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REVIEW

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Targeting kinases that regulate programmed cell death: a new therapeutic strategy for breast cancer

Yun Hong[1,2], Jun He[3], Dan Deng[1,2], Qinyue Liu[1,2], Xuyu Zu[1,4*] and Yingying Shen[1,4*]

Abstract

Breast cancer is one of the most prevalent malignant tumors among women and ranks as the second leading cause of cancer-related deaths in females, primarily due to delays in diagnosis and shortcomings in treatment strategies. Consequently, there is a pressing need to identify reliable therapeutic targets and strategies. In recent years, the identification of effective biomarkers-particularly novel molecular therapeutic targets-has become a focal point in breast cancer research, aimed at predicting disease aggressiveness and monitoring treatment responses. Simultaneously, advancements in understanding the molecular mechanisms underlying cellular programmed death have opened new avenues for targeting kinase-regulated programmed cell death as a viable therapeutic strategy. This review summarizes the latest research progress regarding kinase-regulated programmed death (including apoptosis, pyroptosis, autophagy, necroptosis, and ferroptosis) in breast cancer treatment. It covers the key kinases involved in this mechanism, their roles in the onset and progression of breast cancer, and strategies for modulating these kinases through pharmacological interventions.

Keywords Apoptosis, Pyroptosis, Autophagy, Necroptosis, Ferroptosis, Kinase, Breast cancer

Introduction

Human protein kinases (PKs) represent a substantial enzyme family known as the human kinome, comprising approximately 1.7% of all human genes [1]. Members of the protein kinase superfamily are primarily classified into two major categories based on their phosphorylation targets: serine/threonine kinases, which phosphorylate serine or threonine residues, and tyrosine kinases (TKs), which specifically phosphorylate tyrosine residues [2]. A third category includes dual-specificity protein kinases, which are capable of phosphorylating both tyrosine and serine/threonine residues. Among these, tyrosine kinases are particularly well characterized. They can be further subdivided based on their cellular localization into receptor tyrosine kinases (RTKs), which possess a ligand-binding extracellular domain and a catalytic intracellular kinase domain, and non-receptor tyrosine kinases

*Correspondence:
Xuyu Zu
zuxuyu@ucs.edu.cn
Yingying Shen
shenyingying1113@usc.edu.cn
[1]Cancer Research Institute, The First Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang, Hunan 421001, PR China
[2]Department of Clinical Laboratory Medicine, Institution of Microbiology and Infectious Diseases, Hunan Province Clinical Research Center for Accurate Diagnosis and Treatment of High-Incidence Sexually Transmitted Diseases, The First Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang, Hunan, China
[3]Department of Spine Surgery, The Nanhua Affiliated Hospital, Hengyang Medical School, University of South China, Hengyang 421002, China
[4]Hunan Provincial Clinical Medical Research Center for Drug Evaluation of major chronic diseases, Hengyang, China

(nRTKs), which lack a transmembrane domain and are found in the cytosol, nucleus, or inner surface of the plasma membrane. In terms of catalytic domain sequence comparison, protein kinases are categorized into eight main families: AGC (A, G, and C kinases), CAMK (Ca^{2+}/calmodulin-dependent kinases), CK1 (casein kinase 1), CMGC (cyclin-dependent kinases), MAP kinases, glycogen synthase kinase-3, CLK (cdc2-like kinases), RGC (receptor guanylate cyclases), STE (homologs of yeast sterile 7, 11, and 20 kinases), TK (tyrosine kinases), and TKL (tyrosine kinase-like proteins) [3]. This classification also encompasses many atypical kinases that lack sequence similarity to conserved eukaryotic protein kinase catalytic domains. Together with phosphatases, protein kinases are part of the phosphotransferase group, which includes enzymes that catalyze the reversible transfer of phosphate groups between substrates. Surface receptor kinases are typically activated by their ligands or other stimuli, often necessitating the activation of non-receptor kinase cascades that facilitate signal transduction across the cytoplasm and nucleus. Thus, gene activation and cellular responses are coordinated through the phosphorylation states of protein kinases, phosphatases, and their substrate proteins. The phosphorylation process can induce conformational changes in substrate proteins, leading to either the disruption or formation of protein-protein interaction surfaces [4]. These conformational changes ultimately dictate the protein's activity, cellular localization, and interactions with other proteins. Although protein kinase genes constitute only about 2% of the genomes of most eukaryotes, they have the capacity to phosphorylate over 30% of cellular proteins, underscoring their vital role in regulating post-translational modifications. Since the characterization of protein kinase activity in the 1950s, these enzymes have been demonstrated to regulate molecular pathways that are critical for a wide range of cellular processes, including proliferation, metabolism, migration, survival, and apoptosis [5]. The overexpression of kinase activity is frequently observed in human cancers, resulting in abnormal cell proliferation and the inhibition of both cell differentiation and apoptosis. This dysregulated kinase activity generally facilitates the growth and survival of tumor cells by activating downstream signaling pathways, which in turn drives the initiation and progression of cancer [6, 7]. The pathogenesis of breast cancer is a complex, multi-layered process influenced by genetic, environmental, hormonal, and immune factors [8, 9]. Key contributors to breast cancer development include genetic mutations, DNA damage, and the dysregulation of cell signaling pathways. Research has identified several critical signaling pathways, such as PI3K/Akt, MAPK, and STAT, which are closely linked to the onset and progression of breast cancer [9]. Consequently, the search for new therapeutic strategies to intervene in signaling pathways has become a central focus of current research. Among these strategies, kinases play a crucial role as enzymes that regulate cell survival and apoptosis through phosphorylation [10]. However, abnormal activation of kinases can sometimes inhibit programmed cell death, thereby creating a conducive environment for cancer development [10]. Consequently, modulating kinase activity-particularly within programmed cell death pathways-may represent a promising therapeutic approach for breast cancer. Numerous studies have demonstrated that the expression levels of tyrosine kinases in breast cancer tissues are closely correlated with patient prognosis [11]. High expression of tyrosine kinases is generally associated with increased malignancy and metastatic potential. Thus, the expression levels of tyrosine kinases not only serve as markers for breast cancer initiation and progression but may also become significant indicators for predicting disease progression and treatment outcomes in patients [12]. In recent years, a variety of kinase inhibitors have been developed, and these drugs have been extensively studied and applied in the treatment of breast cancer [13]. Some inhibitors function by disrupting the phosphorylation activity of kinases, thereby inhibiting their signaling pathways and inducing tumor cell death. This development brings new hope for breast cancer treatment, while also raising a series of clinical and pharmacological questions that warrant further investigation. Despite extensive research on the aberrant expression and development of kinases in breast cancer, several challenges persist. First, the mechanisms of action of different kinases need further clarification across various breast cancer subtypes. Second, issues related to drug tolerance and resistance continue to challenge current treatments, necessitating additional research to uncover their underlying mechanisms (Fig. 1).

Kinases and breast cancer

Association of abnormal kinase expression with breast cancer

Alterations in the PI3K/AKT/mTOR pathway are particularly prevalent in breast cancer, with estimates indicating that up to 70% of tumors exhibit some form of genetic mutation that leads to the hyperactivation of this pathway. The PI3K/AKT/mTOR pathway is responsible for regulating numerous critical cellular functions, including metabolism, growth, survival, and proliferation. This pathway transmits various extracellular signals through phosphoinositide 3-kinase (PI3K) via a signaling cascade. In mammals, class I PI3Ks are further categorized into IA and IB subclasses based on their regulatory mechanisms. Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit and a p85 regulatory subunit. The genes *PIK3CA*, *PIK3CB*, and *PIK3CD* encode three highly

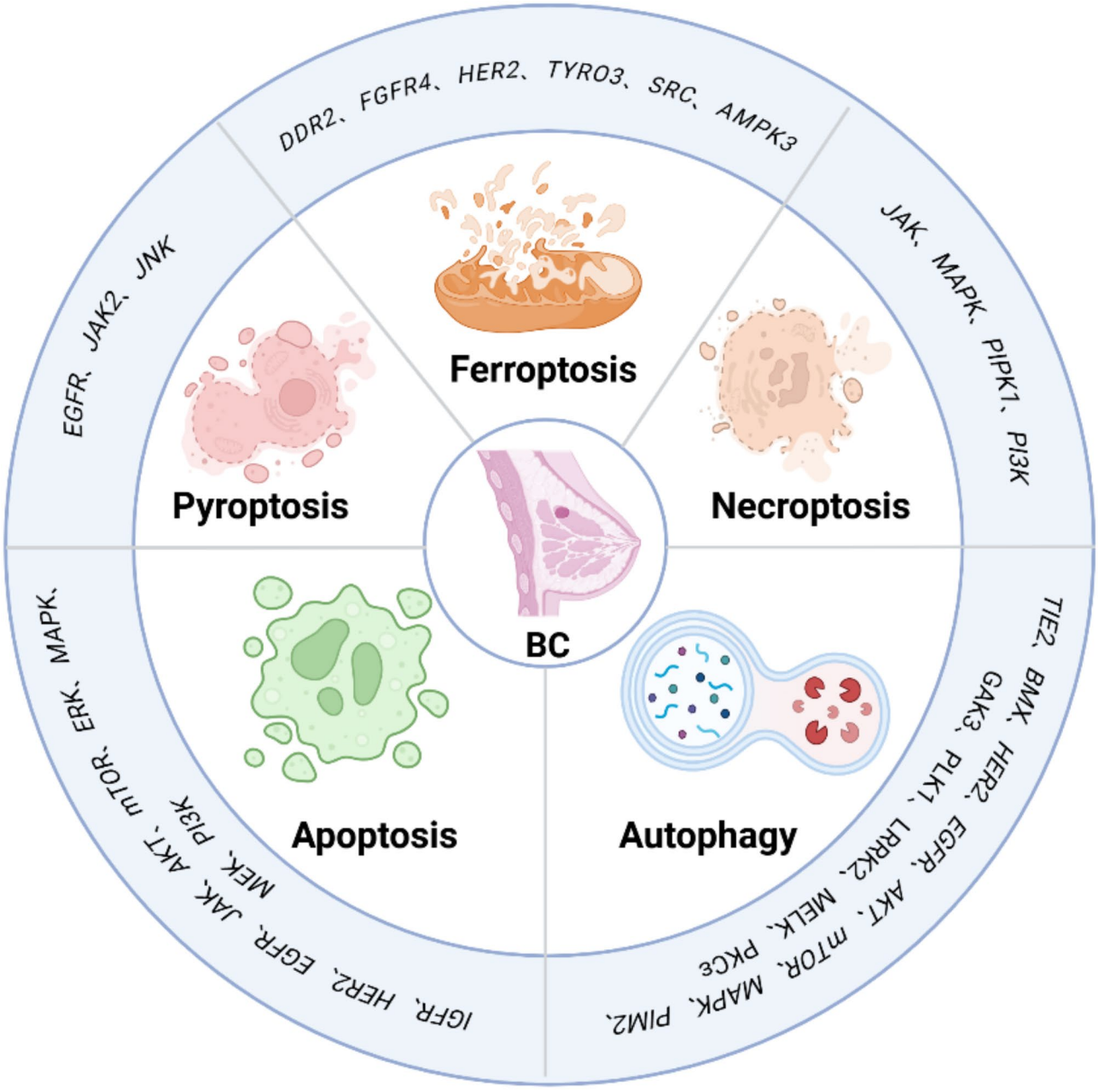

Fig. 1 Kinases play a crucial role in the regulation of programmed cell death, subsequently impacting the progression and treatment of breast cancer

