Phosphorylation beyond the plasma membrane: How secretory pathway kinases sculpt the cellular dialogue in cancer (Review)

ZIANG GENG[1*], SHU GUAN[2*], SHAOQI DU[3*], SHUO ZHANG[3], JIAXIN HAO[4], SIYUAN TIAN[4], CHEN ZHU[5] and SHAONAN DU[4]

[1]Department of Neurosurgery, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100005, P.R. China; [2]Department of Surgical Oncology and Breast Surgery, First Hospital of China Medical University, Shenyang, Liaoning 110001, P.R. China; [3]Department of Operating Room, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, P.R. China; [4]Department of Neurosurgery, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110004, P.R. China; [5]Department of Neurosurgery, The First Hospital of China Medical University, Shenyang, Liaoning 110001, P.R. China

Received November 4, 2025; Accepted April 21, 2026

DOI: 10.3892/ijmm.2026.5845

Abstract. Intercellular communication is critical for tissue homeostasis, development and immune responses, with its disruption often implicated in various diseases, particularly cancer. Secretory pathway kinases and kinase-like proteins (SPKKPs) constitute a distinctive enzyme class operating within the luminal secretory pathway or extracellular space, positioning them as pivotal regulators of cellular communication. This review consolidates current insights into the role of SPKKPs, including the FAM20 family, four-jointed box kinase 1 (FJX1) and others, in orchestrating intercellular interactions through the phosphorylation of secreted proteins, extracellular matrix components and extracellular vesicle (EV) cargo. The molecular mechanisms by which SPKKPs modulate key oncogenic signaling pathways, such as PI3K/AKT, ERK/MAPK and SMAD family member 2, across diverse cancer types are examined. Additionally, their involvement in EV-mediated signaling, extracellular matrix remodeling and regulation of fundamental biological processes, including development, tissue homeostasis and immune coordination, is explored. The review further addresses SPKKP dysregulation in a range of pathologies, from nervous system tumors to gastrointestinal and reproductive cancers, and discusses emerging therapeutic strategies. These strategies include specific kinase inhibitors, FJX1-targeted peptide vaccines and innovative approaches targeting exosomes carrying SPKKPs substrates. Ultimately, this work highlights the essential role of SPKKPs in intercellular communication networks and their promising potential as diagnostic biomarkers and therapeutic targets, particularly in cancer.

Correspondence to: Dr Shaonan Du, Department of Neurosurgery, Shengjing Hospital of China Medical University, 36 Sanhao Street, Heping, Shenyang, Liaoning 110004, P.R. China
E-mail: sndu@cmu.edu.cn

Dr Chen Zhu, Department of Neurosurgery, The First Hospital of China Medical University, 155 Nanjing Street, Heping, Shenyang, Liaoning 110001, P.R. China
E-mail: cmu_zhuchen@126.com

*Contributed equally

Abbreviations: CAF, cancer-associated fibroblast; ECM, extracellular matrix; EV, extracellular vesicle; FCS, fluorescence correlation spectroscopy; GAG, glycosaminoglycan; HSC, hepatic stellate cell; INSPs, inositol polyphosphates; NPC, nasopharyngeal carcinoma; NPTN, neuroplastin; OPN, osteopontin; PALM, primary acral lentiginous melanoma; PK, protein kinase; POMK, protein O-mannosyltransferase; PV1, peptide vaccine; SPKKPs, secretory pathway kinases and kinase-like proteins

Key words: intercellular communication, SPKKPs, tumor microenvironment, signaling pathway, therapeutic targets, extracellular vesicles

Contents

1. Introduction

In multicellular organisms, precise intercellular communication is essential for maintaining tissue homeostasis,

coordinating physiological functions and ensuring proper development (1). This intricate dialogue, facilitated through direct contact or secreted signaling molecules, underpins processes from immune surveillance to neural transmission (1,2). Dysregulation of these networks is a hallmark of numerous pathologies (3,4), with cancer exemplifying how disrupted signals between tumor cells and the surrounding microenvironment drive uncontrolled proliferation, invasion and metastasis (5). Secretory pathways (6), the cellular machinery responsible for synthesizing, modifying and transporting proteins destined for the cell surface or extracellular space, play a central role in intercellular signaling (6,7). Through the coordinated action of the endoplasmic reticulum (ER) and Golgi apparatus (GA) (8-10), cells release a variety of bioactive molecules, including growth factors (GFs), cytokines and extracellular matrix (ECM) components (1,11), which govern both autocrine and paracrine communication (11,12). The integrity of this secretory process is crucial, as it directly influences the composition of the cellular secretome and, consequently, the nature of signals received by neighboring and distant cells.

Within this context, protein kinases (PKs) serve as key regulators, modulating secretory and signaling events through the reversible process of phosphorylation (13-15). While the functions of intracellular kinases are well-documented, a distinct class of enzymes-secretory pathway kinases and kinase-like proteins (SPKKPs)-operates within the luminal compartments of the secretory pathway or the extracellular space itself. These kinases phosphorylate a broad range of secreted proteins and extracellular domains, directly influencing the activity, stability and interactions of critical signaling molecules (1,16,17).

SPKKPs occupy a unique and pivotal role at the nexus of secretory pathway function and intercellular communication. By phosphorylating substrates such as GFs, chemokines and ECM proteins, they serve as master regulators of cellular interactions. Dysregulation of these enzymes disrupts this finely tuned communication, contributing to disease pathogenesis, particularly in cancer. This narrative review synthesizes literature from PubMed (https://pubmed.ncbi.nlm.nih.gov/), Scopus (https://www.scopus.com/pages/home) and Web of Science (https://webofscience.clarivate.cn/wos/woscc/smart-search), searched up to October 2025, focusing on how SPKKPs govern intercellular communication, molecular mechanisms, biological functions in health and disease, and their promising potential as novel therapeutic targets.

2. Relationship between SPKKPs and intercellular communication

Overview of SPKKPs. PKs are enzymes that catalyze the transfer of the γ-phosphate group from adenosine triphosphate (ATP) to specific amino acid residues in proteins, playing a central role in cellular signal transduction. Over 500 human PKs have been identified to date (18). By promoting protein-protein interactions, protein translocation, and conformational changes, PKs phosphorylate substrate proteins, influencing key biological processes such as the cell cycle, proliferation, differentiation, metabolism and apoptosis (19). In recent years, a subset of PKs localized to the ER, GA lumen and extracellular space has attracted increasing attention. These kinases phosphorylate substrates within the secretory pathway, contributing to the maturation of secretory proteins and intercellular signal transduction; hence, they are termed secretory pathway kinases (20). Additionally, certain proteins with kinase-like structures but distinct catalytic properties or functions also regulate secretory pathway-related processes. Collectively, these are referred to as SPKKPs.

The identification of new SPKKP family members continues, with classification primarily based on sequence homology and functional characteristics. As of now, 13 SPKKPs have been characterized, grouped into four categories: i) The Similar Sequence Secretory Kinase family, which includes family with sequence similarity 20, member A (FAM20A), FAM20B, FAM20C, Golgi-associated kinase 1A (GASK1A) (FAM198A) and GASK1B (FAM198B) (21); ii) the Remote Secretory Kinase family, comprising divergent protein kinase domain 2A (DIPK2A) (DIA1), DIPK2B (DIA1R), and protein kinase domain containing, cytoplasmic (PKDCC) (22); iii) the Secretory Pathway Kinase-like Protein family, including DIPK1A (FAM69A), DIPK1B and DIPK1C (FAM69C) (23); iv) the Unclassified Secretory Kinase family, consisting of four-jointed box kinase 1 (FJX1) (24) and protein O-mannosyltransferase (POMK) (25). Different SPKKPs phosphorylate distinct protein substrates. For example, Tagliabracci *et al* (26) identified >100 secretory phosphoproteins as authentic substrates of FAM20C using CRISPR/Cas9 gene editing, mass spectrometry and biochemical methods (Table SI). Furthermore, phosphorylation levels were found to be elevated at 64 sites on 56 proteins in cells overexpressing DIPK2A, with 8 serine sites and 56 threonine sites (22) (Table SII). A proteomics study revealed that PKDCC-dependent phosphorylation sites on >40 proteins are associated with the secretory pathway (27) (Table SIII).

To provide a structured overview of the SPKKPs, their subcellular localization, representative substrates and functional roles in intercellular communication are summarized in Fig. 1 and Table I. Of note, while certain members such as FAM20A, FAM20B and FAM20C have been well characterized with defined substrates and roles in ECM remodeling and secretome regulation; other SPKKPs, including members of the GASK and DIPK families, remain less well understood, particularly regarding their specific substrates and contributions to extracellular vesicle (EV)-mediated signaling and tumor microenvironment (TME) modulation. This highlights the heterogeneity within the SPKKP family and underscores the need for further mechanistic studies.

Modes of intercellular communication relevant to SPKKPs. Intercellular communication is essential for growth, development and tissue homeostasis in multicellular organisms, facilitating the exchange of information through diverse mechanisms (28). Traditional forms of communication include direct cell-cell contact and long-range signaling mediated by soluble signaling molecules (1). Among these, protein secretion plays a pivotal role in intercellular communication. This process, driven by the ER and GA apparatus, involves protein synthesis, post-translational modification and vesicular transport, ultimately releasing functional proteins into the extracellular space via exocytosis (29). These secreted

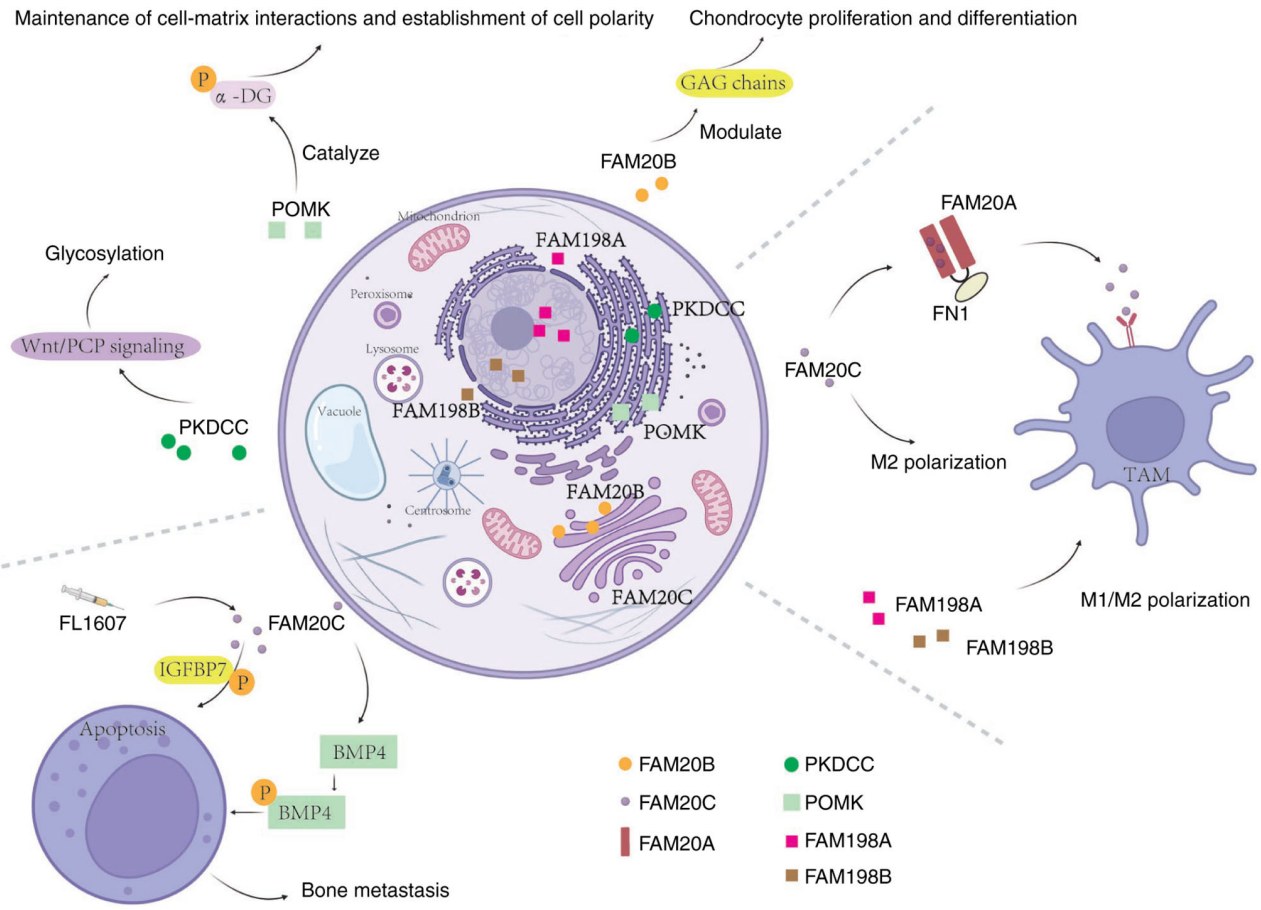

Figure 1. The landscape of SPKKPs. FAM20C is localized to the Golgi apparatus, targets a wide range of secreted proteins, promotes invasion and metastasis in various cancers, and influences the TME. FAM20A is a type II transmembrane protein localized to the Golgi apparatus that does not directly phosphorylate substrates; it regulates the activity and localization of FAM20C by activating it. FAM20B is localized to the Golgi apparatus and targets xylose residues on proteoglycan core proteins; it influences ECM structure and function by regulating proteoglycan synthesis. PKDCC and POMK are localized to the endoplasmic reticulum and are associated with neurodevelopment and glycosylation. FAM198A and FAM198B localize to the nucleus and cytoplasm and may be associated with cancer progression; they promote the polarization of colorectal cancer TAMs and may influence the TME. α-DG, α-dystroglycan; GAG, glycosaminoglycan chains; FN1, fibronectin 1; BMP4, bone morphogenetic protein 4; IGFBP7, insulin like growth factor binding protein 7; TAM, tumor-associated macrophage; TME, tumor microenvironment.

proteins mediate critical biological functions, such as hormone regulation, immune responses and microenvironmental remodeling (30). Notably, the secretory pathway not only facilitates the output of signaling molecules but also serves as a critical site for their post-translational modifications. Recent studies have highlighted the importance of phosphorylation modifications in the ER, GA lumen and extracellular space in regulating the function of secreted proteins.

