, Tang et al. developed a polysaccharide-based calcification agent in 2020, modifying polysialic acid with folic acid to induce tumor-specific calcification under physiologic calcium and phosphate levels *via* systemic administration. This calcification suppressed glycolysis,

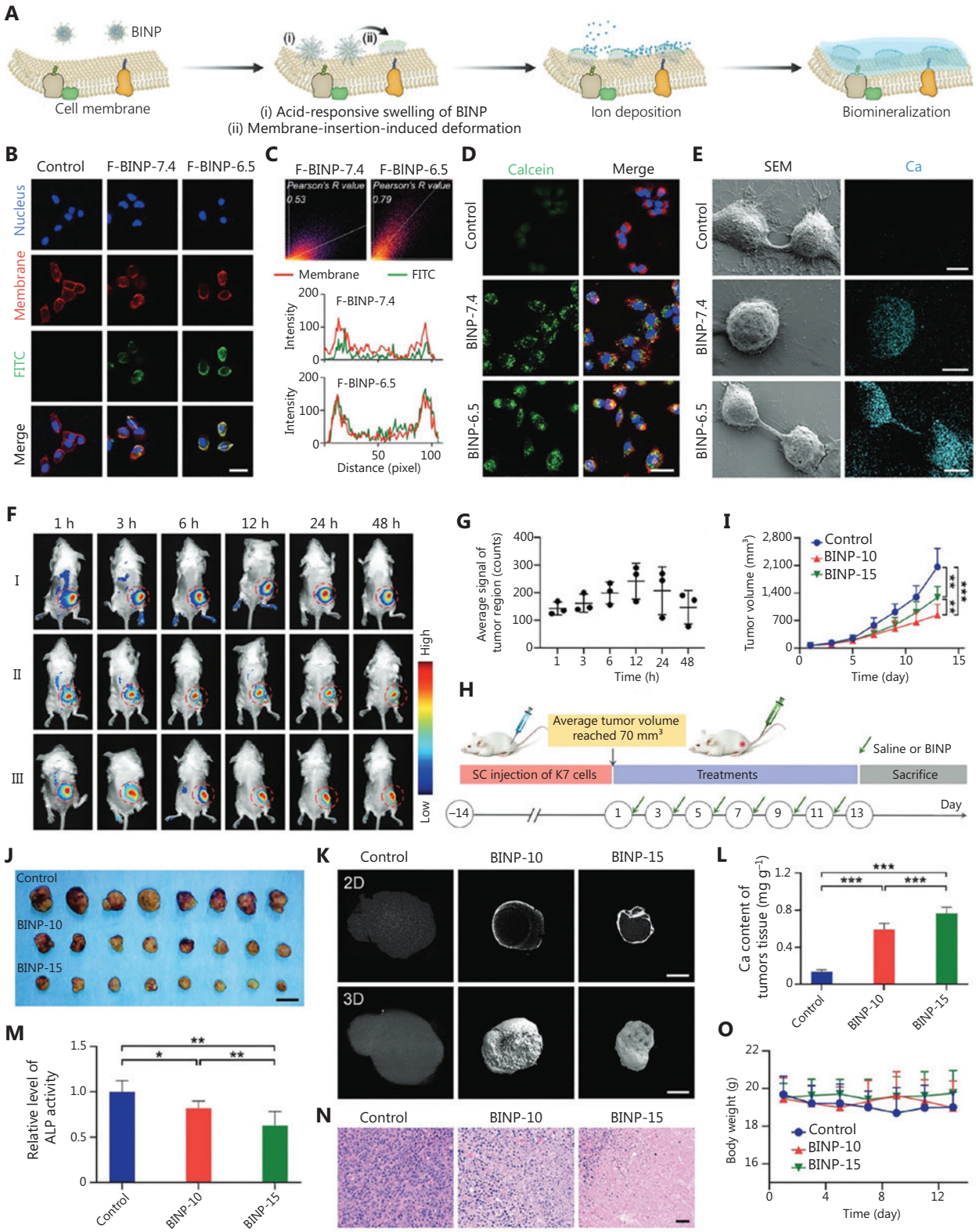


Figure 8 Cytomembrane insertion and *in vitro* biomineralization induction by BINP and influence of mineralization on cell properties. (A) Schematic illustration of BINP-induced biomineralization. (B) Evaluation of cytomembrane-insertion ability of BINP at pH 7.4 or 6.5 by CLSM.

The green fluorescence was emitted by F-BINP. The nucleus and cytomembrane were fluorescently stained with Hoechst 33342 (blue) and DiD (red), respectively. Scale bar = 40 μm. (C) Co-localization of cytomembrane and F-BINP at pH 7.4 and 6.5. ImageJ was used to assess the co-localization of captured images to obtain the firework images and Pearson's correlation coefficient. (D) Evaluation of biomineral deposition on cytomembrane surface of 143B cells after treatment with BINP at pH 7.4 or 6.5 by CLSM. The cells in the control group were treated with normal saline. The green fluorescence emitted by calcein indicates the presence of CaP. Scale bar = 50 μm. (E) Characterization of mineralized 143B cells treated with BINP at pH 7.4 or 6.5 by SEM and Ca elemental mapping. Scale bar = 5 μm. Growth inhibition of subcutaneous osteosarcoma allograft by BINP-induced biomineralization. (F) *In vivo* biodistribution of BINP in BALB/c mice bearing K7M2 osteosarcoma tumors after intravenous injection of Cy-BINP. (G) Average Cy-BINP signals from tumor regions at various times after injection. (H) Therapeutic schedule for subcutaneous osteosarcoma allograft model. (I) Volume of subcutaneous tumor region during administration of normal saline (control) or various doses of BINP. (J) Representative tumor images over six treatments with normal saline or BINP. Scale bar = 2 cm. (K) Evaluation of biomineral deposition in subcutaneous tumor region after administration of various doses of BINP by micro-CT scanning. The upper and lower panels show 2D cross-sectional views and the 3D images, respectively. Scale bar = 5 cm. (L) Ca content of tumor tissues from various groups. (M) Relative serum ALP levels of mice bearing subcutaneous osteosarcoma allografts in treatment groups compared with that in control group. (N) H&E staining of tumor sections after treatment with various doses of BINP. Scale bar = 50 μm. (O) Body weight changes of mice over six treatments with normal saline or BINP[19]. Reproduced with permission[19]. Copyright 2023 Wiley-VCH GmbH.