homologous IA class catalytic isoforms: p110α, p110β, and p110δ. The p110α subunit, which is the catalytic component of the phosphoinositide 3-kinase α (PI3Kα) complex, is essential for normal growth and proliferation. This subunit is particularly critical for the signaling and growth of tumors driven by *PIK3CA* mutations or receptor tyrosine kinases. The downstream target of the PI3K pathway is the serine/threonine kinase AKT, which has three isoforms *(AKT1*, *AKT2*, and *AKT3*) and plays a central role in glucose metabolism, cell survival, growth, and proliferation [14]. Tyrosine kinases, a broadly expressed class of protein kinases, demonstrate significantly elevated expression levels in breast cancer tissues compared to normal tissues. This abnormal expression may involve multiple isoforms, with the epidermal growth factor receptor (EGFR) and *SRC* family kinases (SFKs) being extensively studied in the context of breast cancer. The overexpression of EGFR is linked to tumor proliferation, invasion, and metastasis. Moreover, the oncogenic overexpression or activation of *SRC*, a proto-oncogene in mammalian cells, has been shown to play a crucial role in various aspects of breast cancer progression, including tumor initiation, growth, metastasis, and drug resistance. The abnormal activation of tyrosine kinases can lead to

the dysregulation of multiple signaling pathways, thereby facilitating the development of breast cancer. Among these pathways, the PI3K/Akt and MAPK pathways are considered the most critical. Tyrosine kinases promote cell survival and proliferation through the activation of the PI3K/Akt pathway, while also regulating cell differentiation and invasion via the MAPK pathway. The aberrant activation of these signaling pathways collectively drives breast cancer progression. Additionally, signal transduction induced by RTK activation can lead to the emergence of cancer stem cell (CSC) phenotypes, which are characterized by resistance to therapeutic interventions [15, 16].

The role of apoptosis in breast cancer

Apoptosis

Apoptosis represents a significant form of programmed cell death and has increasingly emerged as a critical target in the screening of anticancer drugs. This highly regulated process serves to eliminate unnecessary or unwanted cells. Various conditions can activate apoptotic pathways, including DNA damage and uncontrolled cell proliferation [17]. Apoptosis can be initiated by both intracellular and extracellular signals, leading to two primary pathways: the intrinsic and extrinsic pathways, which are distinguished by the nature of the activating signal. The intrinsic apoptotic pathway is triggered by internal stresses such as hypoxia, cell cycle arrest, metabolic stress, endoplasmic reticulum stress, cytokine deprivation, genomic stress, and oncogenic signals [18, 19]. This pathway is tightly regulated by members of the B-cell lymphoma 2 (*BCL2*) family, which comprises both pro-apoptotic and anti-apoptotic factors that govern the release of mitochondrial pro-apoptotic factors [20]. When cellular homeostasis is disrupted by toxic agents or DNA damage, the pore-forming proteins Bax and BAK facilitate mitochondrial outer membrane permeabilization (MOMP). This permeabilization allows for the release of cytochrome c, which triggers the formation of apoptotic bodies and the activation of caspase-3, along with the release of pro-apoptotic factors Smac and Omi [21]. Additionally, MOMP induces the formation of apoptotic bodies. The apoptosome, a large complex, consists of cytochrome c, apoptotic protease activator 1 (Apaf-1), dATP, and procaspase-9 [22]. Following the formation of a dimer between caspase-9 and the adaptor protein APAF1, downstream activation of the effector caspases (caspase-3, -6, and – 7) ensues. Caspases cleave a variety of critical substrates, including actin and cadherin, activate nucleases to degrade DNA, and promote self-cleavage, thereby establishing an irreversible positive feedback loop. Interestingly, members of the BCL-2 family, specifically *BCL-2* and *BCL-XL*, play a crucial role in inhibiting apoptosis. Notably, some anti-apoptotic proteins possess two *BCL-2* homology (BH3) domains, which create a binding groove that sequesters either the activator or sensitizer *BCL-2* protein or the BAX and BAK complex [23]. This balance is further influenced by post-translational modifications and cytoplasmic localization of BCL-2 proteins [24]. In contrast, intrinsic apoptosis is triggered by cytochrome c released from mitochondria, whereas extrinsic apoptosis is mediated by extracellular death receptors. The extrinsic apoptotic pathway is activated by extracellular stimuli that engage death receptors (DRs), including members of the tumor necrosis factor receptor (TNFR) superfamily, such as TNFR1, CD95/Fas, and TNF-related apoptosis-inducing ligand receptor-1 (TRAIL) [25]. Upon activation by their respective ligands, the oligomerization of death receptors facilitates the recruitment of adaptor proteins, specifically TNFR1-associated death domain protein (TRADD) and Fas-associated death domain protein (FADD). This process subsequently activates caspases-8 and – 10, culminating in the formation of the death-induced signal transduction complex (DISC). The activation of caspase-8 and – 10 is modulated by the caspase-like protein FLIP, which is also found within the DISC. Additionally, receptor-interacting protein kinase 1 (RIPK1) serves as another regulatory protein for the activation of the extrinsic apoptotic pathway. Notably, even prior to the identification of necroptosis, it was established that RIPK1 is recruited to the DISC, where it interacts by binding to the death domains of FADD and TRADD. RIPK1 can promote both a prosurvival NFκB-mediated pathway and a prodeath pathway either through apoptosis or, in the absence of active caspase-8, through necroptosis, as described earlier [26, 27].

Kinases in apoptosis

Apoptosis is a fundamental process that occurs during development and aging to preserve cellular homeostasis. A critical factor contributing to the onset and progression of breast cancer is the disruption of apoptotic pathways. Cancer cells frequently develop the capacity to evade apoptosis, which significantly contributes to their resistance to therapeutic agents in breast cancer [28].

HER2 is a receptor tyrosine kinase situated on the cell membrane, with its extracellular domain (HER2/neu) commonly involved in signaling pathways that facilitate cell growth and differentiation [29]. The phosphorylation of HER2 can activate the PI3K/AKT/mTOR pathway. Constitutive activation of PI3K, resulting from either *PIK3CA* mutations or loss of PTEN, is linked to resistance against HER2-directed therapies and may indicate a subset of patients with poor prognoses following trastuzumab treatment. These alterations can trigger sequential signaling pathways that initiate mechanisms of therapeutic escape despite HER2 blockade. Numerous researchers

have assessed the efficacy of trastuzumab in patients participating in clinical trials across various disease contexts, focusing on alterations within the PI3K pathway. However, most studies have failed to establish a significant correlation between *PIK3CA* mutations and the therapeutic benefits of trastuzumab. For instance, the FinHER adjuvant phase III trial, which genotyped 687 patients with HER2-positive BC, found no statistically significant association between *PIK3CA* mutations and trastuzumab efficacy or survival outcomes [30]. A recent meta-analysis similarly concluded that neither PTEN loss nor PIK3CA mutations were associated with response rates to trastuzumab-based neoadjuvant therapy. Additional analyses from other trials have also not demonstrated a relationship between PIK3CA or PTEN status and the benefits of adjuvant trastuzumab. Conversely, a novel small molecule, SLLN-15, has exhibited anticancer activity by inhibiting the growth and proliferation of TNBC cells. This activity is associated with the induction of autophagy, achieved through the downregulation of Aurora kinase A (AURKA) and the inhibition of the AKT-mTOR signaling pathway. Additionally, compound 9 m, a novel mTOR inhibitor, has been shown to inhibit the phosphorylation of Akt, 4E-BP1, and S6, leading to G0/G1 phase arrest and the induction of autophagy. Furthermore, HER3 acts as a vital partner to HER2 in tumorigenesis, enhancing processes such as cell proliferation, transcription, migration, and the inhibition of apoptosis [31, 32].

The type I insulin-like growth factor receptor (IGFIR), a member of the tyrosine kinase receptor family, is activated upon binding to its ligands, IGF1 and IGF2. This activation initiates two primary downstream pathways: PI3K/Akt and Ras/MEK/ERK, which regulate apoptosis, cell growth, and differentiation. A substantial body of evidence suggests that IGFIR is implicated in various cancer types, with notably elevated expression levels observed in breast and colorectal cancers [33]. Additionally, IGFIR has been identified as a critical mediator in models of brain metastasis associated with breast cancer [34]. A recently developed analogue, NVP-AEW541, serves as an anti-IGFIR agent. When combined with trastuzumab, it can synergistically enhance therapeutic efficacy [35]. NVP-AEW541 not only decreases pAkt expression but also increases p27 expression, leading to reduced cell proliferation and increased apoptosis. Furthermore, genomic studies have identified several genes whose mRNA is modified by this combined treatment, with PIP emerging as the most upregulated. Although its function in the breast remains unclear, PIP has been reported as a small glycoprotein secreted by breast cancer cells [36, 37]. Another noteworthy finding is the significant effect on the mRNA levels of actin bundle-like protein homolog 1, which is organized into filaments. Actin is present in bundles within microspikes, membrane ruffles, and stress fibers. Fascin homolog 1 has been shown to be overexpressed in breast cancer, with elevated levels associated with poor prognosis [38]. Additionally, the combination treatment with p8, a small protein involved in stress-induced apoptosis, also led to upregulation of apoptotic markers. Other genes implicated in cell cycle progression that interact with p130 and regulate RB function, such as E2F-4, E2F-5, and TFDP1 (which may also influence apoptosis), were specifically modified by the combination treatment [39]. Interestingly, several proteins involved in the proteolytic process were also affected by this combined modification, including several upregulated cystatins. Cystatin S, SN, and SA are secreted proteins that inhibit the function of cysteine proteases. Cystatin-based therapies are reported to modulate various processes, including cell proliferation and apoptosis, and may serve as effective antitumor agents [40]. Collectively, these biochemical and genomic findings suggest that trastuzumab and NVP-AEW541 may exert their effects in breast cancer cells through a complex interplay of transcriptional and regulatory proteins that post-transcriptionally modify cellular dynamics.

SHP-1 is a non-receptor protein tyrosine phosphatase (PTP) that plays an inhibitory role in cancer progression across various types. It possesses an N-terminal *SRC* homology 2 (N-SH2) domain, a C-terminal catalytic PTP domain, and a C-terminal SH2 domain (C-SH2) [41]. SHP-1 can inhibit its own expression through interactions between its N-SH2 and C-terminal domains. Furthermore, SHP-1 plays a critical role in signaling pathways associated with cell growth and survival, particularly those activated by the insulin receptor and lymphocyte-specific protein tyrosine kinases. This is achieved by dephosphorylating several key kinases, including BCR-ABL and PI3K, which in turn influences the JAK/STAT pathway [42]. Nintedanib, a multi-target vascular kinase inhibitor, acts on various growth factor receptors, including PDGFR, FGFR, VEGFR, and the oncogenes *RET*, *FLT3*, and *SRC*, exhibiting anti-angiogenic activity [43]. Reports indicate that nintedanib can activate SHP-1 by directly relieving its autoinhibition, significantly inducing apoptosis in TNBC cells through SHP-1-dependent inhibition of p-STAT3 [44]. Current strategies targeting oncogenic STAT3 activity involve several designated drugs or natural compounds, such as JAK inhibitors or small molecules that directly block functional STAT3 dimerization via the SH2 domain. Enhancing SHP-1, a negative regulator of STAT3 phosphorylation, presents an alternative approach independent of JAK inhibition, thereby providing potential therapeutic targets for the treatment of TNBC.