As key regulatory elements in this process, SPKKPs phosphorylate various substrates within the secretory pathway, thereby influencing protein stability, spatial distribution and receptor interactions. SPKKPs exert their effects through multiple pathways integral to intercellular communication. Studies have shown that SPKKPs can modulate ligand-receptor signaling by regulating the phosphorylation status of secreted proteins (20), influence the extracellular microenvironment by modifying ECM-associated proteins (31) and affect long-range signaling by regulating the phosphorylation of protein components in EVs (32). Although these mechanisms are progressively understood, a comprehensive understanding of the specific contributions of different SPKKPs to various communication modes remains lacking.

Roles of SPKKPs in physiological intercellular communication. Under physiological conditions, SPKKPs are essential regulators of intercellular communication, controlling protein phosphorylation within secretory pathways. During development and morphogenesis, SPKKPs modulate cell differentiation and tissue formation by regulating the function of secreted proteins. FAM20C, a prototypical GA-associated PK, phosphorylates various secretory proteins and plays a pivotal role in biomineralization processes, such as human bone and tooth enamel formation (33,34). *In vitro* studies demonstrate that *FAM20C* promotes the differentiation and mineralization of mouse mesenchymal cells and odontoblasts (35). FAM20A, acting as a pseudokinase, allosterically activates FAM20C, facilitating mineralization-related signaling (36). In zebrafish models, knockout of *fam20a* or *fam20c* results in severe enamel defects and skeletal developmental abnormalities (37). Additionally, FAM20B, a recently identified xylokinase, regulates chondrocyte proliferation and differentiation by modulating the synthesis of glycosaminoglycan (GAG) chains in proteoglycans (38). These findings highlight the indirect role of SPKKPs in cell fate determination and tissue organization through the regulation of secreted

Table I. Overview of SPKKPs: Subcellular localization, substrates and roles in regulating the secretome, ECM, EVs and TME.

SPKKPs	Subcellular localization	Substrates	Roles in secretome/ECM/EVs/TME	(Refs.)
FAM20C	Golgi-associated secretory pathway	Broad range of secreted proteins, including OPN, fibronectin, collagen, 7B2 and multiple mineralization-related proteins	Broadly regulates secreted protein function and extracellular phosphorylation; controls bone and tooth biomineralization; remodels ECM by altering adhesion, stiffness and receptor interactions; activates integrin-FAK, MAPK-ERK, PI3K/AKT and JAK/STAT signaling; modulates extracellular vesicle cargo and immune cell communication; promotes proliferation, migration, invasion and TME remodeling in multiple cancers.	(26,31,33, 34,44,57, 75,76,80)
FAM20A	Golgi apparatus (type II transmembrane protein)	Does not directly phosphorylate canonical substrates; allosterically activates FAM20C and regulates its localization and activity	Indirectly enhances phosphorylation of secreted and ECM proteins; essential for enamel formation and mineralization; its deficiency leads to amelogenesis imperfecta and nephrocalcinosis; influences secretory pathway homeostasis and tissue microenvironment.	(36,51-55)
FAM20B	Secretory pathway/ Golgi apparatus	Xylose residues within the tetrasaccharide linker region of proteoglycans	Regulates GAG chain initiation and elongation, thereby shaping ECM structure and function; involved in cartilage formation, skeletal development and tooth morphogenesis; may influence tumor-related signaling via ERK/MMP1 axis.	(38,52)
GASK1A (FAM198A)	Golgi apparatus, nucleus, cytoplasm	ECM proteins and secreted signaling molecules	Enhances ECM-integrin interactions and activates integrin-FAK, FAK/Src and PI3K/AKT pathways; promotes cytoskeletal remodeling, adhesion, migration and survival; regulates phosphorylated proteins and non-coding RNAs in EVs; facilitates long-range communication, dendritic cell maturation, antigen presentation and T-cell activation; contributes to TME adaptation.	(41-43,45, 58,59)
GASK1B (FAM198B)	Nucleus and cytoplasm	Not clearly defined	Limited direct evidence in the secretory pathway; primarily implicated in tumor-related signaling: Activates PI3K/AKT/BCL-2 signaling in gastric cancer; regulates SMAD2-mediated macrophage polarization in colorectal cancer; associated with ERK/MAPK signaling in lung adenocarcinoma and ovarian cancer.	(27,85,107, 121,123)
DIPK2A (DIA1)	Not clearly defined	Proteomics evidence shows increased phosphorylation at 64 sites across 56 proteins upon overexpression	Substrate spectrum identified by proteomics; functional mechanisms in secretome/ECM/EV/TME regulation remain largely unclear.	(22)
DIPK2B (DIA1R)	Not clearly defined	Not clearly defined	Not clearly characterized in the current literature.	
PKDCC	Endoplasmic reticulum	More than 40 secretory pathway-associated proteins, including LGALS3BP, SPP1 and MINPP1	Associated with neurodevelopment; regulates EV-mediated communication: Exosomal LGALS3BP activates PI3K/AKT/VEGFA signaling to promote proliferation, migration and angiogenesis in endometrial cancer; SPP1 enhances oxidative phosphorylation and proliferation in ovarian cancer; exosomal MINPP1 contributes to TME formation.	(27,32, 73,74)
DIPK1A (FAM69A)	Not clearly defined	Not clearly defined	Associated with lipid metabolism and endothelial-mesenchymal transition in colorectal cancer; serves as a component of prognostic models; lacks direct mechanistic evidence in secretome/ECM/EV/TME regulation.	(23,103)

Table I. Continued.

SPKKPs	Subcellular localization	Substrates	Roles in secretome/ECM/EVs/TME	(Refs.)
DIPK1B	Not clearly defined	Not clearly defined	Not clearly characterized.	
DIPK1C (FAM69C)	Not clearly defined	Not clearly defined	Not clearly characterized.	
FJX1	Not clearly defined	Not clearly defined	Functions primarily as a secreted tumor-associated antigen and signaling regulator; promotes proliferation, invasion, angiogenesis and immune evasion across multiple cancers (NPC, HNSCC, gastric, colorectal, melanoma); serves as a target for peptide vaccines that enhance tumor-specific T-cell responses and modulate the TME.	(24,87-90, 94-97,111)
POMK	Endoplasmic reticulum	Ser734 of α-dystroglycan	Regulates glycosylation and laminin-binding of α-dystroglycan; Influences receptor-ligand interactions, cell-matrix adhesion and membrane protein glycosylation; in breast cancer, glycosylated POMK suppresses migration and metastasis via the PI3K/AKT/GSK-3β/Snail pathway.	(119)

AKT, protein kinase B; BCL-2, BCL2 apoptosis regulator; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; EV, extracellular vesicle; FAK, focal adhesion kinase; GAG, glycosaminoglycan; GSK-3β, glycogen synthase kinase-3β; HNSCC, head and neck squamous cell carcinoma; JAK, Janus kinase; LGALS3BP, lectin galactoside-binding soluble 3-binding protein; MAPK, mitogen-activated protein kinase; MINPP1, multiple inositol polyphosphate phosphatase 1; MMP1, metallopeptidase 1; NPC, nasopharyngeal carcinoma; OPN, osteopontin; PI3K, phosphatidylinositol 3-kinase; POMK, protein O-mannosyltransferase; STAT, signal transducer and activator of transcription; SMAD2, SMAD family member 2; SPP1, secreted phosphoprotein 1; TME, tumor microenvironment; VEGFA, vascular endothelial growth factor A.

protein structure and function, thus supporting coordinated intercellular communication.

In tissue homeostasis, SPKKPs contribute to intercellular signaling via multiple mechanisms. The ECM, serving as a critical physical and signaling platform, relies on post-translational modifications within the secretory pathway to maintain its function. SPKKPs influence ECM integrity and tissue function by phosphorylating ECM proteins (39). FAM20C regulates calpain activity through the phosphorylation of calpastatin, maintaining the balance between cell migration, proliferation and adhesion (40). Additionally, FAM20C modulates cell-matrix interactions by altering the phosphorylation status of ECM proteins, ensuring tissue homeostasis and intercellular coordination (34). GASK1A promotes the integrin-focal adhesion kinase (FAK) signaling pathway by phosphorylating ECM proteins, playing a pivotal role in cytoskeletal reorganization and intercellular coordination (41-43).

In immune homeostasis and immune responses, SPKKPs enhance long-range intercellular communication by regulating the secretion of components within EVs. FAM20C influences distant T cells, natural killer (NK) cells or dendritic cells (DCs) by modulating the phosphorylation status of cytokines, chemokines and the composition of antigenic fragments and regulatory signaling molecules within exosomes. This modulation impacts immune cell localization and function, amplifying the immune response (44). Similarly, GASK1A enhances DC maturation, antigen presentation and T-cell proliferation and activation through similar regulatory mechanisms (45).

3. Mechanisms of SPKKP-mediated intercellular communication

Extracellular phosphorylation and ligand-receptor signaling modulation. SPKKPs play a critical role in regulating extracellular phosphorylation and ligand-receptor signaling, though not all kinases have clearly defined functions. During skeletal development and mineralization, FAM20C phosphorylates Ser146 within the RGDSVVYGLR motif of osteopontin (OPN), inhibiting the interaction between OPN and αvβ3 integrin (46,47). This phosphorylation by FAM20C is essential for the formation of calcified tissues, such as enamel, bone and dentin (48,49). Studies have also demonstrated that the threonine residue in the N-terminal domain of neuroendocrine companion protein 7B2 is phosphorylated by FAM20C (50). FAM20A acts as an activator of FAM20C, enhancing its phosphorylation activity on ECM substrates (51-53). Loss-of-function mutations in FAM20A lead to enamel hypoplasia and renal calcification (54,55), though it is a type II transmembrane protein localized to the GA and is not secreted into the extracellular space (54). FAM20B regulates the initiation and delivery of GAG chains by phosphorylating tetrasaccharide linkers on xylose residues in proteoglycans (52). POMK phosphorylates Ser734 on α-dystroglycan, which is required for LARGE xylosyl- and glucuronyltransferase 1-mediated laminin-binding glycosylation, facilitating receptor-ligand interactions in muscle and brain (51).

SPKKP-mediated ECM remodeling. The ECM not only provides structural support but also serves as a critical platform for intercellular communication. By integrating mechanical and biochemical signals, the ECM plays a central role in cell adhesion, migration and signal transduction (56). SPKKPs remodel the ECM by modulating the structural properties and signaling functions of ECM proteins through phosphorylation. FAM20C phosphorylates several ECM-associated proteins, including OPN, fibronectin and collagen (31). These modifications alter ECM adhesion and stiffness while regulating its binding capacity to cell surface receptors, thereby influencing cell-matrix interactions. In the TME, this regulation enhances ECM signaling capacity and activates pathways such as integrin-FAK and mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK), promoting tumor cell proliferation, migration and invasion (57). Furthermore, SPKKPs amplify their effects by phosphorylating ECM-associated soluble signaling molecules. Phosphorylation of GFs and chemokines secreted by tumor cells by FAM20C enhances their stability and binding affinity to receptors, intensifying signal transduction and promoting cell survival and proliferation through pathways such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and Janus kinase (JAK)/signal transducer and activator of transcription (STAT).

GASK1A also plays a critical role in ECM remodeling. As a GA-localized serine/threonine kinase, GASK1A enhances interactions between the ECM and integrins by phosphorylating ECM proteins and secreted signaling molecules. It activates the FAK/Src and PI3K/AKT signaling pathways, promoting cell adhesion, migration and survival (58). Additionally, GASK1A coordinates intercellular communication processes on a larger spatial scale by regulating the composition of phosphorylated proteins and non-coding RNAs in exosomes, enabling distant cells to sense and respond to microenvironmental signals (59). Overall, SPKKPs play a pivotal role in intercellular communication by regulating the composition, structure and interactions of the ECM with cells, providing fine-tuned control of cellular behavior and the microenvironment. Under pathological conditions, such as cancer, abnormal activation of these regulatory mechanisms may further amplify imbalances in intercellular communication, promoting disease progression.