disrupted cellular metabolism, and reduced migration and tumorigenicity, ultimately leading to tumor necrosis.

Ding and Chen et al. developed pH-responsive polypeptide-based biomineralization-initiating nanoparticles (BINPs) to enhance tumor-specificity (**Figure 8**)[19]. BINPs, which are self-assembled fromdodecylamine-poly[(γ-dodecyl-L-glutamate)-co-(L-histidine)]-block-poly(L-glutamate-graft-alendronate), undergo structural transformation in the weakly acidic TME. Protonation of histidine residues disrupts the assembly, exposing dodecyl chains and bisphosphonate moieties, which insert into tumor membranes and trigger sustained ion deposition. This biomineralized layer increases membrane rigidity, reduces fluidity, and impairs tumor cell activity and invasiveness. After intravenous injection in osteosarcoma-bearing mice, BINPs accumulate at tumor sites *via* the EPR effect and bisphosphonate-mediated bone targeting, achieving tumor inhibition rates of 59.3% and 52.1% in subcutaneous and orthotopic models, respectively. BINP treatment also suppressed lung metastasis, reduced bone destruction, and showed no systemic toxicity, highlighting the potential as a selective osteosarcoma therapy.

Challenges in the clinical translation of induced tumor biomineralization

As previously noted, tumor biomineralization holds considerable diagnostic and therapeutic potential. However, current research is confined to mechanistic investigations and the synthesis of existing findings. The clinical translation is impeded by several critical bottlenecks. A primary challenge is the paucity of clinical evidence. Most studies focus on basic research with no large-sample clinical trials to validate the clinical utility. While imaging modalities can detect intratumoral calcification, there is no unified standard to differentiate pathologic calcification from incidental deposits. In addition, therapeutic strategies targeting tumor biomineralization remain untested in clinical settings, further hindering the translation into practice.

Targeted therapies also pose significant safety concerns. Non-specific targeting may induce abnormal mineral deposition in healthy tissues (e.g., vascular calcification) and the long-term safety of biomineralization-based delivery materials remains unproven. Furthermore, tumor heterogeneity and inefficient targeting ligands impede the effective delivery and bioavailability of therapeutic agents, thereby reducing the therapeutic efficacy.

Another major bottleneck is the absence of standardized evaluation systems and clinical guidelines. There are no unified clinical criteria for the detection and classification of intratumoral calcification, while inconsistent evaluation indicators in basic research complicate result integration. The lack of clinical guidelines also leads to inconsistent clinical practices, hampering the promotion of translational research. In short, the clinical translation of tumor biomineralization is hindered by interconnected challenges, including insufficient clinical evidence, poor target and biomarker specificity, safety risks, inadequate delivery strategies, and the absence of standardized systems. Future research should prioritize addressing these gaps to fully unlock the diagnostic and therapeutic potential of tumor biomineralization.

Despite the potential, induced biomineralization faces considerable challenges prior to clinical translation. Key concerns include achieving sufficient selectivity to restrict mineral deposition to the acidic or enzyme-rich TME, thereby minimizing the risk of ectopic calcification in healthy tissues, elucidating the pathways of degradation and clearance of the resultant mineralized residues and determining the immunologic consequences of mineralized tumor fragments, specifically whether the mineralized tumor fragments function as adjuvants that potentiate

anti-tumor immunity, or conversely, impede immune recognition. Nevertheless, induced tumor biomineralization represents a paradigm shift toward mechanical and structural strategies in oncology, exploiting the intrinsic metabolic signatures of tumors to initiate localized mineral growth and offering a potentially biocompatible, low-resistance alternative to conventional pharmacotherapeutic modalities.

Conclusions

Tumor-associated calcification is an underrecognized yet biologically rich feature of cancer that integrates organic cellular processes with inorganic mineral formation across multiple spatial and temporal scales. Rather than a passive pathologic byproduct, emerging evidence indicates that calcification reflects key aspects of tumor biology, including cell proliferation, differentiation, death, metabolic dysregulation, and microenvironmental remodeling. Advances in analytical technologies, multi-omics approaches, and biomimetic cell models are rapidly overcoming previous methodologic limitations, enabling mechanistic exploration of tumor-associated biomineralization. Notably, induced and targeted biomineralization has revealed new opportunities for cancer diagnosis and therapy, particularly when coupled with materials science as driven strategies and advanced medical imaging. However, clinical data remain fragmented, underscoring the need for systematic reporting and integrative analysis of calcification patterns across tumor types and disease stages. Progress in this field will depend on coordinated efforts spanning materials science, pathology, cytology, imaging, and molecular biology. With continued interdisciplinary integration, tumor-associated calcification has the potential to yield novel biomarkers, deepen understanding of cancer mechanisms, and inspire innovative therapeutic approaches.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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Wrote the paper: Fan Liu, Qiqi Cao, Liyan Miao, Huabing Chen.

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