The epidermal growth factor receptor (EGFR) family, also referred to as human epidermal growth factor receptors (HER) or the erythroblastic leukemia viral oncogene

homolog (ERbB) family, is RTK superfamily and includes 58 transmembrane RTK proteins [45]. In TNBC, EGFR is frequently amplified, making it a significant therapeutic target. The activation of EGFR initiates various downstream signaling pathways, including PI3K/AKT/mTOR and ERK/ MAPK pathways, which play crucial roles in cell proliferation, cell cycle progression, and the development and metastasis of primary tumors [46]. Currently, several EGFR inhibitors are employed in the clinical treatment of TNBC, including tyrosine kinase inhibitors such as afatinib, erlotinib, and lapatinib, as well as monoclonal antibodies like cetuximab and panitumumab [47]. Notably, the EGFR inhibitor gefitinib has been demonstrated to inhibit breast cancer cell proliferation and enhance sensitivity to carboplatin and docetaxel. However, the standalone application of EGFR inhibitors is often ineffective; therefore, they are typically used as adjunctive therapy alongside chemotherapy. Previous studies have elucidated various mechanisms of action for cantharidin, including its ability to induce autophagy and apoptosis in breast cancer through the modulation of the miR-106b-93/p21-PTEN axis. Additionally, cantharidin has been shown to induce apoptosis in leukemia cells via the regulation of p38 MAPK, JNK, p53, and caspase-3 [48, 49]. A recent report has identified miR-607 as a novel regulator of EGFR, which influences the proliferation and apoptosis of TNBC cells. Furthermore, cantharidin significantly inhibits TNBC cell proliferation and induces apoptosis by targeting miR-607. It also suppresses downstream signaling events related to PI3K/AKT/mTOR and ERK/MAPK, as well as apoptosis-related proteins, underscoring its therapeutic potential as an inhibitor for TNBC [50] (Table 1). Moreover, dipyridamole has been shown to attenuate the Wnt signaling pathway by reducing β-catenin activation and to inhibit the ERK1/2-MAPK signaling pathway by decreasing ERK1/2 phosphorylation. This is achieved by increasing IkBα expression levels, reducing p65 phosphorylation, and diminishing the systemic production of inflammatory cytokines, including IL-1β, which is activated by NF-κB through a feedback loop. This study provides further insight into the molecular mechanisms underlying dipyridamole's action, demonstrating its ability to inhibit primary tumor growth and metastasis formation in triple-negative breast cancer [51] (Fig. 2).

Table 1 Programmed death-associated kinase inhibitors

Drugs	Mechanism in RCD	target	Outcome	Ref
Nintadanib	Apoptosis	SHP-1	In clinical use	[43]
Cantharidin	Apoptosis	EGFR(miR-607)	In clinical use	[50]

The role of pyroptosis in breast cancer

Pyroptosis

Pyroptosis, a form of inflammatory cell death, plays a crucial role in the transformation and progression of malignant tumors. Increasing evidence suggests that pyroptosis is implicated in all stages of carcinogenesis, positioning it as a potential target for therapeutic intervention [52]. This process is mediated by inflammasomes, which facilitate the cleavage of gasdermin family proteins and activate previously inactive cytokines, such as IL-1β and IL-18 [53]. The morphological and biochemical characteristics of pyroptosis include the formation of membrane pores, loss of ionic homeostasis, release of inflammatory mediators, and the emergence of large bubbles in the plasma membrane [54]. In 2015, Shao et al. first identified GSDMD as a target of caspase-1, elucidating the downstream mechanisms of pyroptosis [55]. Furthermore, accumulating evidence indicates that gasdermin E (GSDME), activated by caspase-3, can also initiate pyroptosis [56]. Currently, six types of human gasdermins have been classified based on differences in conserved domains: gasdermin A (GSDMA), gasdermin B (GSDMB), gasdermin C (GSDMC), GSDMD, gasdermin E/DFNA5 (which is associated with autosomal dominant deafness), and DFNB59/Pejvakin [57]. The GSDM family members (A-E) predominantly feature an N-terminal pore-forming domain (PFD) and a C-terminal repressor domain. In response to various stimuli, GSDM undergoes cleavage by inflammatory caspases at the linker region, resulting in the release of the PFD from the repressor domain. The oligomerization of the N-terminal PFD and subsequent pore formation in the cell membrane induce cell swelling, chromatin degradation, and the efflux of pro-inflammatory components [58]. Additionally, pyroptosis can be induced by various caspases, including caspase-3, caspase-8, and caspase-9, with death receptor-mediated or mitochondrial apoptotic pathways activating caspase-3 [59–61]. In the context of chemotherapy or viral infections, caspase-3 selectively cleaves GSDME to release GSDME-N, which subsequently stimulates the activation of pyroptosis [62]. Zhang et al. discovered that extracts from Spatholobus suberctus Dunn elevate the levels of caspase-4 and caspase-9, which cleave GSDME, thereby inducing cell membrane permeabilization and pyroptosis [60]. Furthermore, antibiotic chemotherapy drugs can induce caspase-8-mediated pyroptosis through GSDMC [63]. Recent studies have demonstrated that cytosolic caspase-8 can be activated by ligands of Toll-like receptors 3 and 4 (TLR3 and TLR4) or tumor necrosis factor (TNF), resulting in GSDMD cleavage and subsequent pyroptosis [64, 65]. Interestingly, apoptosis can also be triggered by granzyme (GZM) released from natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). Liu et al. initially

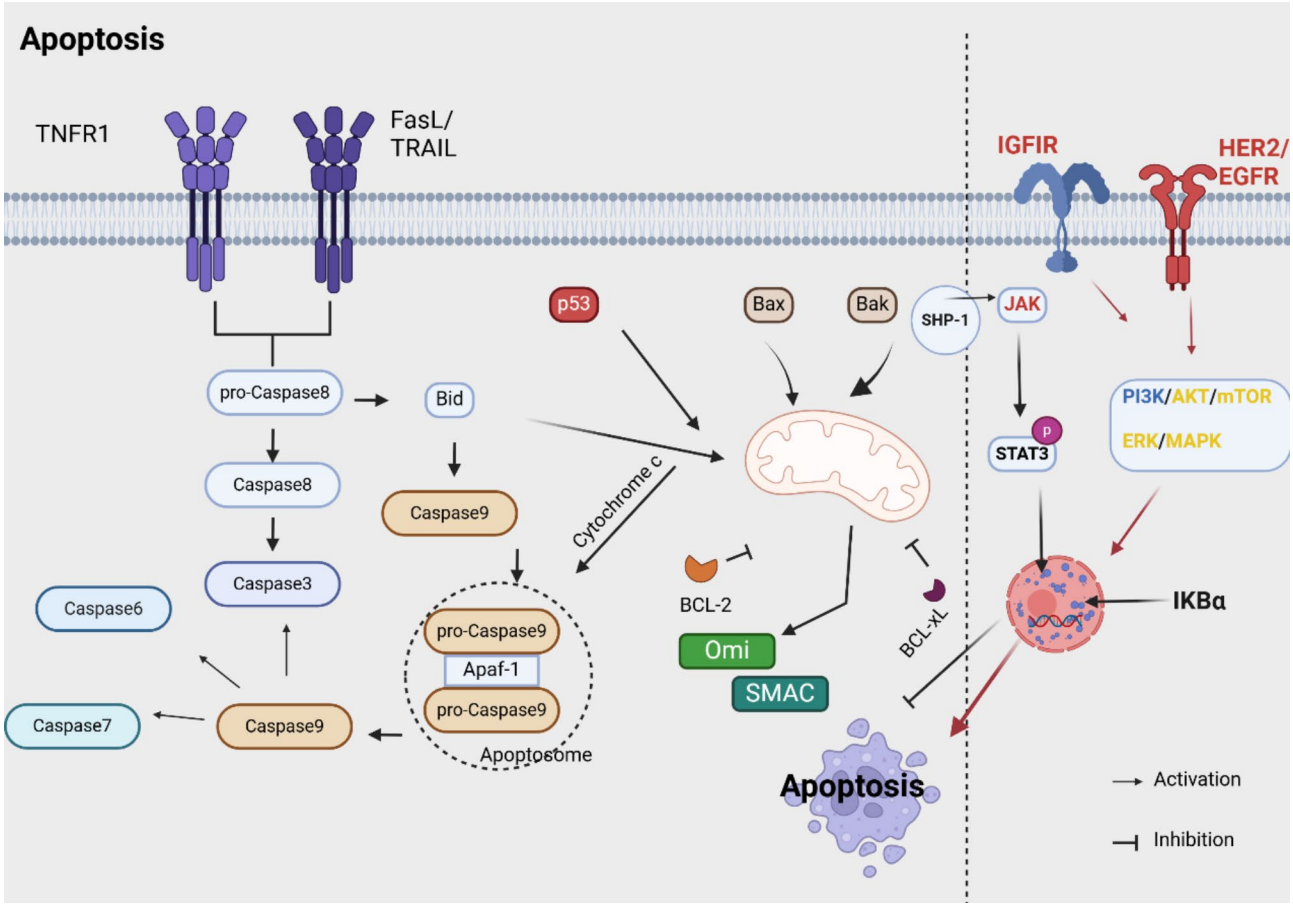

Fig. 2 The core mechanism of apoptosis and related kinases affect the signaling pathway. In apoptosis, the outer membrane permeability induced by pore-forming molecules Bax and BAK leads to the release of cytochrome c and pro-apoptotic factors, such as Smac and Omi. The dimerization of caspase-9 with APAF1 facilitates the activation of downstream caspases, specifically caspase-3, -6, and −7. Extracellular stimulation activates DRs, including members of the TNFR superfamily, such as TNFR1, CD95/Fas, and TRAIL which can initiate exogenous apoptosis. Additionally, HER2 phosphorylation activates the PI3K/AKT/mTOR and ERK/MAPK signaling pathways. The activation of IGF1R occurs upon binding with ligands IGF1 and IGF2, subsequently triggering two major pathways, PI3K/Akt and Ras/MEK/ERK, which regulate apoptosis. Notably, Shp-1-dependent inhibition of p-STAT3 significantly enhances apoptosis in triple-TNBC cells. Furthermore, EGFR activation can initiate various downstream signaling pathways, including PI3K/AKT/mTOR and ERK/MAPK, which are implicated in cell proliferation, cell cycle progression, primary tumor initiation, and metastasis. (Red: Tyrosine kinase; Yellow: Serine-threonine kinases; Blue: Lipid kinases.)

reported that granzyme B (GZMB), released from chimeric antigen receptor T cells, can briefly stimulate caspase-3 in leukemia cells, leading to GSDME-dependent pyroptosis [66]. Additionally, it has been shown that lymphocyte-derived granzyme A (GZMA) can hydrolyze GSDMB at the Lys229/Lys244 site, thus inducing pyroptosis in tumor cells [67].