SPKKP-associated EV communication. EVs are membrane-bound vesicles secreted by cells, including exosomes, microvesicles and apoptotic bodies. These vesicles carry bioactive molecules such as proteins, nucleic acids and lipids, which allow them to transmit biological information from donor to recipient cells, influencing the recipient's function and phenotype (60-69). As key carriers of intercellular communication, EVs are formed through various intracellular pathways and can be internalized by target cells via mechanisms like membrane fusion, endocytosis, phagocytosis and pinocytosis (70-72). At the molecular level, SPKKPs regulate the selective loading of secreted proteins into EVs by phosphorylating them. During the progression of endometrial cancer (EC), elevated levels of the PKDCC substrate lectin galactoside-binding soluble 3-binding protein (LGALS3BP) are found in plasma exosomes. By activating the PI3K/AKT/VEGFA signaling pathway, it promotes the proliferation and migration of EC cells and is closely linked to tumor angiogenesis (73) (Fig. 2). Another classic PKDCC substrate, secreted phosphoprotein 1, enhances oxidative phosphorylation and the proliferative capacity of

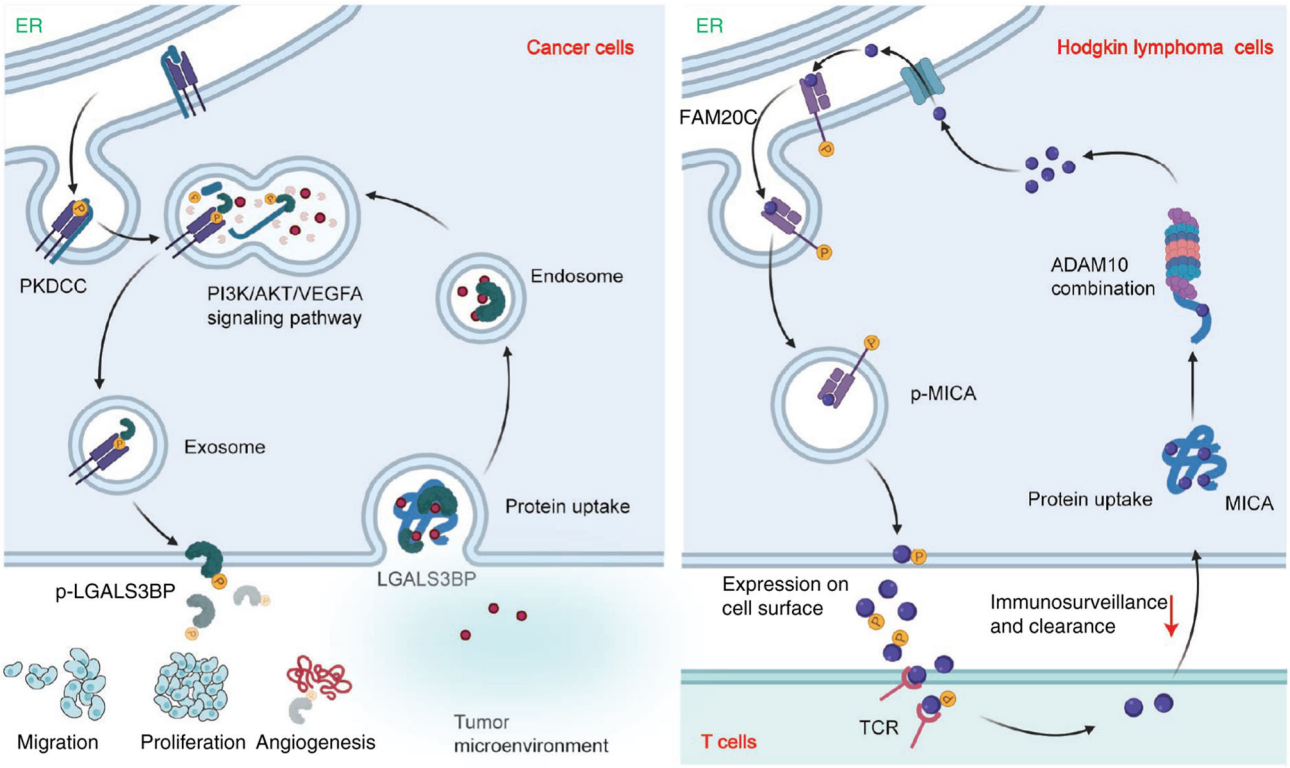

Figure 2. Substrate proteins in exosomes are secreted outside tumor cells. Left panel: The substrate LGALS3BP of PKDCC activates the PI3K/AKT/VEGFA pathway through the autocrine signaling loop of tumor cells, promoting tumor proliferation, migration and angiogenesis in endometrial cancer. Right panel: FAM20C substrate MICA, present in exosomes, is released into the tumor microenvironment to disrupt cytotoxic T-cell surveillance and inhibit the immune clearance of Hodgkin lymphoma cells, thereby impairing the patient's response to immunotherapy. AKT, protein kinase B; ER, endoplasmic reticulum; ADAM10, A disintegrin and metalloproteinase domain-containing protein 10; LGALS3BP, lectin galactoside-binding soluble 3-binding protein; MICA, MHC class I polypeptide-related sequence A; PI3K, phosphatidylinositol 3-kinase; TCR, T cell receptor; VEGFA, vascular endothelial growth factor A.

ovarian cancer (OC) cells by interacting with integrins and the CD44 receptor (74) (Table SIV). Similarly, FAM20C substrate cytoskeleton-associated protein 4 (CKAP4) can be released from lung cancer cells via exosomes. Its exosomal transport is regulated by palmitoylation, and its high expression is associated with tumor proliferation and poor prognosis (75). Additionally, another FAM20C substrate, α-2-HS glycoprotein, is highly expressed in lung cancer-derived EVs, showing potential as a diagnostic biomarker (76).

EVs not only mediate local intercellular communication but also facilitate long-distance signaling through the circulatory system, influencing physiological and pathological processes at the systemic level. In the TME, EVs regulate tumor growth, immune evasion and angiogenesis by transmitting signals between tumor cells, immune cells and stromal cells. Inositol polyphosphates (INSPs) are critical signaling molecules involved in functions like calcium homeostasis, cell survival and apoptosis. Multiple inositol polyphosphate phosphatase 1 (MINPP1), a substrate of PKDCC, regulates intracellular INSP levels and their downstream functions. MINPP1 exists in two subtypes: Type 1, primarily intracellular near the nucleus, is associated with ER stress, while Type 2 is secreted extracellularly via exosomes, contributing to the formation of the TME (32). Exosomes derived from colorectal cancer (CRC) cells can promote immune evasion by upregulating programmed cell death ligand 1 expression in tumor-associated macrophages (77). In Hodgkin's lymphoma, exosomal ADAM metallopeptidase domain 10 (ADAM10) and its substrate MHC class I polypeptide-related sequence A, secreted by tumor and lymphoid stromal cells, interfere with immune surveillance by cytotoxic T cells, inhibiting the response to immunotherapy (78) (Fig. 2).

The composition of EV cargo is closely linked to tumor histological types. In neuroblastoma, the FAM20C substrate fibronectin 1 (FN1) is significantly elevated in patient-derived EVs and correlates with tumor cell proliferation, migration and invasiveness (79). A differential proteomic analysis of exosomes from lung squamous cell carcinoma and lung adenocarcinoma revealed that the FAM20C substrate MFGE8 (Lactadherin) is associated with the progression of lung squamous cell carcinoma and may regulate cholesterol metabolism pathways in lung cancer cells (80). This differential expression across histological subtypes suggests a role in subtype-specific regulatory processes. By carrying specific kinase substrates, EVs contribute to tumor progression. These substrate proteins, modified by phosphorylation, play a pivotal role in driving tumor cell proliferation, immune evasion and angiogenesis, and defining tumor histological subtypes. As such, they offer significant potential for both basic research and clinical applications.

4. Dysregulation of SPKKP-mediated intercellular communication in cancer

Tumors of the nervous system. Glioblastoma multiforme (GBM) is the most common primary malignant tumor of the central nervous system and the deadliest form of brain cancer.

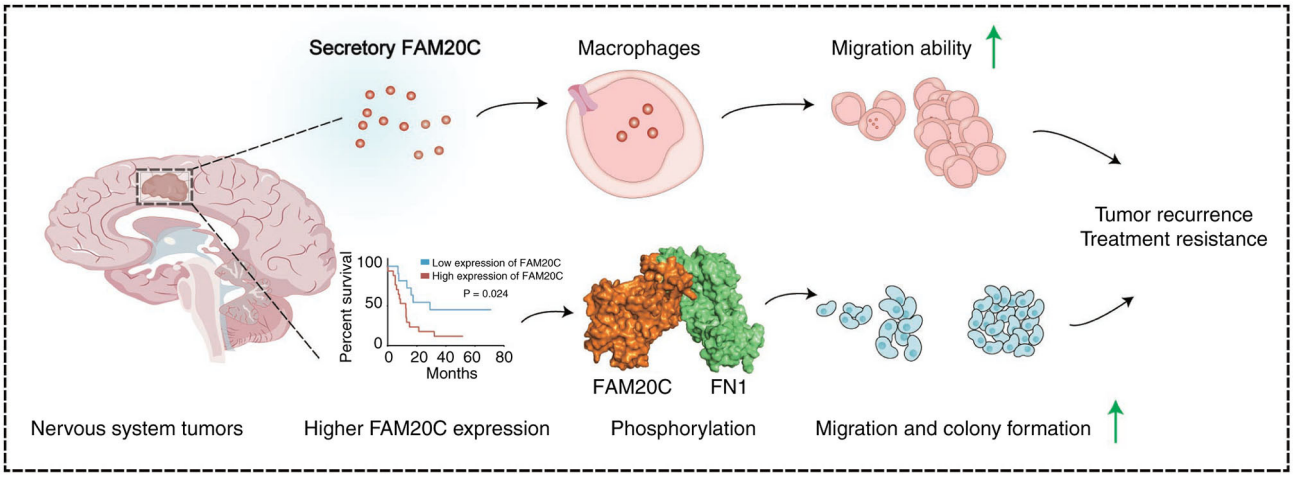

Figure 3. Role of FAM20C in GBM progression. High FAM20C expression in GBM is associated with poor prognosis. FAM20C promotes tumor cell migration and clonogenicity by binding to FN1 and activating the TNF signaling pathway. Additionally, FAM20C secreted by tumor cells enhances the migration of tumor-associated macrophages, which contribute to tumor progression, neoangiogenesis, immune suppression, and metastasis, potentially leading to tumor recurrence and resistance to treatment. FN1, fibronectin 1.

Despite extensive treatment involving surgical resection, chemotherapy and radiation therapy, the median survival of patients with GBM remains limited to only 15 months. With the urgent need for new therapeutic targets, researchers have intensively explored the mechanisms driving glioma development. FAM20A plays a pivotal role in GBM progression. An analysis of the human brain glioma transcriptomic dataset GSE108474 revealed that *FAM20A* is highly expressed in GBM, potentially influencing cell proliferation, adhesion, signal transduction and immune microenvironment establishment (81). Furthermore, FAM20A can form a complex with FAM20C, activating it through allosteric regulation, thereby promoting the phosphorylation of its substrates (82). Assay for Transposase-Accessible Chromatin with high-throughput sequencing-sequencing data further identified *FAM20C* and neuroplastin (*NPTN*) as hub genes associated with chromatin accessibility and differential expression. Studies in mouse xenograft models demonstrated that FAM20C enhances the proliferation and migration of glioma cells (83). By contrast, the recently identified tumor suppressor NPTN counteracts FAM20C by inhibiting glioma cell proliferation and migration. In the glioma cell lines LN229 and U251, NPTN overexpression significantly attenuates FAM20C-mediated cell proliferation. Transwell assays further show that *FAM20C* overexpression promotes cell invasion, while *NPTN* overexpression effectively inhibits it. Neutralizing antibodies targeting FAM20C lead to tumor regression in xenograft models. Additionally, MYC associated transcriptional regulator X, bromodomain containing 4 (BRD4), MYC proto-oncogene, bHLH transcription factor and RE1 silencing transcription factor (REST) were identified as potential transcriptional regulators of *FAM20C* (83), with BRD4 and REST also regulating *NPTN* expression. A previous study has developed signature models based on secreted kinases or kinase-like proteins to predict survival in patients with GBM. Further research indicated that FAM20C modulates immune responses in human GBM and facilitates immune cell migration (84) (Fig. 3). Furthermore, GASK1A is a key regulatory factor in GBM development, with its expression regulated by cavin-1. Database analyses from The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas and Gene Expression Omnibus suggest that cavin-1 promotes GBM progression by facilitating the maturation and secretion of GASK1A precursor (85). As a potential therapeutic target, cavin-1 is also considered a prognostic biomarker for GBM (85). However, there remains a significant bias between large-scale transcriptomic data and the inference of mechanisms in small-sample phosphoproteomics.

Head and neck tumors. FAM20A contributes to lymph node metastasis in thyroid cancer. Analysis of the TCGA database revealed that *FAM20A* expression is upregulated in thyroid cancer tissues with lymph node metastasis compared to those without. Protein-protein interaction analysis identified FAM20A as a hub gene among differentially expressed genes. Immune infiltration analysis further showed that *FAM20A* expression positively correlates with M1-type macrophages and NK cells in the thyroid cancer immune microenvironment, while negatively correlating with $CD4^+$ T cells. This suggests that FAM20A may play a role in shaping the immune microenvironment and facilitating lymph node metastasis in thyroid cancer (86). Additionally, FJX1 plays a significant oncogenic role in nasopharyngeal carcinoma (NPC). Gene expression profiling revealed that *FJX1* is significantly upregulated in primary NPC tissues compared to non-malignant tissues. Validation experiments using primary NPC tissue samples confirmed elevated *FJX1* mRNA levels, with some NPC tissues showing significantly higher FJX1 protein expression compared to normal epithelial tissues. Experimental findings indicate that FJX1 promotes the proliferation, adhesion-dependent growth and invasiveness of NPC cells. Following *FJX1* expression, mRNA levels of Cyclin D1 and Cyclin E1 increase, suggesting that FJX1 enhances cell proliferation by regulating key cell cycle proteins (87). Specific peptides targeting FJX1 have been shown to induce an immune response in NPC, exhibiting immunogenicity by binding to the major histocompatibility complex (MHC), class I, A (HLA-A)2 molecule on T cells and triggering cytotoxic activity against FJX1-expressing tumor cells. Furthermore, MHC class II peptides can stimulate T-cell

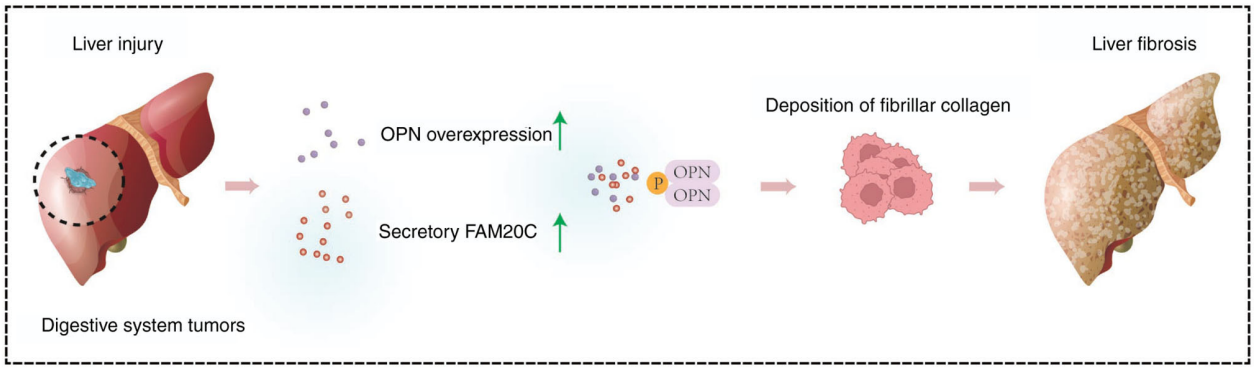

Figure 4. Mechanism of FAM20C-mediated liver fibrosis progression. Liver injury leads to overexpression of OPN, which is phosphorylated by secretory FAM20C within a multiprotein complex in activated hepatic stellate cells. Phosphorylated OPN is secreted into the extracellular matrix, promoting collagen deposition and advancing liver fibrosis. OPN, osteopontin.

proliferation (88). Leveraging these immunological properties, researchers developed a dual-antigen peptide vaccine (PV1) targeting both FJX1 and MAGE family member D4B (MAGED4B). After PV1 stimulation, T cells from patients with head and neck squamous cell carcinoma (HNSCC) secreted cytotoxic cytokines upon exposure to target cells expressing the corresponding antigens. Patients with high tumor expression of *MAGED4B* and *FJX1* showed a more pronounced response to PV1 stimulation, indicating the vaccine's specificity (89). In the context of anti-programmed cell death 1 (PD-1) immune checkpoint inhibitor therapy for HNSCC, the overall response rate is ~16%. Tumor-infiltrating T cells are critical to the efficacy of immune checkpoint inhibitors. A DNA vaccine targeting FJX1 can increase the number of reactive T cells and enhance tumor cell clearance in synergy with PD-1 inhibitors (90).

Tumors of the digestive system. FAM20A plays a critical role in the development and progression of hepatocellular carcinoma (HCC). Gene expression analyses of human HCC cells have revealed that *FAM20A* interacts with α-fetoprotein and is involved in alternative splicing processes in HCC. FAM20A contributes to several key cellular processes, including cell migration, growth-cone collapse and the regulation of insulin-like growth factor (IGF) transport and uptake via IGF-binding proteins (91). OPN, a phosphoglycoprotein secreted into the ECM during liver injury, acts as a cytokine to promote fibrous collagen deposition during liver fibrosis. In mouse livers with ligated bile ducts and in activated hepatic stellate cells (HSCs) cultured *in vitro*, OPN was found to be both upregulated and significantly phosphorylated by FAM20C. By contrast, FAM20C activity is minimal in the livers of sham-operated animals and in quiescent HSCs, but it is markedly enhanced upon binding to a multiprotein complex of ~500 kDa. Knocking out FAM20C not only confirmed its role in OPN phosphorylation but also revealed the importance of phosphorylation in OPN secretion. However, incubating HSCs with phosphorylated or unphosphorylated recombinant OPN increased collagen-I expression, suggesting that OPN's pro-fibrotic function is independent of its phosphorylation status. These findings highlight OPN's role in promoting liver fibrosis and highlight FAM20C as a key regulatory factor that drives this process by enhancing OPN secretion by HSCs, offering new avenues for research on the mechanisms underlying liver fibrosis (92) (Fig. 4). Pan-cancer analysis also revealed that *FAM20C* is upregulated in stomach adenocarcinoma (STAD), and its expression correlates positively with immune cell infiltration, including $CD4^+$ T cells, macrophages, neutrophils, DCs and other immune cells (93).

FJX1 is also implicated in gastric cancer (GC) onset and progression. Detailed analysis of tumor and non-tumor tissues from patients with GC revealed significantly elevated mRNA and protein levels of FJX1 in GC tissues compared to normal gastric mucosal tissue. Furthermore, high FJX1 expression at both the mRNA and protein levels is associated with poor prognosis in patients with GBM, making it an independent risk factor for adverse outcomes. FJX1 is predominantly localized in the cytoplasm of GC cells and its expression level positively correlates with Ki67 expression. Additionally, *FJX1* expression is closely associated with CA19-9 levels, tumor invasion depth and lymph node metastasis. FJX1 promotes the expression of Ki67 and proliferating cell nuclear antigen, which are linked to cell proliferation, and regulates GC-cell proliferation. The PI3K/AKT signaling pathway may underlie FJX1's function, as *FJX1* enhances the expression of phosphorylated proteins in the PI3K and AKT pathways, thereby promoting GC cell proliferation (94).

FJX1 has been identified as an oncogene in colorectal tumors and is regulated by multiple upstream microRNAs (miRNAs). In a competitive endogenous RNA network associated with colorectal adenocarcinoma, *FJX1* was found to be regulated by miRNA-17 and miRNA-106a, influencing the expression of downstream genes such as solute carrier family 16 member 9, cytochrome b reductase 1 and RNA binding motif protein 20. Survival analyses confirmed that FJX1 is associated with poor prognosis in patients with CRC (95). Compared to traditional CRC biomarkers such as CEA and CA19-9, FJX1 shows considerable potential in predicting patient prognosis. Serum FJX1 displays high sensitivity and specificity in distinguishing patients with CRC from controls, and its concentration is correlated with distant metastasis. Furthermore, serum FJX1 levels decrease significantly following surgery in patients with CRC (96). FJX1 enhances the expression of angiogenesis-related genes, including hypoxia-inducible factor 1, VEGF, Fms related receptor tyrosine kinase and kinase insert domain receptor, promoting the formation of epithelial luminal structures in CRC. This suggests that FJX1 acts as a novel regulator

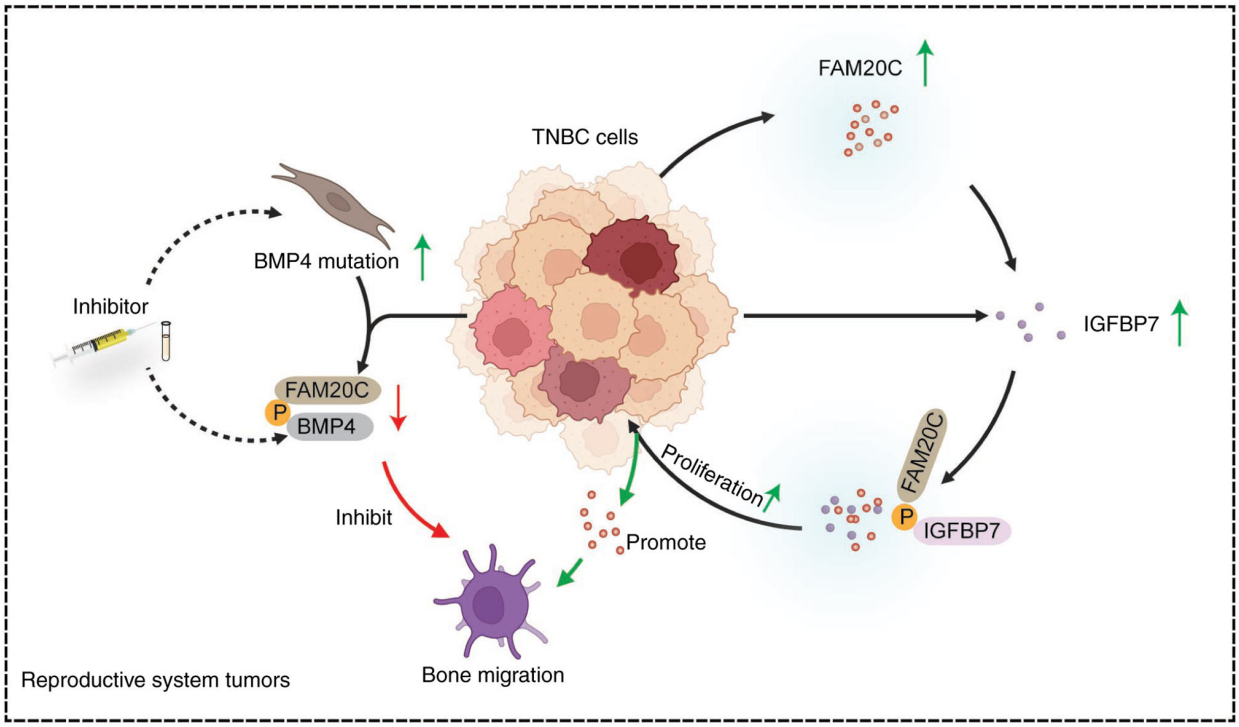

Figure 5. FAM20C-mediated mechanisms promoting bone metastasis in TNBC. Mutations in BMP4 phosphorylation sites enhance BMP4 degradation and reduce its secretion, while BMP4 signaling inhibitors accelerate these effects. FAM20C also phosphorylates IGFBP7, further enhancing cell migration and metastatic spread to bone. TNBC, triple-negative breast cancer; BMP4, bone morphogenetic protein 4; IGFBP7, insulin-like growth factor binding protein 7.

of tumor progression, particularly in the process of tumor angiogenesis (97). Long non-coding RNA plasmacytoma variant translocation 1 (PVT1) has also been implicated in CRC progression. MiRNA-106b-5p, a target of PVT1, is suppressed by PVT1 in CRC tissues, and this suppression leads to increased expression of FJX1. Upregulation of PVT1, achieved by down-regulating miRNA-106b-5p and restoring *FJX1* expression, promotes the proliferation, migration, invasion and tumor growth of CRC cells (98). Additionally, miRNA-532-3p (99) and miRNA-1249 (100) have been identified as important regulators of FJX1, further influencing CRC progression. Further study has demonstrated that FJX1 is closely associated with liver metastasis in CRC through its modulation of the Notch signaling pathway. FJX1 is also thought to impact chromatin-modifying enzymes, the Notch signaling system, cellular senescence and other signaling pathways (101).

In addition, FAM198A and FAM69A are involved in the progression of colorectal adenocarcinoma. An RNA analysis of a patient with CRC with peritoneal metastasis revealed a *FAM198A-RAF1* fusion event (102). Furthermore, FAM69A has been shown to regulate lipid metabolism in colorectal adenocarcinoma. *FAM69A* expression is downregulated in CRC and serves as a prognostic marker, forming part of a risk scoring model alongside seven other genes. Gene enrichment analysis revealed that FAM69A is closely linked to lipid metabolism and the endothelial-mesenchymal transition (103).

Tumors of the reproductive system. Current research indicates that FAM20C can phosphorylate IGFBP7, thereby promoting the proliferation and migration of human triple-negative breast cancer (TNBC) cells. Both FAM20C and IGFBP7 are highly expressed in breast cancer cells (33). The FAM20C inhibitor FL-1607 effectively suppresses the migratory capacity of TNBC cells and acts as a novel targeted agent that promotes TNBC cell apoptosis (104) (Fig. 5). In myeloid cells, FAM20C inhibits osteoclastogenesis and bone resorption. OPN, the most abundant phosphorylated protein secreted in a FAM20C-dependent manner, is essential in this process. FAM20C-mediated OPN phosphorylation reduces its secretion, and neutralizing OPN diminishes osteoclast differentiation and bone metastasis induced by FAM20C deficiency. In breast cancer cells, FAM20C enhances osteoclastogenesis by promoting the phosphorylation and secretion of bone morphogenetic protein 4 (BMP4), thereby facilitating bone metastasis. Mutations in BMP4 phosphorylation sites increase degradation of its fragments and reduce BMP4 secretion. BMP4 deficiency or treatment with BMP4 signaling inhibitors in cancer cells reduces osteoclast differentiation and bone metastasis, eliminating FAM20C's regulatory role in these processes (105) (Fig. 5). Furthermore, studies indicate that elevated *FAM20C* expression correlates with reduced sensitivity to cisplatin in testicular cancer (106).

By contrast, FAM198B appears to have a protective effect in uterine corpus endometrial carcinoma (UCEC). Transcriptomic data analysis, including univariate Cox and Least Absolute Shrinkage and Selection Operator (LASSO) regression, revealed that in UCEC, the hazard ratio for FAM198B was <1, with the LASSO regression coefficient also below zero. Immunohistochemical analysis showed that FAM198B expression in tumor tissue was significantly lower than in adjacent tissues. Furthermore, FAM198B, along with six other kinases, co-regulates the immune microenvironment of UCEC, promoting the infiltration of immunosuppressive cells (107).

Malignant neoplasm of mesenchymal tissue. DIPK2A has been implicated in melanoma development, with abnormal DNA methylation being a significant epigenetic alteration. Unsupervised hierarchical clustering and multidimensional parametric analysis revealed distinct methylation profiles between primary acral lentiginous melanoma (PALM) and primary non-lentiginous acral melanoma, with *DIPK2A* exhibiting low methylation in PALM. High methylation of *DIPK2A* was found to be associated with poorer disease-specific survival (108,109). The effects of DIPK2A on melanoma cell proliferation and invasion are regulated by the microphthalmia-associated transcription factor (Mitf). Reduced Mitf levels downregulate the expression of DIPK2A, actin and Rho associated coiled-coil containing protein kinase, enhancing cellular invasiveness and affecting cell cycle progression (110). In melanoma, *FJX1* overexpression promotes tumor progression, but this effect can be inhibited by miRNA-127-3p, which binds to FJX1 and negatively regulates its expression. Additionally, *FOXD3-AS1* further influences melanoma development by regulating the miRNA-127-3p/FJX1 axis (111).

5. Molecular pathways mediating SPKKP-regulated intercellular communication

SPKKPs are integral to cell-cell interactions, regulating cellular communication through various mechanisms, including direct cell contact, secretion of signaling molecules and the exchange of substances via EVs and the ECM. Phosphorylation, a pivotal event in signal transduction, is central to these processes. When extracellular signaling molecules bind to their receptors, they trigger intracellular PK cascades, such as the MAPK and PI3K-AKT pathways. These pathways amplify and organize signals within the cell, allowing a small number of ligands to influence the activity of numerous intracellular molecules (52,53,112). Consequently, intercellular communication extends beyond mere signal reception and transmission to a complex, multilevel network regulated by protein modifications and processing mechanisms. In this context, SPKKPs are increasingly recognized as key regulatory factors that influence intercellular communication, owing to their distinct roles in the secretory pathway and the assembly of signaling complexes (34,51,113-115).

PI3K-AKT-mTOR signaling pathway. The PI3K/AKT pathway is a critical cellular signaling cascade that responds to extracellular signals, promoting processes such as metabolism, cell proliferation, survival, growth and angiogenesis. This pathway involves serine/threonine phosphorylation of downstream substrates, with key components including PI3K and AKT, which give the pathway its name. The PI3K/AKT signaling pathway can be divided into three primary components: Upstream, core and downstream. Its involvement in tumor progression has been extensively validated by numerous studies (116). Activation of this pathway results in the phosphorylation of downstream substrates, initiating cascades that trigger various cellular responses. Kinases within the secretory pathway also regulate the PI3K/AKT signaling axis, influencing multiple biological processes in different tumor types. This complex signaling network is essential for understanding the molecular mechanisms underlying tumorigenesis and has become a central focus in cancer research.