Kinases in pyroptosis

Cancer cells are characterized by their limitless proliferation, prompting the organism to employ normal cellular protective mechanisms to regulate this process and inhibit tumor development under physiological conditions [68]. The relationship between pyroptosis and cancer is complex, as the effects of pyroptosis can vary based on the specific tissue and genetic composition of the individual. Pyroptosis influences cancer through two primary mechanisms: it alters TME, which affects tumor formation and progression, including growth, invasion, and metastasis [69]. Chronic pyroptosis results in the release of inflammatory cytokines such as IL-1, IL-18, LPH, and HMGB1, thereby creating an inflammatory microenvironment that can initiate tumorigenesis. Evidence suggests that NLRP3, IL-1β, and IL-18 may promote the development of lung cancer, melanoma, and breast cancer. As a form of inflammatory cell death, the immune responses elicited by pyroptosis can have both pro-tumor and anti-tumor effects throughout all stages of tumor development [70]. The activation of pyroptosis and the secretion of related cytokines can either modify the TME to accelerate cancer progression through immune evasion strategies or stimulate the immune system by activating immune cells that generate immune memory, potentially leading to tumor regression and

reduced resistance to immunotherapy. One study demonstrated that pyroptosis induced by NLRP3 and IL-1β secretion may adjust the TME to an immunosuppressive environment, promoting tumor proliferation and invasion in both mouse and human breast cancer models.

Janus kinase 2 (JAK2) is a non-receptor tyrosine kinase frequently amplified or hyperactivated in triple-negative and HER2-enriched breast cancers. JAK2 serves as a central signaling hub, linking the signals from oncogenic receptor tyrosine kinases and interleukin receptor activities to the transcription factor signal transducer and STAT3 [71, 72]. JAK2 phosphorylates STAT3 at the Y705 residue, which facilitates STAT3 dimerization, nuclear translocation, and activation of its transcriptional activity, thereby promoting the expression of target genes associated with cell proliferation, differentiation, survival, and migration [73, 74]. Given that the JAK2-STAT3 signaling pathway is often upregulated in aggressive breast cancers, and its activation correlates with poor overall clinical outcomes, it is regarded as a critical target for cancer drug development. Previous studies have underscored the significant role of the JAK/STAT axis in cellular metabolism and breast cancer cell proliferation [75]. Additionally, stimulation of JAK2/STAT3 has been shown to induce the expression of breast cancer stem cell markers, which may serve as a novel prognostic indicator for breast cancer metastasis [76]. Recently, Liu et al. hypothesized that polydatin induces pyroptosis via the JAK2/STAT3 pathway, demonstrating anti-cancer effects in TNBC mice subjected to a high-fat diet [77]. In experimental models, polydatin was found to downregulate the phosphorylation of STAT3 and JAK2 while simultaneously enhancing the expression of NLRP3, caspase-1, IL-1β, and IL-18, thereby contributing to the activation of pyroptosis. Pyroptotic cells are characterized by the secretion of inflammatory cytokines IL-1β and IL-18 through pores formed by GSDMD, leading to inflammatory responses mediated by inflammasomes [78]. Numerous studies have established that IL-1β and IL-18 play critical roles within the immune system [79]. Their functions in breast cancer are complex; on one hand, the expression of these inflammatory cytokines recruits immune cells to the TME, thereby stimulating breast cancer progression. Conversely, these cytokines can also inhibit tumorigenesis, growth, and metastasis of breast cancer cells.

In many malignant tumors, including breast cancer, elevated levels of IL-1β are observed within the TME, where it can promote tumor proliferation while also exhibiting anti-tumor effects [80]. Furthermore, IL-1β influences immune responses by enhancing adaptive T cell-mediated immunity and facilitating the maturation of CD4 + and CD8 + T cells [81]. Initially identified as a factor that induces IFN-γ production in anti-CD3-stimulated T cells [82]. IL-18 is widely recognized as a crucial mediator of anti-cancer immune functions, regulating immune components through the attraction and differentiation of NK cells, T cells, and monocytes [83]. Mesenchymal stem cells expressing IL-18 have been shown to suppress the growth, invasion, and metastasis of breast cancer cells in vitro, reducing the proliferation index marked by Ki-67 and halting tumor progression [84]. However, IL-18 may also contribute to cancer immune evasion; in the context of breast cancer, IL-18 has been shown to enhance PD-1 expression in NK cells, thereby increasing their immunosuppressive profile, which correlates with poor outcomes in patients with TNBC [85].

Endothelial cells (ECs) are pivotal in regulating the development of endothelial dysfunction. The ligands of EGFR secreted by ECs phosphorylate EGFR when co-cultured with MDA-MB-231 breast cancer cells (HU-231), thereby playing a crucial role in cancer progression. Additionally, photodynamic therapy (PDT) employs mechanisms that inhibit breast cancer progression, including the suppression of EGFR signaling in direct co-culture systems. AE-PDT not only induces apoptosis and pyroptosis to facilitate HU-231 cell death but also inhibits angiogenesis. Notably, EGFR inhibition further enhances AE-PDT-induced apoptosis via mitochondrial pathways and promotes pyroptosis through modulation of the caspase-1/GSDMD axis. Furthermore, EGFR inhibitors can be effectively combined with AE photodynamic therapy for the treatment of breast cancer. These findings necessitate further investigation in larger human cohorts and diverse clinical contexts to uncover potential molecular mechanisms. JNK, a crucial component of the serine/threonine protein MAPK signaling cascade, has been implicated in mediating pyroptosis in human breast cancer cells by promoting JNK phosphorylation and activating the downstream NF-KB/caspase-1/GSDMD signaling pathway. This presents promising therapeutic targets for breast cancer treatment. Additionally, the caspase-GSDME pathway is frequently associated with intracellular ROS production in cancer cells. Numerous researchers have examined the interplay between the caspase-GSDME pathway and ROS signaling, yielding a variety of insightful results. DOX treatment induces ROS accumulation, which subsequently promotes JNK phosphorylation to p-JNK, further activating the key regulatory factor caspase-3 through a series of cascading reactions. Conversely, ROS activated by DOX can influence the cleavage of caspase-8; c-Caspase-8 facilitates the cleavage of caspase-3, which in turn induces the cleavage of GSDME and triggers pyroptosis in breast cancer cells [86] (Fig. 3).

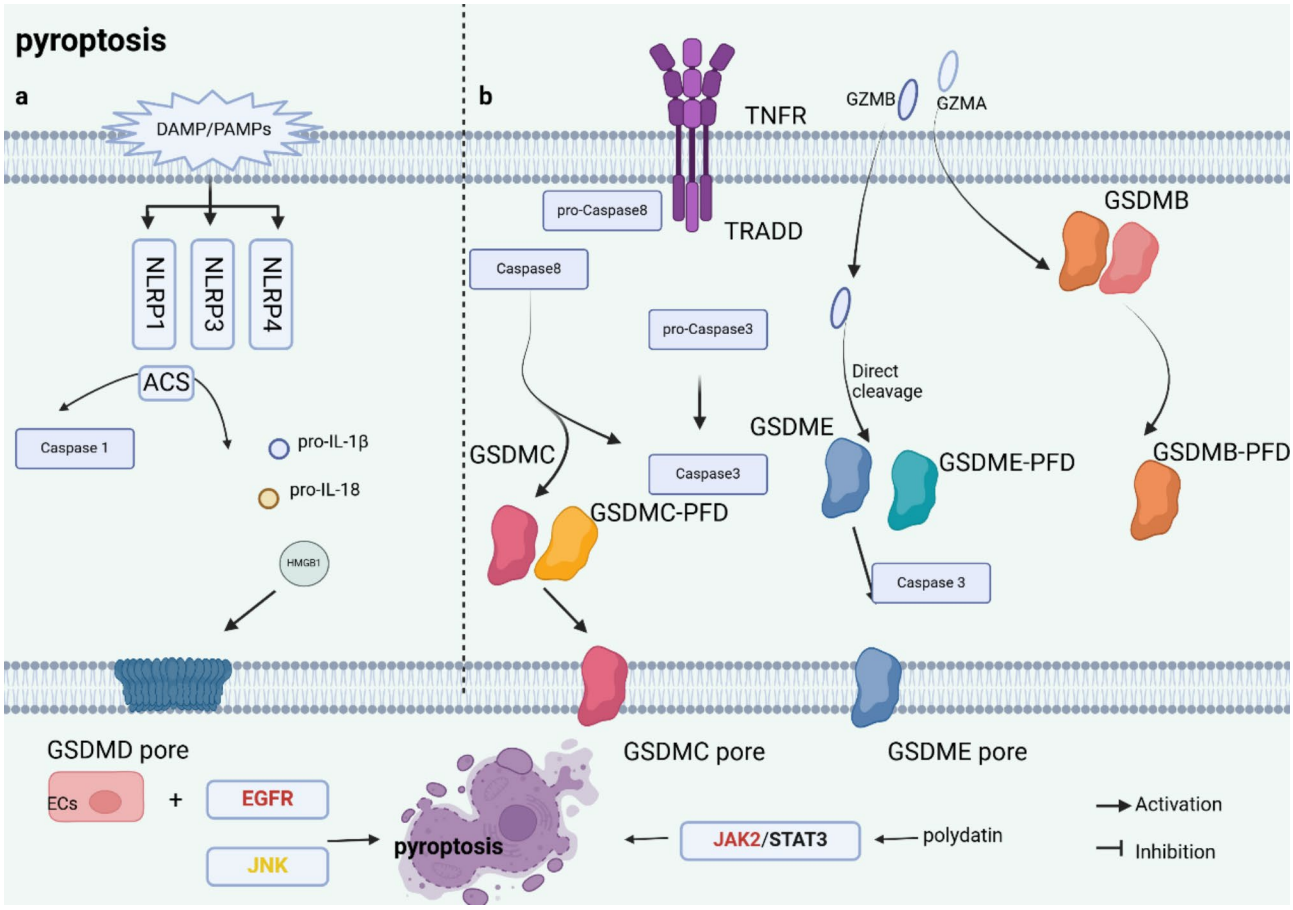

Fig. 3 The core mechanism of pyrotosis and related kinases affect the signaling pathway. Pyroptosis is initiated when cytoplasmic sensor proteins, specifically members of the NOD-like receptor family (NLRP1, NLRP3, and NLRP4), recruit and activate CASPASE1 through ASC in response to PAMPs or DAMPs. This process leads to the secretion of the precursor interleukin IL-1β. Subsequently, the high mobility group box 1 protein (HMGB1) facilitates the cleavage and production of GSDMD-N, which triggers pyroptotic cell death by activating both canonical and non-canonical inflammasomes. Additionally, the release of GZMB by T cells can transiently stimulate caspase-3 in leukemia cells, resulting in the pyroptosis of GSDME. Lymphocyte-derived GZMA hydrolyzes GSDMB at the Lys229/Lys244 sites, leading to the pyroptotic death of tumor cells. TNFR activation induces pyroptosis of GSDMC via caspase-8. Furthermore, JAK2 interacts with the STAT3 transcription factor, phosphorylating the Y705 residue of STAT3, which promotes its dimerization and nuclear translocation. This activation enhances STAT3's transcriptional activity and the expression of its target genes. Inhibition of the EGFR further enhances apoptosis induced by AE-PDT through the mitochondrial pathway, while also promoting AE-PDT-mediated focal cell death by modulating the caspase-1/GSDMD axis. Given that JAK2-STAT3 signaling is frequently upregulated in aggressive breast cancer and is associated with poor clinical outcomes, this pathway represents a significant target for cancer drug development. Moreover, JNK, a critical component of the serine/threonine MAPK signaling pathway, inhibits MFHAS1 to induce pyroptosis in human breast cancer cells by promoting JNK phosphorylation and activating the downstream NF-κB/caspase-1/GSDMD signaling cascade. (Red: Tyrosine kinase; Yellow: Serine-threonine kinases.)

The role of autophagy in breast cancer

Autophagy

Autophagy is a crucial physiological mechanism that regulates various biological processes and maintains dynamic homeostasis [87, 88]. It facilitates the degradation of microbes, including viruses and bacteria, as well as damaged organelles and proteins, via lysosomal and proteasomal pathways. Autophagy can be categorized into three primary types: macroautophagy, microautophagy, and chaperone-mediated autophagy, each distinguished by its unique mechanisms for delivering cargo to the lysosome [89–92]. The predominant form, macroautophagy, is characterized by the formation of autophagosomes, which begins with the generation of phagophores. During the elongation phase, the membrane of the phagophore expands, engulfing cytoplasmic cargo to create the autophagosome, a double-membraned structure. Once the autophagosome is formed, it fuses with lysosomes, where enzymes, including tissue proteins and acidic hydrolases, facilitate the degradation of the cargo. After degradation within the autolysosome, the resulting contents are released into the cytoplasm to initiate biosynthetic processes. Numerous regulatory factors that influence the cellular autophagy mechanism have been identified. Under conditions of low energy and amino acid levels, such as

during starvation, the mTORC1 complex is inhibited and remains free in the cytoplasm, unable to regulate autophagy. Conversely, when energy and amino acid levels are elevated, v-ATPase is activated through interactions with the lysosome and its membrane, which recruits mTORC1 to inhibit autophagy. A complex comprising ULK1/2, Beclin-1, and Vps34 plays a critical role in the induction of autophagy [93–96]. The VPS34 complex acts as a phosphatidylinositol 3-phosphate kinase to generate phosphatidylinositol 3-phosphate (PI3P), which acts as a scaffold to recruit PI3P-binding molecules to form a detached preautophagic structure called phagosome. Collectively, PI3P recruits and asseminates two ubiquitin-like coupling systems involved in LC3 lipidation and autophagosome formation [97]. In addition, Upon activation of the mTOR signaling pathway, autophagy is inhibited through the impairment of ULK1/2. ULK1/2, via the overexpression of LC3B, is associated with the elongation of phagophores and the formation of autophagosomes [98, 99]. Additionally, upregulation of mTORC1 can inhibit the fusion of lysosomes and autophagosomes by phosphorylating UVRAG, thereby modulating autophagy within the cell [100]. Additionally, TRAIL can recruit autophagy proteins to facilitate death mechanisms during apoptosis [101]. The regulation of autophagy mechanisms in cancer, along with their therapeutic modulation, is both crucial and diverse [102–106]. Inhibiting the PIKfyve and p38 MAPK pathways can impede autophagy and slow tumor cell progression by suppressing protein degradation via autophagy inhibition [107]. In addition to apoptosis, ferroptosis mechanisms can also be regulated through autophagy, involving various pathways such as TMEM164, Gpx4, and HPCAL1 [108–110]. Moreover, drug repurposing is regarded as one of the targeted strategies for modulating autophagy in cancer treatment.

Tyrosine in autophagy

The capacity of breast cancer cells to transition between epithelial and mesenchymal states significantly enhances their metastatic potential. Consequently, inducing tumor cells to adopt a stable mesenchymal state, rather than completely eradicating them, may limit the efficacy of therapeutic agents and create opportunities for disease progression. One study proposed that cytokine-induced epithelial-mesenchymal transition (EMT) is sufficient to confer resistance to lapatinib, establishing both stable and reversible EMT states induced by lapatinib and TGF-β in a human mammary epithelial cell (HME2) model of HER2 transformation. This model system aimed to test the hypothesis that metastasis necessitates epithelial-mesenchymal plasticity (EMP), identifying spleen tyrosine kinase (SYK) as a critical molecular mediator of EMP. Furthermore, SYK activity is essential for the autophagy-mediated clearance of P bodies during mesenchymal-epithelial transition (MET). Pharmacological inhibition of SYK may offer a novel therapeutic strategy to mitigate breast cancer metastasis, not by eradicating tumor cells but by maintaining disseminated cells in an asymptomatic dormant state [111]. Despite extensive research on the role of HER2 in mediating extracellular signal transmission to intracellular matrices, the nuclear function of HER2 remains largely unexplored. Nuclear HER2 directly binds to consensus sequences in the DEPTOR promoter, thereby inhibiting its transcription. The kinase activity of HER2 is required for its nuclear translocation and DEPTOR transcriptional repression. Additionally, nuclear ErbB2 inhibits DEPTOR, which suppresses autophagy induction by activating mTORC1. This study uncovers a novel mechanism through which functional ErbB2 regulates autophagy by translocating to the nucleus and acting as a transcriptional regulator that inhibits DEPTOR transcription. This process leads to the activation of the PI3K/AKT/mTOR pathway, ultimately suppressing autophagy. Additionally, DEPTOR serves as a direct inhibitor of both mTORC1 and mTORC2. The induction of DEPTOR following HER2 inactivation not only promotes autophagy but may also influence cell proliferation, survival, and drug resistance. These findings suggest that targeting DEPTOR's inhibition of mTOR could offer therapeutic benefits in strategies aimed at modulating nuclear HER2 [112].

Leucine-rich repeat kinase 2 (LRRK2), a member of the leucine-rich repeat kinase family, is implicated in the regulation of autophagy. The modulation of autophagy represents a promising target for the development of anti-TNBC drugs, which has significant implications for breast cancer research. Breast cancer exhibits histological diversity, characterized by multiple TNBC molecular subtypes and complex tumor features, coupled with aggressive behavior that results in high recurrence rates. TNBC is driven by various signaling pathways that involve multiple kinases. Zhou et al. identified KIN-281, a small molecule based on quinazoline, which inhibits several kinases, including maternal embryonic leucine zipper kinase (MELK) and BMX. Structure-based molecular docking screening was performed to identify compounds that inhibit MELK as potential therapeutic targets. MELK overexpression has been linked to poor cancer prognosis due to its role in promoting cancer cell survival and significantly regulating the cell cycle by inducing G2/M arrest [113–115]. Additionally, KIN-281 has been demonstrated to inhibit several kinases in TNBC cells, including the tyrosine kinases BMX/ETK and TIE2/TEK, with IC50 values ranging from 1 to 4 mM to 42.7 mM. Following treatment with KIN-281, an upregulation of p21 WAF1/CIP1 was observed, which corresponded with reduced levels of cyclin A2.

Moreover, breast cancer tumors often depend on HER2 signaling, which can activate PI3K [116]. Young and colleagues utilized SAR405, a pyrimidine-based small molecule, to target class I (p110α) and class III (vacuolar protein sorting 34, Vps34) PI3K, effectively inhibiting these kinases, reducing tumor growth, and inducing autophagy. They demonstrated that a locked nucleic acid antisense oligonucleotide (LNA-ASO) named EZN4150 could sensitize HER2; however, it did not induce autophagy and even inhibited autophagy induction in response to catalytic class I PI3K inhibitors. The combination of small molecules and oligomers is believed to inhibit Vps34-dependent pathways through tumor cell apoptosis, although the specific molecular mechanisms warrant further investigation.

Autophagy functions as a critical survival pathway for cancer cells that are subjected to genotoxic stress and is activated by oncogenic signals. Various oncogenic proteins, including class I phosphoinositide 3-kinase, protein kinase B, the mammalian target of rapamycin, *BCL-2*, and mitogen-activated protein kinases, have been shown to inhibit autophagy. In contrast, other proteins, such as class III PI3K (PI3KCIII), PTEN, death-associated protein kinases (DAPKs), Beclin-1, Bax-interacting factor 1 (Bif-1), and p53 (depending on the specific cancer type), can promote autophagy [117–119].

Protein kinase C (PKC) comprises a family of serine/threonine protein kinases that are pivotal in signal transduction and cellular regulation [120]. This family is classified into three distinct classes based on structural variations and biochemical properties: conventional (α, βI, βII, and γ), novel (δ, ε, η, and θ), and atypical (λ/ι and ξ). Recent research indicates that the PKC isoform PKCε is linked to autophagy, with its knockout mitigating the increased autophagy resulting from the depletion of Raptor and Rictor. Overexpression of PKCε in MCF-7 cells activates mTORC1 and elevates levels of LC3-I, LC3-II, and p62. Furthermore, the knockout of mTOR and Rictor, or the induction of starvation, enhances autophagy in cells that overexpress PKCε. Although PKCε overexpression suppresses apoptosis in MCF-7 cells, it simultaneously triggers autophagy in response to tumor necrosis factor-α. Consequently, PKCε contributes to the survival of breast cancer cells not only by inhibiting apoptosis but also by promoting autophagy. Given that PKCε overexpression leads to chemotherapy resistance, targeting autophagy in combination with standard treatment may offer an effective strategy for managing breast cancers characterized by elevated PKCε levels [121].

Polo-like kinase 1 (PLK1) is a serine/threonine protein kinase that is overexpressed in breast cancer, making it a promising therapeutic target. Inhibition of PLK1 has been demonstrated to enhance the radiosensitivity of breast cancer cells, a phenomenon associated with the suppression of radiation-induced autophagy. This underscores its potential as a crucial target for sensitizing breast cancer to radiation treatment [122]. Furthermore, previous research has established that PIM-2, one of the three PIM kinases, plays a significant role in autophagy regulation by activating the mTOR pathway. Abnormal expression of PIM-2 has been identified in various malignancies. Evidence indicates that PIM-2 can directly phosphorylate TSC2 at the Ser1798 site, thereby alleviating TSC2's inhibition of mTORC1. Additionally, PIM-2 promotes autophagy and can inhibit the reduction of autophagic responses, preventing the dissociation of *BCL-2* from Beclin-1 and enhancing lysosomal acidification. Other studies have revealed that during glucose starvation, the phosphorylation of hexokinase-II by PIM-2 is essential for the autophagic process [123].

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase that is widely expressed and was originally identified as a regulator of glycogen synthesis; however, it also plays a significant role in the regulation of autophagy. In MCF-7 cells, the overexpression of GSK-3α/β activates mTORC1 and inhibits autophagy through the phosphorylation of Ser859 on Raptor. This phosphorylation event results in decreased phosphorylation of both p70S6K1 and ULK1, ultimately leading to an increase in autophagic flux. In prostate cancer cell models, GSK-3β has been demonstrated to regulate autophagy via the LKB1-AMPK pathway. Specifically, the inhibition of GSK-3β results in a rapid decline in cellular ATP levels, which is followed by LKB1-dependent AMPK activation and the inactivation of the mTOR pathway, both of which are associated with the induction of autophagy [123].

Extracellular signal-regulated kinase 1 (ERK1), also referred to as p44MAPK or MAPK3, is a subtype of ERK that is part of the mitogen-activated protein kinase family and is involved in the regulation of autophagy in various tumor cells. ERK1 is phosphorylated and activated via the RAS-RAF-MEK signaling pathway to modulate autophagy. Additionally, research has suggested that non-classical activation of the MEK/ERK pathway can influence Beclin-1 expression, thereby stimulating autophagy. Acute activation of MEK/ERK promotes protective autophagy by inhibiting mTORC1 or mTORC2, accompanied by moderate increases in Beclin-1 expression. Conversely, prolonged activation of MEK/ERK leads to the dual inhibition of both mTORC1 and mTORC2, resulting in a substantial increase in Beclin-1 expression and the induction of destructive autophagy [124] (Fig. 4).