The PI3K/AKT pathway plays a pivotal role in the development of TNBC (117). Additionally, POMK, associated with muscular dystrophy and congenital glycosylation disorders, has been implicated in breast cancer metastasis. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses have revealed that POMK's interacting proteins are significantly enriched among membrane proteins and metabolic pathway-related proteins (118). In a basal-like breast cancer cell model, N-glycosylated POMK was identified as a suppressor of metastasis, primarily through the AKT-glycogen synthase kinase (GSK)3β pathway. High POMK mRNA expression in patients with breast cancer was found to be associated with prolonged survival. Critical glycosylation sites (Asn67, Asn165, Asn220 and Asn235) are essential for POMK's role in inhibiting breast cancer cell migration and metastasis. The inhibitory effect is mediated via the PI3K/AKT/glycogen synthase kinase-3β (GSK-3β)/Snail pathway. POMK deficiency leads to AKT and GSK-3β activation, upregulating Snail levels and promoting cancer progression (119).

The PI3K/AKT pathway also has a significant role in GC progression (120). In STAD, FAM198B is highly expressed and associated with poor prognosis. FAM198B contributes to GC progression by upregulating the PI3K/AKT/BCL2 apoptosis regulator (BCL-2) signaling pathway. This includes phosphorylation of PI3K, AKT and cyclin-dependent kinase 1, as well as increased BCL-2 expression and reduced BCL2-associated agonist of cell death and BCL2-associated X protein levels. Knockdown of FAM198B using small inhibitory RNA reverses these effects, inhibiting GC progression. High expression of FAM198B is linked to processes such as myogenesis, angiogenesis, epithelial-mesenchymal transition and cytokine binding. Notably, silencing FAM198B significantly inhibits the proliferation and migration of GC cells (121) (Fig. 6).

ERK/MAPK signaling pathway. The MAPK pathway is a critical intracellular signaling cascade comprising a three-tiered structure and four major branches, one of which is the ERK. ERK regulates various essential physiological and pathological processes, including cell growth, differentiation, stress responses, and inflammatory reactions. Extensive research has highlighted the role of the MAPK/ERK signaling pathway in tumorigenesis. Upon the binding of an extracellular stimulus, such as a GF, to a receptor tyrosine kinase on the cell surface, GF receptor-bound protein 2 (GRB2) is activated. GRB2, in turn, activates Ras, which further activates Raf kinase. Raf then phosphorylates and activates MAPK kinase, which ultimately phosphorylates and activates ERK (122). Despite these insights, further research is needed to fully elucidate the detailed mechanisms underlying this pathway.

FAM198B has been implicated in survival outcomes in patients with lung adenocarcinoma. Elevated FAM198B expression in lung adenocarcinoma tissues enhances cancer cell invasiveness, migration, motility and proliferation. Furthermore, matrix metalloproteinase 1 (MMP1) has been identified as a key downstream target of FAM198B. Differential gene and pathway analyses revealed that FAM198B inhibits both the mRNA and protein expression of MMP1 by reducing ERK phosphorylation levels and suppressing the ERK/MAPK signaling pathway (123). FAM20B, like FAM198B, can also reduce ERK phosphorylation and subsequently inhibit

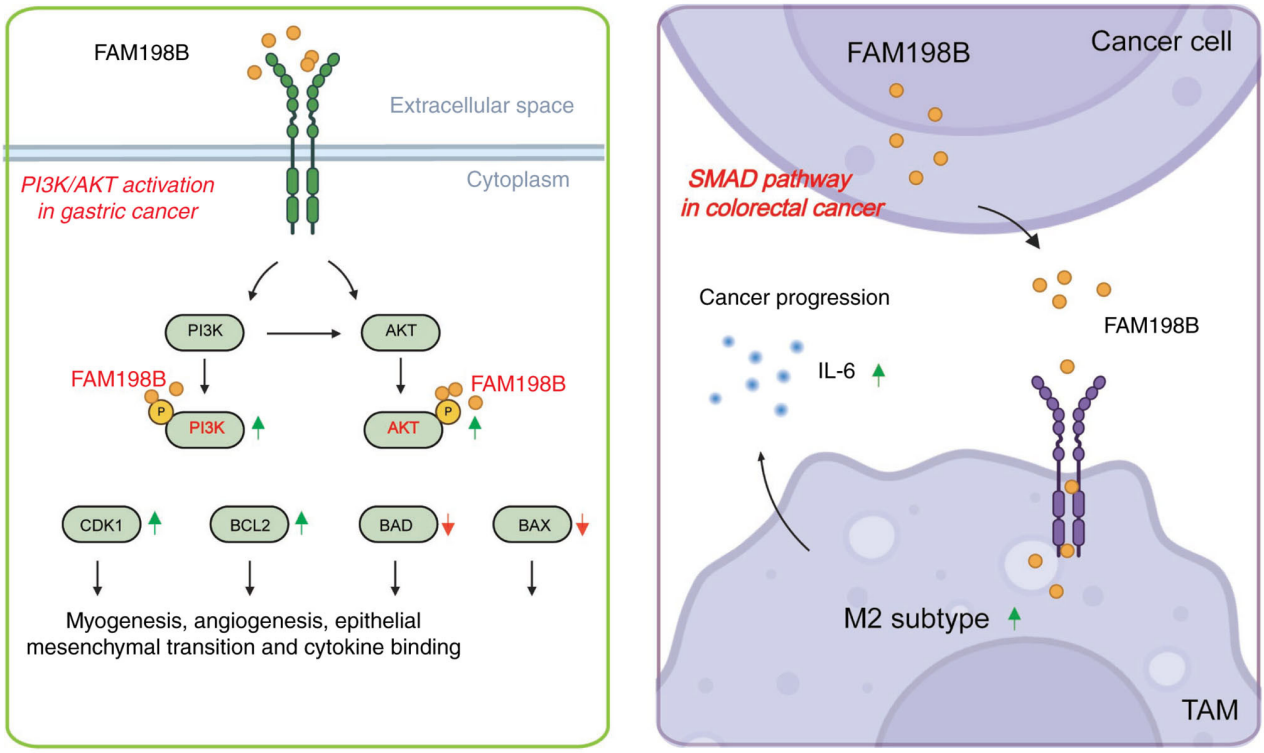

Figure 6. FAM198B regulates tumor progression through various signaling pathways. Left panel: FAM198B activates the PI3K/AKT pathway, upregulating CDK1 and BCL-2, while inhibiting BAD and BAX to promote malignant phenotypes such as cell proliferation, migration and angiogenesis in gastric cancer. Right panel: In colorectal cancer, FAM198B targets the SMAD signaling pathway in tumor-associated macrophages, driving M2 polarization and promoting metastasis. AKT, protein kinase B; BCL2, B-cell lymphoma 2; BAD, BCL2-associated agonist of cell death; BAX, BCL2-associated X protein; CDK1, cyclin-dependent kinase 1; PI3K, phosphatidylinositol 3-kinase; SMAD, SMAD family member.

MMP1 expression. However, N-glycosylated FAM20B fails to suppress ERK phosphorylation, resulting in increased MMP1 expression. FAM198B also exerts a protective role in OC. CUGBP Elav-like family member 2 (CELF2), an RNA-binding protein, is downregulated in OC and inhibits tumor cell proliferation, migration and invasion. FAM198B has been identified as a downstream target of CELF2, and CELF2 enhances FAM198B stability by binding to AU/U-rich elements in its 3' untranslated region. The CELF2/FAM198B axis regulates tumorigenesis and response to cisplatin therapy through the ERK/MAPK signaling pathway (124).

SMAD2 signaling pathway. In CRC, FAM198B promotes tumor progression by regulating the SMAD2 signaling pathway, which influences tumor-associated macrophage polarization. Elevated FAM198B expression in tumor-associated macrophages correlates with poor prognosis in patients with CRC. By targeting SMAD2, FAM198B regulates macrophage polarization toward the M2 phenotype, thereby promoting metastasis (27) (Fig. 6).

6. Therapeutic targeting of SPKKPs in intercellular communication across different cancer microenvironments

Given the pivotal role of SPKKPs in cancer progression and intercellular communication, targeting their activity offers significant therapeutic potential. Inhibiting key SPKKPs can disrupt pro-tumor signaling pathways within the TME, potentially halting disease progression.

FAM20C inhibitors (FL-1607). Targeting SPKKPs is anticipated to modulate the TME by altering the tumor cell secretome, ECM remodeling and paracrine signaling networks. Through systems biology network integration, molecular docking and molecular dynamics simulations, researchers identified FL-1607, a FAM20C inhibitor, from a panel of candidate compounds. FL-1607 demonstrates potent antiproliferative effects in TNBC cells. By inhibiting FAM20C-mediated phosphorylation of secreted protein substrates, FL-1607 induces apoptosis and suppresses cell migration in MDA-MB-468 cells. This compound impedes metastasis-promoting exosome signaling and ECM-receptor interaction pathways, thereby inhibiting tumor cell survival and motility (33). FAM20C catalyzes the serine phosphorylation of various secreted proteins, and in numerous tumors, upstream oncogenic signaling pathways typically enhance tumor growth, angiogenesis and immune evasion by boosting secretome activity and ECM remodeling. FL-1607, by inhibiting FAM20C, significantly reduces the phosphorylation levels of substrates rich in S-x-E/pS motifs, thereby decreasing the pathogenic modifications and stability of pro-tumor cytokines, chemokines and ECM-associated proteins. Consistent with findings in TNBC models, FL-1607 inhibited proliferation, promoted apoptosis and suppressed migration. Although current research on FL-1607 relies primarily on *in vitro* tumor cell experiments and lacks systematic pharmacokinetic studies and *in vivo* validation, it represents the first reported FAM20C inhibitor. This provides a promising development path for future molecular optimization and expansion to other tumor types.

FJX1-targeting peptide vaccines. FJX1, a non-Epstein-Barr virus-derived tumor-associated antigen, is highly expressed in NPC and HNSCC, where it plays a role in tumor immune evasion and microenvironmental remodeling. Due to its enrichment at secretory sites and antigen-presenting potential, researchers have developed a short-peptide vaccine targeting FJX1. This vaccine aims to disrupt pro-tumor communication between tumor cells and immune cells by inducing FJX1-specific T-cell responses. The FJX1 peptides typically consist of 9-20 amino acids. After processing by DCs, they are presented primarily to $CD8^+$ T cells via the HLA-A2-restricted MHC-I pathway, while some HLA-DR-restricted peptides promote the expansion of $CD4^+$ T helper cells. This process enhances the expression of cytotoxic effector molecules, such as IFN-γ and granzyme B, in the TME, leading to the recognition and lysis of tumor cells with upregulated FJX1 expression (88).

In a patient population study, researchers conducted *in vitro* validation of the FJX1 peptide vaccine in patients with HNSCC. They combined FJX1 with another tumor antigen, MAGED4B, to develop the dual-antigen peptide vaccine PV1 and evaluated its immunogenicity and association with immune responses in patients with HNSCC. Immunohistochemistry and transcriptomic analyses revealed that 94.8% of patients with HNSCC co-express MAGED4B and FJX1, indicating high applicability for PV1. In HLA-A2 dimer and ELISPOT assays, pre-existing antigen-specific T cells, which could be further expanded by PV1, were found in patients' peripheral blood mononuclear cells. After vaccination, these T cells released higher levels of IFN-γ and granzyme B when exposed to target cells expressing the corresponding antigens. Notably, patients with high FJX1/MAGED4B expression in tumor tissue exhibited stronger cytotoxic responses. Additionally, FJX1 and MAGED4B expression was observed in cancer cell lines from breast, lung, colon, prostate and rectal cancers, as well as in TCGA data, providing preliminary evidence for expanding the FJX1 peptide vaccine's application beyond HNSCC (88). However, the paper conflates the mere qualitative detection of a protein with its functional relevance as an immunogenic target amenable to vaccine-mediated targeting, thereby overlooking critical biological variables, including antigen processing and presentation, tumor-driven immune evasion mechanisms, and intra- and inter-tumoral heterogeneity.

The potential of targeting exosomes carrying SPKKP substrates. Targeting exosomes carrying SPKKP substrate proteins presents a novel and promising therapeutic strategy in oncology. As previously discussed, exosomes are not merely passive vehicles but active participants in tumor progression, mediating essential processes like immune evasion, angiogenesis and metastasis. Therapeutic approaches focused on these exosomes are founded on two key principles. First, the specific phosphorylated proteins they carry, such as FN1, ADAM10, LGALS3BP and CKAP4, serve as distinct 'molecular signatures' of the tumor cells from which they originate. This specificity allows for highly targeted interventions, potentially minimizing off-target effects.

Researchers have isolated exosomes from the serum of patients with pancreatic cancer, identifying 611 proteins, with 141 differentially expressed. Notably, apolipoprotein E (APOE) expression was elevated in these exosomes and closely interacted with other proteins (125). Additionally, APOA2, present in exosomes from pancreatic cancer, shows promise as a biomarker, while APOB is downregulated (126). CKAP4, secreted via exosomes from pancreatic ductal adenocarcinoma (PDAC), may serve as a PDAC biomarker, and anti-CKAP4 monoclonal antibodies are showing promise for PDAC treatment (127). GA membrane protein 1, a serum marker for HCC, is carried in exosomes that activate the GSK-3β/MMP signaling axis in recipient cells, promoting proliferation and migration (128). By contrast, exosomes from hepatitis B virus (HBV)-associated HCC exhibit reduced FN1 expression compared to normal stem cells, influencing HBV-associated pathways, viral activity, invasion, exosome formation, adhesion and protein binding (129). Additionally, GC individuals with exosome phenotype B show poorer prognoses and greater resistance to immunotherapy. Matrilin 3 has been identified as a signature protein for phenotype B, offering potential as a prognosis predictor (130). In CRC, exosomes derived from cancer-associated fibroblasts (CAFs) exhibit elevated quiescin sulfhydryl oxidase 1 (QSOX1) levels compared to CAFs from healthy individuals, indicating that QSOX1 may serve as a biomarker for CRC (131).

Inhibiting the secretion or uptake of these bioactive exosomes presents an additional therapeutic strategy. Small-molecule inhibitors targeting key steps in exosome biogenesis or endocytosis in recipient cells can effectively disrupt this critical intercellular communication network, blocking the transport of oncoprotein SPKKP substrates. Even more promising is the potential to engineer exosomes to carry inhibitors or antagonists targeting these SPKKP substrates, effectively 'hijacking' the pro-tumor system. This strategy could serve as a highly efficient and specific 'Trojan horse' therapy. For aggressive tumors such as glioblastoma, where the TME poses a significant treatment barrier, disrupting the exosomal communication sustaining this adverse environment could substantially enhance the efficacy of existing treatments, including immunotherapy and anti-angiogenic therapies.

7. New technologies for detecting and studying SPKKPs

Developing a novel, unified and high-throughput approach for the *in situ* measurement of SPKKPs in living cells remains a major challenge. Current studies have largely focused on individual kinases and relied heavily on *in vitro* biochemical assays or the detection of specific substrates. For instance, FAM20C activity has been measured *in situ* in living cells using fluorescence correlation spectroscopy (FCS). By analyzing fluorescence fluctuations generated by the movement of fluorescently labeled molecules within a defined detection volume, FCS provides information on molecular concentration, diffusion coefficients and molecular interactions (132). Although this method shows promise for studying low-abundance secreted proteins under physiological conditions, its throughput and its capacity to simultaneously detect multiple kinases remain limited (132). In the case of FAM20A and POMK, their activities have been directly measured using *in vitro* reconstituted secretory pathway membrane vesicles combined with radioactive ATP pulse-tracking assays (52,54). While these approaches provide relatively precise enzymatic activity data, achieving high-throughput and real-time monitoring in

live-cell settings remains challenging. Consequently, current research is still largely confined to a 'single kinase-single substrate' framework, making it difficult to systematically dissect the global regulatory roles of SPKKPs in extracellular signaling networks.

Against this background, recent advances in phosphoproteomics have provided a critical breakthrough for SPKKP research. A landmark study has shown that FAM20C generates the majority of the extracellular phosphoproteome and phosphorylates >100 secreted substrates, underscoring its central role in extracellular signal regulation (26). More recent work has further expanded the substrate spectrum of FAM20C through systematic phosphoproteomic analyses, identifying ~256 proteins and 443 phosphorylation sites, of which nearly 77% were previously unrecognized, thereby providing additional support for its role as a dominant kinase of the secretory pathway (133). Together, these studies indicate that phosphoproteomics has advanced SPKKP research from single-substrate validation to the systematic interrogation of extracellular signaling networks. In addition, the substrates of FAM20C and related SPKKPs are broadly distributed along the continuous secretory axis spanning the ER, GA and extracellular space, and include ECM proteins, secreted factors and cell-surface proteins, highlighting a strong spatial dependency (134). This feature suggests that conventional proteomic approaches alone are insufficient to fully resolve their spatial regulatory mechanisms within tissues.

Nevertheless, most existing studies are still based on bulk-level analyses, which makes it difficult to distinguish SPKKPs and their substrates derived from different cellular sources. Given that, within the TME, SPKKPs and their substrates may originate from tumor cells, fibroblasts, immune cells and other cellular populations, single-cell proteomics may, in the future, enable cell type-specific characterization of SPKKP expression and substrate phosphorylation, thereby providing a more precise understanding of their division of labor in intercellular communication (135). At the same time, available evidence indicates that FAM20C substrates, including ECM proteins such as osteopontin and fibronectin, exhibit marked spatial heterogeneity within tissues, with especially pronounced differences between the invasive front and the tumor core (133). Accordingly, spatially resolved technologies are expected to further illuminate the tissue distribution patterns of ECM phosphorylation and clarify how SPKKP-mediated intercellular signaling is directionally transmitted within the microenvironment. Overall, the development of SPKKP-specific chemical probes and Golgi-targeted kinase activity reporter systems, together with the integration of phosphoproteomics, single-cell approaches and spatially resolved technologies, is expected to overcome the limitations of current methods and enable high-throughput, dynamic and *in situ* monitoring of SPKKP activity. Such advances will provide a more systematic understanding of the roles of SPKKPs in intercellular communication and in the initiation and progression of disease.

8. Conclusion and perspective

This review synthesizes emerging evidence positioning SPKKPs as master regulators of intercellular communication, with profound implications in cancer and other diseases. It outlines how SPKKPs, through their unique localization and ability to phosphorylate a diverse secretome, orchestrate key signaling pathways, modulate the ECM and govern the bioactive cargo of EVs. Their dysregulation directly fuels tumor progression by promoting cancer hallmarks, such as proliferation, metastasis, immune evasion and angiogenesis, across a wide range of malignancies, including glioblastoma, as well as gastrointestinal and reproductive cancers. The development of targeted agents, such as the FAM20C inhibitor FL-1607 and FJX1-directed vaccines, highlights the translational potential of targeting this kinase family.

Despite these advances, substantial gaps remain in the current understanding. A primary challenge is the incomplete mapping of the SPKKP 'phosphoproteome' and its context-dependent variations across different tumor types and cellular states. Key points remaining to be clarified include the precise kinase-specific substrate profiles for less-characterized SPKKPs like the DIPK and GASK families and how the TME influences the substrate preference and activity of these kinases. Addressing these questions is essential for comprehending the full scope of their biological functions and for minimizing off-target effects in therapy.

Furthermore, the complex regulatory networks controlling SPKKP activity remain poorly defined. Current research lacks a deep mechanistic understanding of how SPKKPs are transcriptionally and post-translationally regulated, and how they interact with other major signaling pathways. Future studies should explore the upstream oncogenic signals driving SPKKP overexpression in specific cancers and how SPKKPs integrate with canonical intracellular signaling to create feedback loops that sustain tumor growth. Unraveling these mechanisms will reveal novel regulatory nodes that could be therapeutically targeted.

From a translational perspective, the journey from mechanistic insight to clinical application faces several hurdles. The development of isoform-specific inhibitors remains a challenge due to structural similarities within kinase families. Critical research directions include the design of allosteric inhibitors or proteolysis-targeting chimeras with enhanced specificity and how to effectively deliver therapeutics to block extracellular kinase activity or intercept pathogenic exosomes *in vivo*. Additionally, validating SPKKP substrates in circulating exosomes as reliable, non-invasive biomarkers will require large-scale, multi-center clinical studies.

Looking forward, the future of SPKKP research lies in embracing complexity and innovation. Single-cell multi-omics and spatial transcriptomics are poised to uncover the cell-type-specific roles of SPKKPs within the TME. Combining SPKKP-targeted agents with existing therapies, such as immunotherapy, chemotherapy or radiation, presents a promising strategy to overcome resistance and improve patient outcomes. By systematically addressing these research gaps, it may be possible to fully harness the potential of SPKKPs, paving the way for a new class of targeted therapies and advancing the frontier of precision oncology.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 82103450 to Zhu C) and the Liaoning Provincial Science and Technology Program (grant no. 2024-BS-029 to Du SN).

Availability of data and materials

Not applicable.

Authors' contributions

ZG and ShaonanD were involved in the conception and design of the study. ZG, SG, ShaoqiD and SZ performed the literature search, study selection and data extraction. ZG and ShaonanD collated and analyzed the information from the literature. ZG, SG, ShaoqiD and SZ drafted the manuscript. ST, JH, ShaonanD and CZ edited the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Su J, Song Y, Zhu Z, Huang X, Fan J, Qiao J and Mao F: Cell-cell communication: New insights and clinical implications. Signal Transduct Target Ther 9: 196, 2024.
2. Meng L: Roles of secreted peptides in intercellular communication and root development. Plant Sci 183: 106-114, 2012.
3. Radak M and Fallahi H: Cell-cell communication in stem cells and cancer: Alone but in touch. Fundam Clin Pharmacol 38: 479-488, 2024.
4. Kanno Y: Modulation of cell communication and carcinogenesis. Jpn J Physiol 35: 693-707, 1985.
5. AlMusawi S, Ahmed M and Nateri AS: Understanding cell-cell communication and signaling in the colorectal cancer microenvironment. Clin Transl Med 11: e308, 2021.
6. Martinez J, Marmisolle I, Tarallo D and Quijano C: Mitochondrial bioenergetics and dynamics in secretion processes. Front Endocrinol (Lausanne) 11: 319, 2020.
7. Zhang YN, Wang SM, Ren XR, Duan QY and Chen LH: The transmembrane and cytosolic domains of equine herpesvirus type 1 glycoprotein D determine Golgi retention by regulating vesicle formation. Biochem Biophys Res Commun 702: 149654, 2024.
8. De Craene JO, Bertazzi DL, Bar S and Friant S: Phosphoinositides, major actors in membrane trafficking and lipid signaling pathways. Int J Mol Sci 18: 634, 2017.
9. Yoon S, Choi JH, Shah M, Kwon SM, Yang J, Park YN, Wang HJ and Woo HG: USO1 isoforms differentially promote liver cancer progression by dysregulating the ER-Golgi network. Carcinogenesis 42: 1208-1220, 2021.
10. Vitale A and Pedrazzini E: Endoplasmic reticulum and Golgi apparatus: Old friends, novel intimate relationships. J Exp Bot 68: 3283-3285, 2017.
11. Vizoso FJ, Eiro N, Costa L, Esparza P, Landin M, Diaz-Rodriguez P, Schneider J and Perez-Fernandez R: Mesenchymal stem cells in homeostasis and systemic diseases: Hypothesis, evidences, and therapeutic opportunities. Int J Mol Sci 20: 3738, 2019.
12. Canaider S, Facchin F, Tassinari R, Cavallini C, Olivi E, Taglioli V, Zannini C, Bianconi E, Maioli M and Ventura C: Intracrine endorphinergic systems in modulation of myocardial differentiation. Int J Mol Sci 20: 5175, 2019.
13. Nogues L, Palacios-Garcia J, Reglero C, Rivas V, Neves M, Ribas C, Penela P and Mayor F Jr: G protein-coupled receptor kinases (GRKs) in tumorigenesis and cancer progression: GPCR regulators and signaling hubs. Semin Cancer Biol 48: 78-90, 2018.
14. Khalilimeybodi A, Riaz M, Campbell SG, Omens JH, McCulloch AD, Qyang Y and Saucerman JJ: Signaling network model of cardiomyocyte morphological changes in familial cardiomyopathy. J Mol Cell Cardiol 174: 1-14, 2023.
15. Zou ZG, Rios FJ, Montezano AC and Touyz RM: TRPM7, magnesium, and signaling. Int J Mol Sci 20: 1877, 2019.
16. Hsieh LY, Chiang AWT, Duong LD, Kuo CC, Dong SX, Dohil R, Kurten R, Lewis NE and Aceves SS: A unique esophageal extracellular matrix proteome alters normal fibroblast function in severe eosinophilic esophagitis. J Allergy Clin Immunol 148: 486-494, 2021.
17. Liu Y, Wu H, Sang Y, Chong W, Shang L and Li L: Research progress of exosomes in the angiogenesis of digestive system tumour. Discov Oncol 15: 33, 2024.
18. Manning G, Whyte DB, Martinez R, Hunter T and Sudarsanam S: The protein kinase complement of the human genome. Science 298: 1912-1934, 2002.
19. Fischer EH: Cellular regulation by protein phosphorylation. Biochem Biophys Res Commun 430: 865-867, 2013.
20. Sreelatha A, Kinch LN and Tagliabracci VS: The secretory pathway kinases. Biochim Biophys Acta 1854: 1687-1693, 2015.
21. Du S, Zhu C, Ren X, Chen X, Cui X and Guan S: Regulation of secretory pathway kinase or kinase-like proteins in human cancers. Front Immunol 14: 942849, 2023.
22. Hareza A, Bakun M, Swiderska B, Dudkiewicz M, Koscielny A, Bajur A, Jaworski J, Dadlez M and Pawlowski K: Phosphoproteomic insights into processes influenced by the kinase-like protein DIA1/C3orf58. PeerJ 6: e4599, 2018.
23. Tennant-Eyles AJ, Moffitt H, Whitehouse CA and Roberts RG: Characterisation of the FAM69 family of cysteine-rich endoplasmic reticulum proteins. Biochem Biophys Res Commun 406: 471-477, 2011.
24. Ashery-Padan R, Alvarez-Bolado G, Klamt B, Gessler M and Gruss P: Fjx1, the murine homologue of the Drosophila four-jointed gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain. Mech Dev 80: 213-217, 1999.
25. Yoshida-Moriguchi T, Yu L, Stalnaker SH, Davis S, Kunz S, Madson M, Oldstone MB, Schachter H, Wells L and Campbell KP: O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. Science 327: 88-92, 2010.
26. Tagliabracci VS, Wiley SE, Guo X, Kinch LN, Durrant E, Wen J, Xiao J, Cui J, Nguyen KB, Engel JL, *et al*: A single kinase generates the majority of the secreted phosphoproteome. Cell 161: 1619-1632, 2015.
27. Bordoli MR, Yum J, Breitkopf SB, Thon JN, Italiano JE Jr, Xiao J, Worby C, Wong SK, Lin G, Edenius M, *et al*: A secreted tyrosine kinase acts in the extracellular environment. Cell 158: 1033-1044, 2014.
28. Piperigkou Z, Mangani S, Koletsis NE, Koutsakis C, Mastronikolis NS, Franchi M and Karamanos NK: Principal mechanisms of extracellular matrix-mediated cell-cell communication in physiological and tumor microenvironments. FEBS J 293: 26-41, 2026.
29. Dederer V and Lemberg MK: Transmembrane dislocases: A second chance for protein targeting. Trends Cell Biol 31: 898-911, 2021.
30. Pompa A, De Marchis F, Pallotta MT, Benitez-Alfonso Y, Jones A, Schipper K, Moreau K, Zarsky V, Di Sansebastiano GP and Bellucci M: Unconventional transport routes of soluble and membrane proteins and their role in developmental biology. Int J Mol Sci 18: 703, 2017.
31. Zhang R, Ren Y, Ju Y, Zhang Y, Zhang Y and Wang Y: FAM20C: A key protein kinase in multiple diseases. Genes Dis 12: 101179, 2025.
32. Zubair M, Hamzah R, Griffin R and Ali N: Identification and functional characterization of multiple inositol polyphosphate phosphatase1 (Minpp1) isoform-2 in exosomes with potential to modulate tumor microenvironment. PLoS One 17: e0264451, 2022.