The role of necroptosis in breast cancer

Necroptosis

Necroptosis is distinct from apoptosis in that its progression does not involve the activation of caspases. Instead, necroptosis is mediated by external signals that activate

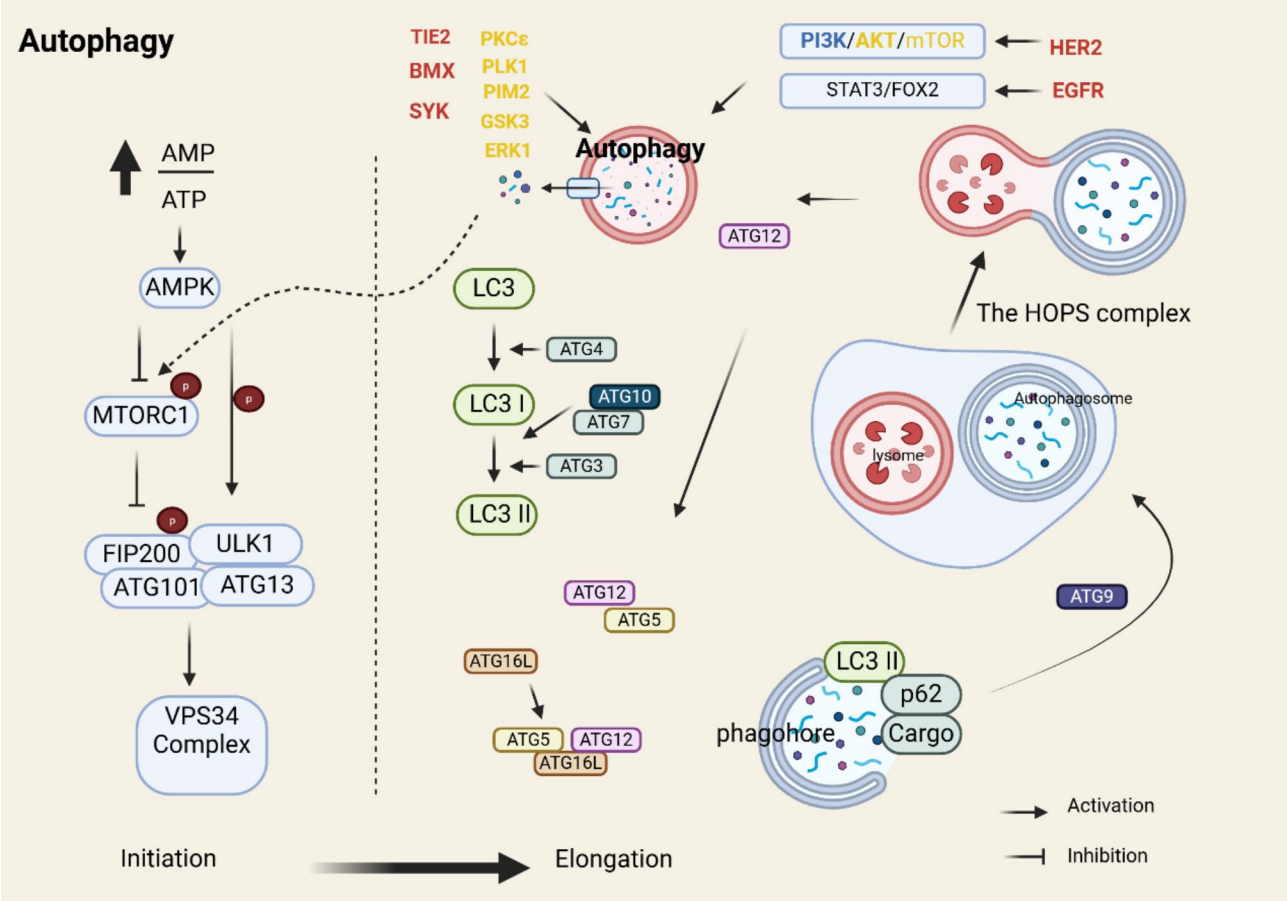

Fig. 4 The core mechanism of autophagy and related kinases affect the signaling pathway. Autophagy is initiated by energy stress signals from AMPK, which ultimately activate VPS34. The VPS34 complex generates PI3P on the membrane, facilitating the recruitment and assembly of the ubiquitin-like coupling system. During the lipidation of LC3, ATG7, ATG3, and the ATG5-ATG12-ATG16L complexes function as ubiquitin enzymes, which can load cargo receptors such as SQSTM1/P62 and NBR. The transport of autophagosomes and late endosomes/lysosomes is closely coordinated with the recruitment of binding factors; HOPS complexes can be recruited by ARL8 or the ORP1L-RAB7-RILP complex, thereby achieving the coupling of anterograde and retrograde transport as well as fusion events. The PKC isoenzyme PKCε is associated with autophagy, and its deletion mitigates the increase in autophagy induced by the depletion of Raptor and Rictor. Conversely, overexpression of PKCε in MCF-7 cells leads to the activation of mTORC1 and an increase in LC3-I, LC3-II, and p62 levels. PLK1 may represent a crucial therapeutic target for radiosensitized breast cancer. PIM2 directly phosphorylates TSC2 at Ser1798, thereby inhibiting TSC2's action at mTORC1. In MCF-7 cells, the overexpression of GSK-3α/β activates mTORC1 and inhibits autophagy through the phosphorylation of Ser859 on Raptor, resulting in decreased phosphorylation of p70S6K1 and ULK1, alongside increased autophagic flux. Non-classical activation of MEK/ERK may also modulate beclin-1 expression to stimulate autophagy. KIN-281 inhibits MELK and BMX. Pharmacological inhibition of SYK may provide a unique therapeutic strategy to limit the metastatic progression of breast cancer. Additionally, DEPTOR, induced following Her2 inactivation, not only promotes autophagy but may also mediate cell proliferation, survival, and drug resistance. Thus, the inhibition of mTOR by DEPTOR could contribute to the therapeutic effects observed when targeting nuclear Her2. (Red: Tyrosine kinase; Yellow: Serine-threonine kinases; Blue: Lipid kinase.)

a signaling cascade involving receptor-interacting protein 1 (RIP1), RIP3, and mixed lineage kinase domain-like (MLKL). The pseudokinase MLKL plays a crucial role in necroptosis, as it can form membrane pores through oligomerization and subsequent insertion into the plasma membrane. In addition, necrotizing apoptosis is promoted by a variety of stimuli, including members of the TNF ligand family, interferon (IFN), TLR ligands that induce interferon-β using the adaptor TRIF (TIR domain-containing adaptor), and viral DNA via Z-DNA-binding protein 1 (ZBP1) [125]. Notably, necroptosis is characterized by the permeabilization of lysosomal membranes, followed by mitochondrial damage, ultimately leading to necrotic-like cell death, both morphologically and biochemically. This process is finely tuned and serves multiple functions; for example, under physiological conditions, necroptosis facilitates the formation of mammalian bone plates, megakaryocyte production, and the maintenance of epithelial hemostasis [126–128]. Furthermore, necroptosis has been shown to exert both pro-tumor and anti-tumor effects within TME. On one hand, low expression levels of necroptotic regulators RIP3 and MLKL are associated with poor prognosis in various types of solid tumors [129, 130]. Specifically, necroptotic

cells have been demonstrated to promote dendritic cell maturation and enhance cross-presentation efficiency, thereby generating anti-tumor immunity in CD8 + T cells through RIPK1 and NF-κB signaling [131, 132]. In contrast, cells undergoing passive necrosis are unable to effectively activate CD8 + T cells in vivo. Notably, the absence of RIP3 in mice diminishes the cytotoxicity of NKT cells against tumors [133]. Consequently, triggering necroptosis in cancer cells while simultaneously activating cytotoxic T cells has emerged as a novel strategy for cancer therapy. Furthermore, necroptotic cancer cells can stimulate dendritic cell maturation and enhance the cross-priming of CD8 + T cells, resulting in increased production of IFN-γ and improved anti-tumor immunity [134]. On the other hand, inhibiting TCR restimulation-induced necroptosis in T cells can rejuvenate their anti-tumor efficacy [135]. Additionally, necroptosis induced in endothelial cells by tumor cells may promote tumor metastasis. In a pancreatic ductal adenocarcinoma (PDA) mouse model, signaling associated with necroptosis has been shown to facilitate macrophage-mediated T cell suppression [136]. Recently, Jiao et al. demonstrated that in late-stage breast tumors, the levels of RIP3-mediated MLKL phosphorylation in necrotic areas are elevated compared to those observed in the early stages of breast cancer [137].

Tyrosine in necroptosis

Necroptosis, typically identified by its necrotic morphology, serves adaptive functions not only in response to stress-induced failures but also in developmental processes, specifically by ensuring the elimination of potentially defective organisms prior to birth, and in the maintenance of adult T cell homeostasis [138–140]. At the molecular level, necroptosis is predominantly dependent on the sequential activation of RIPK3 and MLKL [141, 142].

Recent literature indicates a correlation between necroptosis and tumor growth; however, several fundamental questions regarding its regulation and role in tumorigenesis remain unanswered. In the MVT1 breast cancer model, tumor necroptosis does not depend on RIPK1 and may even exert an inhibitory effect on the induction of necroptosis. This observation is consistent with previous studies that suggest RIPK1 impedes the necroptosis-inducing activity of ZBP1 (Z-DNA binding protein 1) during normal embryonic development. ZBP1 has been identified as a crucial mediator of tumor necroptosis in both the MVT-1 and B16 syngeneic cancer models, where its absence has been shown to inhibit tumor metastasis in the MVT-1 model. Although both RIPK1 and ZBP1 can recruit RIPK3 to facilitate necroptosis, ZBP1, rather than RIPK1, acts as a pivotal upstream mediator of RIPK3 in tumor necroptosis, with glucose deprivation initiating ZBP1-dependent necroptosis in tumor cells. Glucose deprivation results in the release of mitochondrial DNA (mtDNA) into the cytoplasm, which subsequently binds to ZBP1 and activates MLKL through BCL-2 family proteins in an NOXA-dependent manner. These findings provide new insights into the regulation of tumor necroptosis during tumor development.

The binding of death receptors, particularly TNFR1, is the primary trigger for the activation of RIPK3. Death receptors, such as CD95, TNFR1, DR4, DR5, and DR6, are members of the TNF receptor superfamily [143–147]. These type I transmembrane proteins exhibit common characteristics, including an extracellular amino-terminal cysteine-rich domain that facilitates ligand specificity and receptor pre-binding, as well as a death domain (DD) comprised of an 80-amino acid sequence located at the cytoplasmic tail, which transmits apoptotic signals through protein-protein interactions (PPI) [148–151]. While the molecular mechanisms underlying CD95-induced apoptotic signaling have been elucidated, the pathways through which these receptors activate non-apoptotic signaling cascades, such as NF-κB, MAPK, and PI3K, remain unclear. Membrane-bound CD95 ligand (m-CD95L) effectively induces cell death and can be cleaved by various metalloproteinases [152]. In contrast, soluble CD95 ligand (s-CD95L) interacts with the soluble form of CD95 but does not induce cell death [153, 154]; however, it does activate non-apoptotic signaling pathways [155, 156]. To date, the specific interactions between the unique ligand (CD95L) and receptor (CD95) that lead to the activation of these distinct signaling pathways have yet to be fully explained.