33. Qin Z, Wang P, Li X, Zhang S, Tian M, Dai Y and Fu L: Systematic network-based discovery of a Fam20C inhibitor (FL-1607) with apoptosis modulation in triple-negative breast cancer. Mol Biosyst 12: 2108-2118, 2016.
34. Gerson-Gurwitz A, Worby CA, Lee KY, Khaliullin R, Bouffard J, Cheerambathur D, Oegema K, Cram EJ, Dixon JE and Desai A: Ancestral roles of the Fam20C family of secreted protein kinases revealed in C. elegans. J Cell Biol 218: 3795-3811, 2019.
35. Wang X, Wang S, Li C, Gao T, Liu Y, Rangiani A, Sun Y, Hao J, George A, Lu Y, *et al*: Inactivation of a novel FGF23 regulator, FAM20C, leads to hypophosphatemic rickets in mice. PLoS Genet 8: e1002708, 2012.
36. Cui J, Zhu Q, Zhang H, Cianfrocco MA, Leschziner AE, Dixon JE and Xiao J: Structure of Fam20A reveals a pseudokinase featuring a unique disulfide pattern and inverted ATP-binding. Elife 6: e23990, 2017.
37. Vogel P, Hansen GM, Read RW, Vance RB, Thiel M, Liu J, Wronski TJ, Smith DD, Jeter-Jones S and Brommage R: Amelogenesis imperfecta and other biomineralization defects in Fam20a and Fam20c null mice. Vet Pathol 49: 998-1017, 2012.
38. Ma P, Yan W, Tian Y, Wang J, Feng JQ, Qin C, Cheng YS and Wang X: Inactivation of Fam20B in joint cartilage leads to chondrosarcoma and postnatal ossification defects. Sci Rep 6: 29814, 2016.
39. Adams JC: Passing the post: Roles of posttranslational modifications in the form and function of extracellular matrix. Am J Physiol Cell Physiol 324: C1179-C1197, 2023.
40. Liu X, Jiang L, Zhang W, Zhang J, Luan X, Zhan Y, Wang T, Da J, Liu L, Zhang S, *et al*: Fam20c regulates the calpain proteolysis system through phosphorylating Calpasatatin to maintain cell homeostasis. J Transl Med 21: 417, 2023.
41. di Paola G, Albano F, Zoppoli P, Bravoco D, D'Amore T, Amendola E, Lucci V, Roberto L, Brancaccio M, Salerno P, *et al*: Hormonally upregulated neu tumor-associated kinase (HUNK) modulates gastric cancer progression through the regulation of cell homeostasis. Cell Commun Signal 23: 320, 2025.
42. Nishiguchi G, Mascibroda LG, Young SM, Caine EA, Abdelhamed S, Kooijman JJ, Miller DJ, Das S, McGowan K, Mayasundari A, *et al*: Selective CK1α degraders exert antiproliferative activity against a broad range of human cancer cell lines. Nat Commun 15: 482, 2024.
43. Jiang S, Zhang M, Sun J and Yang X: Casein kinase 1alpha: Biological mechanisms and theranostic potential. Cell Commun Signal 16: 23, 2018.
44. Hallal S, Tuzesi A, Grau GE, Buckland ME and Alexander KL: Understanding the extracellular vesicle surface for clinical molecular biology. J Extracell Vesicles 11: e12260, 2022.
45. Tegge AN, Sharp N and Murali TM: Xtalk: A path-based approach for identifying crosstalk between signaling pathways. Bioinformatics 32: 242-251, 2016.
46. Jiang L, Liu X, Liu L, Su L, Lu Z, Zhang H, Guo Y, Zhang W, Zhang S, Xu W, *et al*: Knocking out FAM20C in pre-osteoblasts leads to up-regulation of osteoclast differentiation to affect long bone development. Gene 915: 148396, 2024.
47. Schytte GN, Christensen B, Bregenov I, Kjoge K, Scavenius C, Petersen SV, Enghild JJ and Sorensen ES: FAM20C phosphorylation of the RGDSVVYGLR motif in osteopontin inhibits interaction with the alphavbeta3 integrin. J Cell Biochem 121: 4809-4818, 2020.
48. Zhang H, Lu Y, Kramer PR, Benson MD, Cheng YL and Qin C: Intracranial calcification in Fam20c-deficient mice recapitulates human Raine syndrome. Neurosci Lett 802: 137176, 2023.
49. Govitvattana N, Kaku M, Ohyama Y, Jaha H, Lin IP, Mochida H, Pavasant P and Mochida Y: Molecular cloning of mouse homologue of enamel protein C4orf26 and its phosphorylation by FAM20C. Calcif Tissue Int 109: 445-454, 2021.
50. Ramos-Molina B and Lindberg I: Phosphorylation and alternative splicing of 7B2 reduce prohormone convertase 2 activation. Mol Endocrinol 29: 756-764, 2015.
51. Worby CA, Mayfield JE, Pollak AJ, Dixon JE and Banerjee S: The ABCs of the atypical Fam20 secretory pathway kinases. J Biol Chem 296: 100267, 2021.
52. Zhang H, Zhu Q, Cui J, Wang Y, Chen MJ, Guo X, Tagliabracci VS, Dixon JE and Xiao J: Structure and evolution of the Fam20 kinases. Nat Commun 9: 1218, 2018.
53. Cui J, Xiao J, Tagliabracci VS, Wen J, Rahdar M and Dixon JE: A secretory kinase complex regulates extracellular protein phosphorylation. Elife 4: e06120, 2015.
54. Siddiqui MF, Li J, Wang S, Zhang H, Qin C and Lu Y: FAM20A is a golgi-localized Type II transmembrane protein. Sci Rep 14: 6518, 2024.
55. Simancas Escorcia V, Guillou C, Abbad L, Derrien L, Rodrigues Rezende Costa C, Cannaya V, Benassarou M, Chatziantoniou C, Berdal A, Acevedo AC, *et al*: Pathogenesis of Enamel-renal syndrome associated gingival fibromatosis: A proteomic approach. Front Endocrinol (Lausanne) 12: 752568, 2021.
56. Bres EE and Faissner A: Low density receptor-related protein 1 interactions with the extracellular matrix: More than meets the eye. Front Cell Dev Biol 7: 31, 2019.
57. Wu Y, Wang H and Liu C: From biomineralization to tumorogenesis-the expanding insight of the physiological and pathological roles of Fam20C. Biosci Rep 41: BSR20210040, 2021.
58. Liu S, Zhang Z, Jiang L, Zhang M, Zhang C and Shen L: Claudin-18.2 mediated interaction of gastric cancer cells and Cancer-associated fibroblasts drives tumor progression. Cell Commun Signal 22: 27, 2024.
59. Popova NV and Jucker M: The functional role of extracellular matrix proteins in cancer. Cancers (Basel) 14: 238, 2022.
60. Raposo G and Stoorvogel W: Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol 200: 373-383, 2013.
61. Colombo M, Raposo G and Thery C: Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 30: 255-289, 2014.
62. Koniusz S, Andrzejewska A, Muraca M, Srivastava AK, Janowski M and Lukomska B: Extracellular vesicles in physiology, pathology, and therapy of the immune and central nervous system, with focus on extracellular vesicles derived from mesenchymal stem cells as therapeutic tools. Front Cell Neurosci 10: 109, 2016.
63. Maacha S, Bhat AA, Jimenez L, Raza A, Haris M, Uddin S and Grivel JC: Extracellular vesicles-mediated intercellular communication: Roles in the tumor microenvironment and anti-cancer drug resistance. Mol Cancer 18: 55, 2019.
64. Leidal AM and Debnath J: Unraveling the mechanisms that specify molecules for secretion in extracellular vesicles. Methods 177: 15-26, 2020.
65. Sherman CD, Lodha S and Sahoo S: EV cargo sorting in therapeutic development for cardiovascular disease. Cells 10: 1500, 2021.
66. Salomon C, Das S, Erdbrugger U, Kalluri R, Kiang Lim S, Olefsky JM, Rice GE, Sahoo S, Andy Tao W, Vader P, *et al*: Extracellular vesicles and their emerging roles as cellular messengers in endocrinology: An endocrine society scientific statement. Endocr Rev 43: 441-468, 2022.
67. Amarasinghe I, Phillips W, Hill AF, Cheng L, Helbig KJ, Willms E and Monson EA: Cellular communication through extracellular vesicles and lipid droplets. J Extracell Biol 2: e77, 2023.
68. Fyfe J, Casari I, Manfredi M and Falasca M: Role of lipid signalling in extracellular vesicles-mediated cell-to-cell communication. Cytokine Growth Factor Rev 73: 20-26, 2023.
69. Liu YJ and Wang C: A review of the regulatory mechanisms of extracellular vesicles-mediated intercellular communication. Cell Commun Signal 21: 77, 2023.
70. Bryja A, Zadka L, Farzaneh M, Zehtabi M, Ghasemian M, Dyszkiewicz-Konwinska M, Mozdziak P, Zabel M, Podhorska-Okolow M, Dziegiel P, *et al*: Small extracellular vesicles-A host for advanced bioengineering and 'Trojan Horse' of non-coding RNAs. Life Sci 332: 122126, 2023.
71. Lehmann TP, Golik M, Olejnik J, Lukaszewska M, Markowska D, Drozdzynska M, Kotecki A, Glowacki M and Jagodzinski PP: Potential applications of using tissue-specific EVs in targeted therapy and vaccinology. Biomed Pharmacother 166: 115308, 2023.
72. Xie J, Haesebrouck F, Van Hoecke L and Vandenbroucke RE: Bacterial extracellular vesicles: An emerging avenue to tackle diseases. Trends Microbiol 31: 1206-1224, 2023.
73. Song Y, Wang M, Tong H, Tan Y, Hu X, Wang K and Wan X: Plasma exosomes from endometrial cancer patients contain LGALS3BP to promote endometrial cancer progression. Oncogene 40: 633-646, 2021.
74. Li N, Lin G, Zhang Y, Zhang Q and Zhang H: Exosome-related protein CRABP2 is upregulated in ovarian carcinoma and enhances cell proliferation. Discov Oncol 13: 33, 2022.
75. Nagoya A, Sada R, Kimura H, Yamamoto H, Morishita K, Miyoshi E, Morii E, Shintani Y and Kikuchi A: CKAP4 is a potential exosomal biomarker and therapeutic target for lung cancer. Transl Lung Cancer Res 12: 408-426, 2023.
76. Niu L, Song X, Wang N, Xue L, Song X and Xie L: Tumor-derived exosomal proteins as diagnostic biomarkers in non-small cell lung cancer. Cancer Sci 110: 433-442, 2019.