A study reported that TNBC cells exposed to s-CD95L exhibited no detectable levels of caspase-8 in the membrane immunosuppressive compartment (MISC), although the presence of a trace amount of this protease cannot be entirely ruled out, as it is not detectable by Western blotting [156]. Caspase-8 is recognized for its role in cell migration, primarily through non-enzymatic mechanisms [157]. *SRC* kinase mitigates the proteolytic activity of caspase-8 by phosphorylating tyrosine 380 (Y380), which promotes the recruitment of the p85α subunit of PI3K, thereby activating the PI3K signaling pathway [158]. Concurrently, TNBC cells overexpressing EGFR and exposed to s-CD95L facilitate the binding of CD95 to EGFR, enhancing the migration and metastatic dissemination of these cancer cells. Although the CD95/CD95L interaction is known to eliminate malignant cells by promoting the formation of the death-inducing signaling complex (DISC), the molecular mechanisms that govern the transition between different signaling pathways remain unclear (Fig. 5).

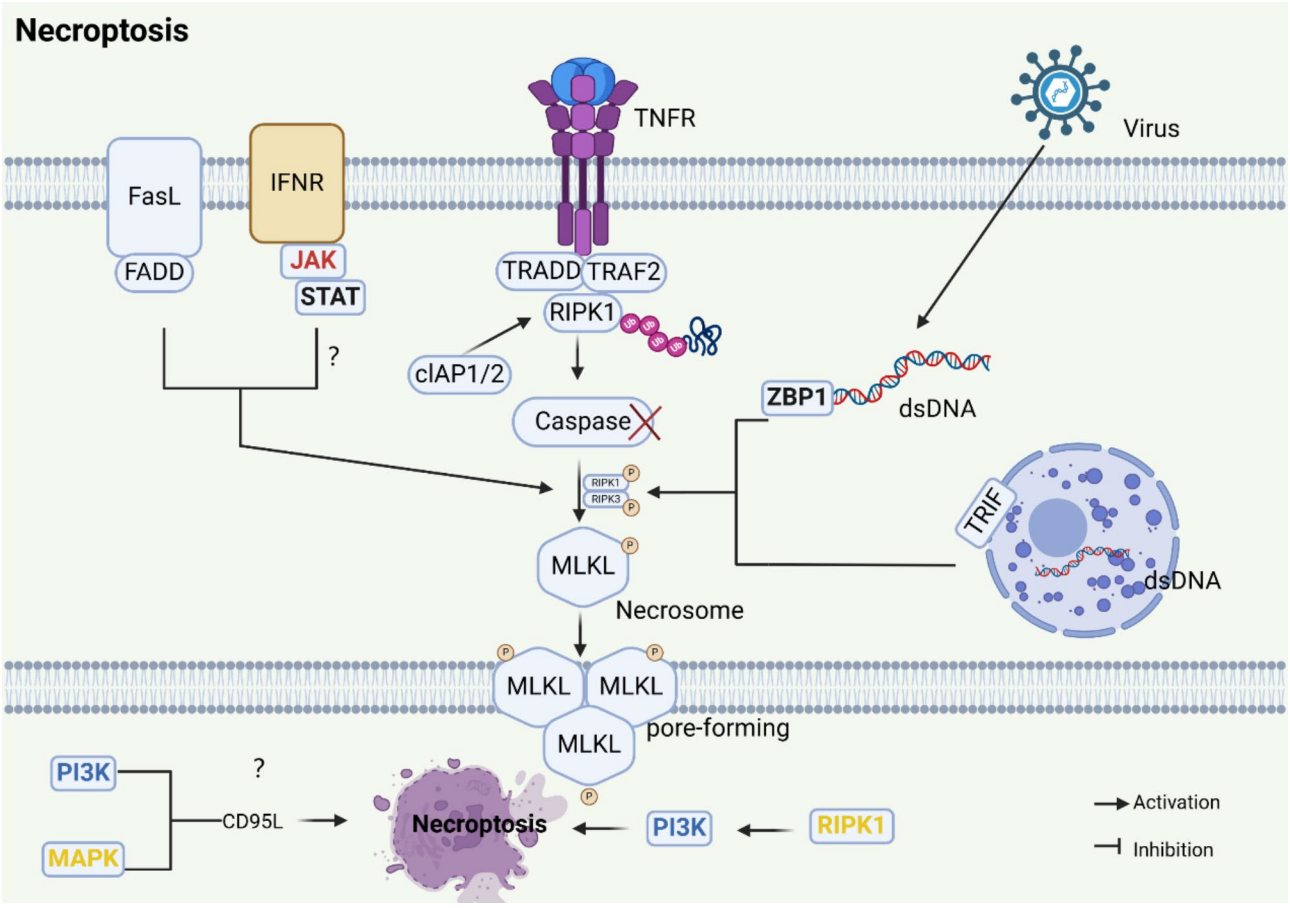

Fig. 5 The core mechanism of necroptosis and related kinases affect the signaling pathway. In necroptosis, the binding of TNFα to its receptor leads to the recruitment of multiple proteins via the intracellular tail of TNFR1, forming complex I. The IAP-mediated Lys63-linked polyubiquitination of RIP1 (Lys63-Ub) is crucial for survival pathways. When CASP8 is inhibited, MLKL, RIPK1, and RIPK3 are recruited to form necrosomes through phosphorylation. The phosphorylation-mediated activation of MLKL, followed by MLKL-induced membrane pore formation, culminates in necroptosis. Further investigation is needed to elucidate the mechanisms and functions of RIPK3, MAPK, and JAK/STAT signaling pathways in the context of breast cancer treatment. (Red: Tyrosine kinase; Yellow: Serine-threonine kinases; Blue: Lipid kinase.)

The role of ferroptosis in breast cancer

Ferroptosis

Ferroptosis is a form of programmed cell death distinguished by three hallmark features: the loss of lipid hydroperoxide repair capability mediated by glutathione peroxidase 4 (GPX4), the presence of redox-active iron, and the oxidation of phospholipids that contain polyunsaturated fatty acids (PUFAs). Morphologically, ferroptosis cells exhibit ultrastructural changes in mitochondria, such as reduced size, increased bilayer density, disruption of the outer mitochondrial membrane (OMM), and disappearance of mitochondrial cristas. In addition, the balloon-like phenotype (that is, the formation of clear, round cells composed mainly of empty cytosol) can be used for microscopic identification of ferroptosis cells [159, 160].

Iron is pivotal in the process of ferroptosis. Within cells, iron exists primarily in two forms: ferritin-bound iron and the free labile iron pool (LIP). Ferritin is composed of two subunits: ferritin heavy chain 1 (FTH1) and ferritin light chain (FTL), which function to sequester iron and thereby inhibit its involvement in deleterious oxidative reactions. The degradation of ferritin occurs predominantly through ferritin autophagy, a mechanism facilitated by nuclear receptor coactivator 4 (NCOA4). This process results in the release of substantial amounts of iron, thereby promoting ferroptosis [161, 162]. Extracellular ferric iron (Fe^{3+}) binds to transferrin (TF) and enters the cell via transferrin receptor 1 (TFR1). Once inside, trivalent iron is reduced to divalent iron (Fe^{2+}) by prostate sextant transmembrane epithelial antigen 3 (STEAP3) [163], which is then released into the cytoplasm through solute carrier family 11 member 2 (SLC11A2), contributing to the formation of the LIP. The divalent iron in the LIP reacts with hydrogen peroxide (H_2O_2) through the Fenton reaction, generating hydroxyl radicals (·OH) and other highly reactive free radicals. These free radicals can initiate lipid peroxidation, resulting in cell membrane damage and promoting

the process of ferroptosis [161]. In addition, excess cytoplasmic ferrous iron can be exported from the cell via solute carrier family 40 member 1 (SLC40A1) to maintain iron homeostasis [164]. A critical factor driving ferroptosis is the excessive production of lipid peroxides (LPO) [165]. Polyunsaturated fatty acids exhibit a high sensitivity to peroxidation due to their multiple double bonds. The oxidation of PUFA-containing phospholipids (PUFA-PL) can occur through both enzymatic and non-enzymatic autoxidation processes, which are often driven by the Fenton reaction. Lipid peroxidation primarily involves lipoxygenases (ALOXs) and cytochrome P450 oxidoreductase (POR). ALOXs are heme iron-free enzymes that facilitate the direct incorporation of oxygen into PUFAs and PUFA-containing lipids found within biofilms. For instance, ALOX12 is crucial for p53-dependent ferroptosis, while ALOX15 specifically interacts with stearoyl-arachidonoyl-phosphatidylethanolamine (stearoyl-AA-PE) to generate lipid peroxides. This interaction leads to the formation of a complex with phosphatidylethanolamine binding protein 1 (PEBP1), which subsequently promotes ferroptosis induced by erastin or RSL3 [166]. GPX4 serves as a critical regulator of ferroptosis. It is the sole member of the glutathione peroxidase family that operates as a phospholipid hydroperoxidase, capable of reducing lipid hydroperoxides (PLOOH) to their corresponding phosphatidyl alcohols (PLOH). The mechanism of GPX4 action is as follows: PLOOH oxidizes the active site of GPX4, specifically the selenyl alcohol (GPX4-SeH), resulting in the formation of a selenic acid intermediate (GPX4-SeOH). This intermediate subsequently reacts with GSH to produce a Se-glutathione adduct (GPX4-Se-SG). The GPX4-Se-SG then interacts with a second GSH molecule, leading to the conversion of GPX4-Se-SG into GPX4-SeH and generating oxidized glutathione (GSSG) [167]. GPX4 is crucial for neutralizing phospholipid hydroperoxides. Research has elucidated the mechanisms underlying ferroptosis induced by erastin and RSL3. Erastin functions by inhibiting System Xc^-, which reduces cellular uptake of cystine and subsequently suppresses the synthesis of GSH. Given that glutathione serves as an essential cofactor for the normal functioning of GPX4, decreased synthesis of GSH results in diminished GPX4 activity. This reduction impairs the enzyme's capacity to effectively eliminate lipid peroxides within the cell, ultimately leading to ferroptosis. In contrast, RSL3 directly binds to GPX4, inhibiting its activity and further compromising the enzyme's ability to clear lipid peroxides, thereby facilitating the occurrence of ferroptosis. SLC7A11, a subunit of the cystine/glutamate antiporter (xCT) system, is recognized as a key regulator of ferroptosis, as it facilitates the import of cysteine necessary for glutathione (GSH) synthesis. GSH serves as an essential substrate for GPX4, which catalyzes the conversion of lipid hydroperoxides to alcohols. Furthermore, NCOA4 promotes ferroptosis by degrading ferritin, which leads to an increase in unstable cellular iron levels [168]. Another significant gene, long-chain acyl-CoA synthetase 4 (ACSL4), enhances ferroptosis by enriching cell membranes with long polyunsaturated n-6 fatty acids, which are particularly prone to oxidation via free radicals or enzyme-mediated processes [169, 170].

Tyrosine in ferroptosis

The TYRO3 protein tyrosine kinase is clinically associated with resistance to anti-PD-1/PD-L1 therapy in cancer patients, indicating its potential as a predictive biomarker for patient stratification aimed at enhancing treatment outcomes. Prior research has established that TYRO3 is crucial for tumor cell proliferation; however, it may also facilitate resistance to anti-PD-1/PD-L1 therapy by inhibiting ferroptosis and fostering a pro-tumor TME. Furthermore, the role of metabolic reprogramming in tumorigenesis and its impact on therapy resistance remains inadequately understood [171, 172]. Alterations in the TME can significantly influence metabolic pathways, presenting challenges for therapies targeting cancer metabolism [173].