77. Yin Y, Liu B, Cao Y, Yao S, Liu Y, Jin G, Qin Y, Chen Y, Cui K, Zhou L, *et al*: Colorectal Cancer-derived small extracellular vesicles promote tumor immune evasion by upregulating PD-L1 expression in tumor-associated macrophages. Adv Sci (Weinh) 9: 2102620, 2022.
78. Tosetti F, Vene R, Camodeca C, Nuti E, Rossello A, D'Arrigo C, Galante D, Ferrari N, Poggi A and Zocchi MR: Specific ADAM10 inhibitors localize in exosome-like vesicles released by Hodgkin lymphoma and stromal cells and prevent sheddase activity carried to bystander cells. Oncoimmunology 7: e1421889, 2018.
79. Morini M, Raggi F, Bartolucci M, Petretto A, Ardito M, Rossi C, Segalerba D, Garaventa A, Eva A, Cangelosi D and Bosco MC: Plasma-derived exosome proteins as novel diagnostic and prognostic biomarkers in neuroblastoma patients. Cells 12: 2516, 2023.
80. Bao M, Huang Y, Lang Z, Zhao H, Saito Y, Nagano T, Kawagoe I, Divisi D, Hu X and Jiang G: Proteomic analysis of plasma exosomes in patients with non-small cell lung cancer. Transl Lung Cancer Res 11: 1434-1452, 2022.
81. Lu CH, Wei ST, Liu JJ, Chang YJ, Lin YF, Yu CS and Chang SL: Recognition of a novel gene signature for human glioblastoma. Int J Mol Sci 23: 4157, 2022.
82. Ohyama Y, Lin JH, Govitvattana N, Lin IP, Venkitapathi S, Alamoudi A, Husein D, An C, Hotta H, Kaku M and Mochida Y: FAM20A binds to and regulates FAM20C localization. Sci Rep 6: 27784, 2016.
83. Gong B, Liang Y, Zhang Q, Li H, Xiao J, Wang L, Chen H, Yang W, Wang X, Wang Y and He Z: Epigenetic and transcriptional activation of the secretory kinase FAM20C as an oncogene in glioma. J Genet Genomics 50: 422-433, 2023.
84. Du S, Guan S, Zhu C, Guo Q, Cao J, Guan G, Cheng W, Cheng P and Wu A: Secretory pathway kinase FAM20C, a marker for glioma invasion and malignancy, predicts poor prognosis of glioma. Onco Targets Ther 13: 11755-11768, 2020.
85. Guo Q, Guan GF, Cheng W, Zou CY, Zhu C, Cheng P and Wu AH: Integrated profiling identifies caveolae-associated protein 1 as a prognostic biomarker of malignancy in glioblastoma patients. CNS Neurosci Ther 25: 343-354, 2019.
86. Wu L, Zhou Y, Guan Y, Xiao R, Cai J, Chen W, Zheng M, Sun K, Chen C, Huang G, *et al*: Seven genes associated with lymphatic metastasis in thyroid cancer that is linked to tumor immune cell infiltration. Front Oncol 11: 756246, 2021.
87. Chai SJ, Ahmad Zabidi MM, Gan SP, Rajadurai P, Lim PVH, Ng CC, Yap LF, Teo SH, Lim KP, Patel V and Cheong SC: An oncogenic role for Four-jointed Box 1 (FJX1) in nasopharyngeal carcinoma. Dis Markers 2019: 3857853, 2019.
88. Chai SJ, Yap YY, Foo YC, Yap LF, Ponniah S, Teo SH, Cheong SC, Patel V and Lim KP: Identification of Four-Jointed Box 1 (FJX1)-Specific Peptides for Immunotherapy of Nasopharyngeal Carcinoma. PLoS One 10: e0130464, 2015.
89. Chai SJ, Fong SCY, Gan CP, Pua KC, Lim PVH, Lau SH, Zain RB, Abraham T, Ismail SM, Abdul Rahman ZA, *et al*: In vitro evaluation of dual-antigenic PV1 peptide vaccine in head and neck cancer patients. Hum Vaccin Immunother 15: 167-178, 2019.
90. Wang C, Zainal NS, Chai SJ, Dickie J, Gan CP, Zulaziz N, Lye BKW, Sutavani RV, Ottensmeier CH, King EV, *et al*: DNA vaccines targeting novel cancer-associated antigens frequently expressed in head and neck cancer enhance the efficacy of checkpoint inhibitor. Front Immunol 12: 763086, 2021.
91. Jin YJ, Aycheh HM, Han S, Chamberlin J, Shin J, Byun S and Lee Y: Differential alternative splicing between hepatocellular carcinoma with normal and elevated serum alpha-fetoprotein. BMC Med Genomics 13 (Suppl 11): S194, 2020.
92. Tibaldi E, Brocca A, Sticca A, Gola E, Pizzi M, Bordin L, Pagano MA, Mazzorana M, Dona G, Violi P, *et al*: Fam20C-mediated phosphorylation of osteopontin is critical for its secretion but dispensable for its action as a cytokine in the activation of hepatic stellate cells in liver fibrogenesis. FASEB J 34: 1122-1135, 2020.
93. Liu X, Zhan Y, Xu W, Liu X, Geng Y, Liu L, Da J, Wang J, Zhang X, Jin H, *et al*: Prognostic and immunological role of Fam20C in pan-cancer. Biosci Rep 41: BSR20201920, 2021.
94. Zhang H, Zhang Z, Wang Q, Wang L, Yang Z, Geng Z, Wang Y, Li J and Zuo L: FJX1 overexpression is associated with poor prognosis and promotes gastric cancer proliferation via the PI3K/AKT signaling pathway. Nan Fang Yi Ke Da Xue Xue Bao 43: 975-984, 2023 (In Chinese).
95. Wang WJ, Li HT, Yu JP, Han XP, Xu ZP, Li YM, Jiao ZY and Liu HB: A competing endogenous RNA network reveals novel potential lncRNA, miRNA, and mRNA biomarkers in the prognosis of human colon adenocarcinoma. J Surg Res 235: 22-33, 2019.
96. Liu L, Huang Y, Li Y, Wang Q, Hao Y, Liu L, Yao X, Yao X, Wei Y, Sun X and Guo Y: FJX1 as a candidate diagnostic and prognostic serum biomarker for colorectal cancer. Clin Transl Oncol 24: 1964-1974, 2022.
97. Al-Greene NT, Means AL, Lu P, Jiang A, Schmidt CR, Chakravarthy AB, Merchant NB, Washington MK, Zhang B, Shyr Y, *et al*: Four jointed box 1 promotes angiogenesis and is associated with poor patient survival in colorectal carcinoma. PLoS One 8: e69660, 2013.
98. Liu F, Wu R, Guan L and Tang X: Knockdown of PVT1 suppresses colorectal cancer progression by regulating MiR-106b-5p/FJX1 axis. Cancer Manag Res 12: 8773-8785, 2020.
99. Cheng T, Zhu X, Lu J and Teng X: MiR-532-3p suppresses cell proliferation, migration and invasion of colon adenocarcinoma via targeting FJX1. Pathol Res Pract 232: 153835, 2022.
100. Dang W and Zhu Z: MicroRNA-1249 targets four-jointed box kinase 1 and reduces cell proliferation, migration and invasion of colon adenocarcinoma. J Gene Med 22: e3183, 2020.
101. Zou J, Zhang H, Huang Y, Xu W, Huang Y, Zuo S, Li Z and Zhou H: Multi-omics analysis of the tumor microenvironment in liver metastasis of colorectal cancer identified FJX1 as a novel biomarker. Front Genet 13: 960954, 2022.
102. Heuvelings DJI, Wintjens A, Moonen L, Engelen SME, de Hingh I, Valkenburg-van Iersel LB, den Dulk M, Beckervordersandforth J, Thijssen SGM, Leunissen DJG, *et al*: Predictive genetic biomarkers for the development of peritoneal metastases in colorectal cancer. Int J Mol Sci 24: 975-984, 2023.
103. Jiang C, Liu Y, Wen S, Xu C and Gu L: In silico development and clinical validation of novel 8 gene signature based on lipid metabolism related genes in colon adenocarcinoma. Pharmacol Res 169: 105644, 2021.
104. Zhao R, Fu L, Yuan Z, Liu Y, Zhang K, Chen Y, Wang L, Sun D, Chen L, Liu B and Zhang L: Discovery of a novel small-molecule inhibitor of Fam20C that induces apoptosis and inhibits migration in triple negative breast cancer. Eur J Med Chem 210: 113088, 2021.
105. Zuo H, Yang D and Wan Y: Fam20C regulates bone resorption and breast cancer bone metastasis through osteopontin and BMP4. Cancer Res 81: 5242-5254, 2021.
106. Trendowski MR, Wheeler HE, El-Charif O, Feldman DR, Hamilton RJ, Vaughn DJ, Fung C, Kollmannsberger C, Einhorn LH, Travis LB and Dolan ME: Clinical and Genome-wide analysis of multiple severe Cisplatin-induced neurotoxicities in adult-onset cancer survivors. Clin Cancer Res 26: 6550-6558, 2020.
107. Wei S, Zhang J, Shi R, Yu Z, Chen X and Wang H: Identification of an integrated kinase-related prognostic gene signature associated with tumor immune microenvironment in human uterine corpus endometrial carcinoma. Front Oncol 12: 944000, 2022.
108. Micevic G, Theodosakis N and Bosenberg M: Aberrant DNA methylation in melanoma: Biomarker and therapeutic opportunities. Clin Epigenetics 9: 34, 2017.
109. Pradhan D, Jour G, Milton D, Vasudevaraja V, Tetzlaff MT, Nagarajan P, Curry JL, Ivan D, Long L, Ding Y, *et al*: Aberrant DNA methylation predicts Melanoma-specific survival in patients with acral melanoma. Cancers (Basel) 11: 2031, 2019.
110. Carreira S, Goodall J, Denat L, Rodriguez M, Nuciforo P, Hoek KS, Testori A, Larue L and Goding CR: Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. Genes Dev 20: 3426-3439, 2006.
111. Wan N, Yang W, Cheng H and Wang J: FOXD3-AS1 contributes to the progression of melanoma via miR-127-3p/FJX1 axis. Cancer Biother Radiopharm 35: 596-604, 2020.
112. Tagliabracci VS, Engel JL, Wen J, Wiley SE, Worby CA, Kinch LN, Xiao J, Grishin NV and Dixon JE: Secreted kinase phosphorylates extracellular proteins that regulate biomineralization. Science 336: 1150-1153, 2012.
113. Zhang J, Zhu Q, Wang X, Yu J, Chen X, Wang J, Wang X, Xiao J, Wang CC and Wang L: Secretory kinase Fam20C tunes endoplasmic reticulum redox state via phosphorylation of Ero1α. EMBO J 37: e98699, 2018.
114. Chen X, Zhang J, Liu P, Wei Y, Wang X, Xiao J, Wang CC and Wang L: Proteolytic processing of secretory pathway kinase Fam20C by site-1 protease promotes biomineralization. Proc Natl Acad Sci USA 118: e2100133118, 2021.
115. Searles SC, Chen WS, Yee JD, Lee P, Lee CK, Caron C, Neto F, Matei I, Lyden D and Bui JD: MAP kinase kinase 1 (MEK1) within extracellular vesicles inhibits tumour growth by promoting anti-tumour immunity. J Extracell Vesicles 13: e12515, 2024.

116. Peng Y, Wang Y, Zhou C, Mei W and Zeng C: PI3K/Akt/mTOR pathway and its role in cancer therapeutics: Are we making headway? Front Oncol 12: 819128, 2022.
117. Zhang T, Zhu X, Wu H, Jiang K, Zhao G, Shaukat A, Deng G and Qiu C: Targeting the ROS/PI3K/AKT/HIF-1α/HK2 axis of breast cancer cells: Combined administration of Polydatin and 2-Deoxy-d-glucose. J Cell Mol Med 23: 3711-3723, 2019.
118. Buljan M, Ciuffa R, van Drogen A, Vichalkovski A, Mehnert M, Rosenberger G, Lee S, Varjosalo M, Pernas LE, Spegg V, *et al*: Kinase interaction network expands functional and disease roles of human kinases. Mol Cell 79: 504-520, 2020.
119. Xu C, Zhang M, Bian L, Li Y, Yao Y and Li D: N-glycosylated SGK196 suppresses the metastasis of basal-like breast cancer cells. Oncogenesis 9: 4, 2020.
120. Wang RG, Zhang D, Zhao CH, Wang QL, Qu H and He QS: FKBP10 functioned as a cancer-promoting factor mediates cell proliferation, invasion, and migration via regulating PI3K signaling pathway in stomach adenocarcinoma. Kaohsiung J Med Sci 36: 311-317, 2020.
121. Chen B, Diallo MT, Ma Y, Yu W, Yao Q, Gao S, Yu Y, Sun Q, Wang Y, Ren J and Wang D: Fam198b as a novel biomarker for gastric cancer and a potential therapeutic target to prevent tumor cell proliferation dysregulation. Transl Oncol 39: 101824, 2024.
122. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y and Hu LL: ERK/MAPK signalling pathway and tumorigenesis. Exp Ther Med 19: 1997-2007, 2020.
123. Hsu CY, Chang GC, Chen YJ, Hsu YC, Hsiao YJ, Su KY, Chen HY, Lin CY, Chen JS, Chen YJ, *et al*: FAM198B is associated with prolonged survival and inhibits metastasis in lung adenocarcinoma via blockage of ERK-mediated MMP-1 expression. Clin Cancer Res 24: 916-926, 2018.
124. Guo Q, Wu Y, Guo X, Cao L, Xu F, Zhao H, Zhu J, Wen H, Ju X and Wu X: The RNA-binding protein CELF2 inhibits ovarian cancer progression by stabilizing FAM198B. Mol Ther Nucleic Acids 23: 169-184, 2021.
125. Tang P, Tao L, Yuan C, Zhang L and Xiu D: Serum derived exosomes from pancreatic cancer patients promoted metastasis: An iTRAQ-based proteomic analysis. Onco Targets Ther 12: 9329-9339, 2019.
126. Marin AM, Batista M, Korte de Azevedo AL, Bombardelli Gomig TH, Soares Caldeira Brant R, Chammas R, Uno M, Dias Araujo D, Zanette DL and Nobrega Aoki M: Screening of Exosome-derived proteins and their potential as biomarkers in diagnostic and prognostic for pancreatic cancer. Int J Mol Sci 24: 12604, 2023.
127. Kimura H, Yamamoto H, Harada T, Fumoto K, Osugi Y, Sada R, Maehara N, Hikita H, Mori S, Eguchi H, *et al*: CKAP4, a DKK1 receptor, is a biomarker in exosomes derived from pancreatic cancer and a molecular target for therapy. Clin Cancer Res 25: 1936-1947, 2019.
128. Gai X, Tang B, Liu F, Wu Y, Wang F, Jing Y, Huang F, Jin D, Wang L and Zhang H: mTOR/miR-145-regulated exosomal GOLM1 promotes hepatocellular carcinoma through augmented GSK-3β/MMPs. J Genet Genomics 46: 235-245, 2019.
129. Todorova VK, Byrum SD, Mackintosh SG, Jamshidi-Parsian A, Gies AJ, Washam CL, Jenkins SV, Spiva T, Bowman E, Reyna NS, *et al*: Exosomal MicroRNA and protein profiles of hepatitis B Virus-related hepatocellular carcinoma cells. Int J Mol Sci 24: 13098, 2023.
130. Lin Y, Huang K, Cai Z, Chen Y, Feng L, Gao Y, Zheng W, Fan X, Qiu G, Zhuang J and Feng S: A Novel Exosome-relevant molecular classification uncovers distinct immune escape mechanisms and genomic alterations in gastric cancer. Front Pharmacol 13: 884090, 2022.
131. Ganig N, Baenke F, Thepkaysone ML, Lin K, Rao VS, Wong FC, Polster H, Schneider M, Helm D, Pecqueux M, *et al*: Proteomic Analyses of Fibroblast- and Serum-Derived Exosomes Identify QSOX1 as a Marker for Non-invasive detection of colorectal cancer. Cancers (Basel) 13: 1351, 2021.
132. Yao J, Huang X and Ren J: In situ determination of secretory kinase Fam20C from living cells using fluorescence correlation spectroscopy. Talanta 232: 122473, 2021.
133. Cesaro L, Noventa F, De Los Angeles Cordero T, Molon B, Bosello Travain V, Aspromonte MC and Salvi M: Comprehensive analysis of the putative substratome of FAM20C, the master serine kinase of the secretory pathway. Biomolecules 15: 1582, 2025.
134. Noventa F and Salvi M: Protein kinase FAM20C-when subcellular localization matters. FEBS Lett 599: 3281-3283, 2025.
135. Brunner AD, Thielert M, Vasilopoulou C, Ammar C, Coscia F, Mund A, Hoerning OB, Bache N, Apalategui A, Lubeck M, *et al*: Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. Mol Syst Biol 18: e10798, 2022.