Discoidin domain receptor tyrosine kinase 2 (DDR2) is implicated in the process of epithelial-mesenchymal transition. Standard treatment for breast cancer typically involves the surgical excision of the primary tumor, followed by various combinations of adjuvant therapies, including radiotherapy, chemotherapy, and hormone therapy. While most patients respond favorably to these interventions and may achieve remission, a subset of patients will experience recurrent breast cancer months to years later [174]. Recent research has identified extensive epigenomic and transcriptional alterations in recurrent tumors, particularly concerning RIPK3, G9a histone methyltransferase, and heightened NRF2 activation, all of which play critical roles in tumor recurrence [175–177]. A notable characteristic of both mouse and human recurrent tumors is the occurrence of EMT, which entails dynamic transitions from epithelial to mesenchymal phenotypes, thereby enhancing cellular migration and invasion [178]. This process also reestablishes metabolic pathways, prompting tumor cells to depend on extracellular cysteine uptake, which increases their vulnerability to ferroptosis [179]. DDR2 facilitates ferroptosis in recurrent tumor cells through *SRC*-mediated activation of YAP/TAZ, suggesting that inducing ferroptosis may offer significant therapeutic opportunities [180]. Additionally, Sirtuin 3 has been shown to positively regulate autophagy by promoting AMPK phosphorylation. The combination of erastin and TGF-β1 treatment leads to a reduction in EMT-related markers, thereby inhibiting breast cancer invasion and metastasis. Furthermore, SIRT3-mediated

autophagy enhances anticancer effects through the BECN1-SLC7A11 complex, representing a promising therapeutic strategy for breast cancer.

Previous studies have identified several mechanisms of resistance to anti-HER2 therapy in HER2-positive breast cancer, including the presence of p95 HER2 truncated proteins and MUC4 overexpression, which result in the loss of trastuzumab binding sites [181]. Additionally, constitutive activation of the HER2 signaling pathway is attributed to the dysregulation of downstream signaling components, such as PIK3CA mutation and PTEN loss [182]. Unfortunately, most inhibitors developed to target these pathways have not succeeded in overcoming anti-HER2 resistance in clinical trials. Furthermore, there is a notable absence of biomarkers capable of accurately predicting treatment responses and relapse risks following neoadjuvant and adjuvant anti-HER2 therapy. Therefore, identifying robust genomic resistance targets is essential for developing new treatment options for patients who do not respond to anti-HER2 therapies. One study suggested that the upregulation of fibroblast growth factor receptor 4 (FGFR4) contributes to anti-HER2 resistance by reducing ferroptosis in breast cancer. The combined inhibition of FGFR4 and HER2 may induce synergistic ferroptosis. Specifically, FGFR4 inhibition significantly diminishes glutathione synthesis and the efficiency of Fe^{2+} efflux through the β-catenin/TCF4-SLC7A11/FPN1 axis, which results in ROS production and the accumulation of an unstable iron pool. These findings shed light on the mechanisms underlying anti-HER2 resistance and propose a potential strategy to overcome resistance in refractory HER2-positive breast cancer through FGFR4 inhibition [183]. Additionally, Circ-BGN is significantly elevated in trastuzumab-resistant breast cancer cells and tissues, and this elevation correlates with poorer overall survival. Silencing circ-BGN inhibits breast cancer cell viability and markedly restores sensitivity to trastuzumab. Furthermore, circ-BGN can directly bind to OTUB1 and SLC7A11, enhancing the deubiquitination of SLC7A11 mediated by OTUB1, which subsequently inhibits ferroptosis. Erastin effectively restores the antitumor effects of trastuzumab. Preclinical in situ tumor models demonstrate that erastin significantly reduces tumor volume in trastuzumab-resistant breast cancer cells, particularly when combined with circ-BGN knockout. These findings reveal a novel circRNA that regulates trastuzumab resistance through the modulation of ferroptosis, thereby providing a promising therapeutic strategy for patients with trastuzumab-resistant breast cancer [184].

A recent report indicates that the breast cancer cell lines MDA-MB-231, MCF-7, ZR-75, and SKBr3 can synergistically induce cell death when treated with a combination of siramizine and lapatinib [185]. This mode of cell death is associated with elevated levels of Lipid-ROS and FeCl3. Furthermore, the application of ferrostatin-1 and DFO was found to reverse the cell death induced by siramizine and lapatinib. Notably, lapatinib, whether administered alone or in conjunction with siramizine, resulted in reduced expression of ferroportin, which in turn decreased the export of iron into the extracellular space. Concurrently, lapatinib enhanced the expression of transferrin, thereby promoting iron transport into the cells, increasing intracellular iron content, and catalyzing the production of iron-dependent Lipid-ROS. The overexpression of ferroportin or the reduction of transferrin expression led to decreased intracellular Lipid-ROS production and inhibited cell death. These findings suggest that the synergistic anticancer effects of siramizine and lapatinib operate through the ferroptosis pathway. Given that lipid-ROS serve as a critical trigger for ferroptosis, the significance of lipid metabolism has been extensively studied. Among the acyl-CoA synthetases, ACSL4 has been specifically linked to ferroptosis due to its association with acylated polyunsaturated fatty acids, particularly arachidonic acid (AA) and adrenic acid (AdA) [186]. These fatty acids are subsequently incorporated into membrane phospholipids by lysophosphatidylcholine acyltransferase 3 (LPCAT3). In the context of breast cancer, ACSL4 is reported to be preferentially expressed in a subset of basal-like breast cancer cell lines, where it promotes ferroptosis by enriching the cell membrane with long-chain polyunsaturated n-6 fatty acids [169].

Current research on ferroptosis in breast cancer has mainly focused on TNBC. Small molecule targeted drug therapy has emerged as a preferred treatment approach. Among the various drug delivery systems, exosomes-micromembrane vesicleshave garnered significant attention due to their potential to transport low-molecular-weight chemotherapy drugs in cancer treatment [187–189]. A novel formulation of exosomes loaded with folic acid (FA), referred to as erastin@FA-exo, has been developed [190]. This formulation operates by inhibiting GPX4 expression and up-regulating the expression of dioxygenase (CDO1), leading to a reduction in GSH levels, an increase in ROS levels, and the promotion of ferroptosis in TNBC cells that overexpress FA receptors. Compared to free erastin, erastin@FA-exo exhibits a significantly stronger capacity to inhibit the proliferation and migration of TNBC cells [191]. These findings suggest that exosome-based drug delivery systems may offer a novel therapeutic option and direction for the treatment of TNBC (Fig. 6).

Targeted therapy of kinases (Brief Introduction)

The human genome encodes 538 protein kinases, which are responsible for transferring γ-phosphate groups from ATP to serine, threonine, or tyrosine residues. Many of these kinases are implicated in the initiation and

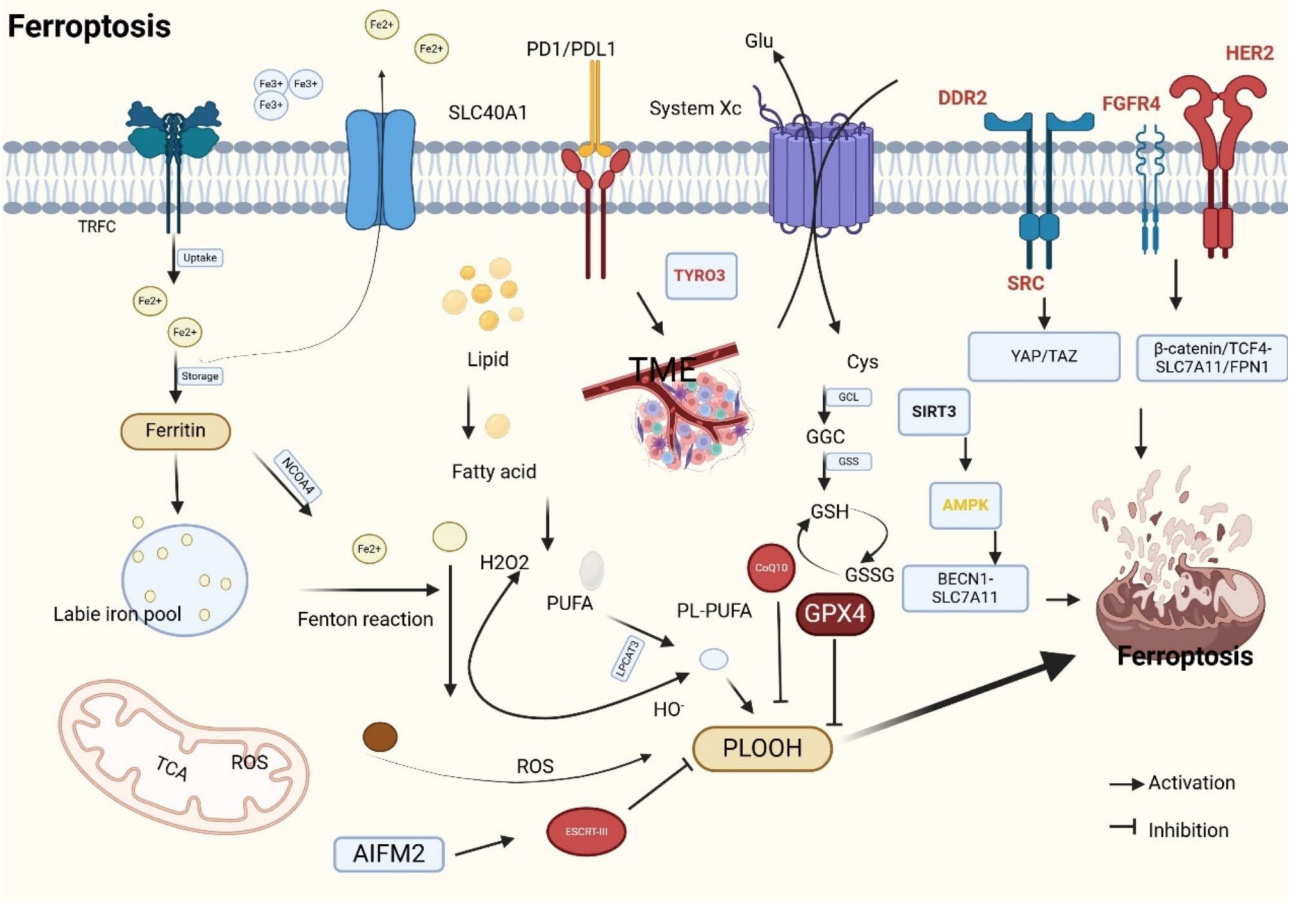

Fig. 6 The core mechanism of ferroptosis and related kinases affect the signaling pathway. Ferroptosis is characterized by the disruption of iron homeostasis, the production of toxic lipid peroxides, and the inhibition of antioxidant systems. Key components that promote ferroptosis include the transferrin-transferrin receptor (TF-TFRC) complex, iron export transporters, and ferritin autophagy, all of which contribute to increased iron accumulation, limited iron efflux, and reduced iron storage, respectively. The overload of divalent iron ions also causes the Fenton reaction, which generates excessive free radicals and then oxidises phospholipids, generating toxic lipid peroxides and triggering ferroptosis. The ACSL4-LPCAT3-ALOXs pathway is crucial for promoting ferroptosis by activating lipid peroxidation, which produces PLOOH from PUFA in conjunction with Rab7A-dependent fat phagocytosis. Various antioxidant systems, including the Xc-system-GSH-GPX4, AIFM2-CoQ10, GTP-GCH1-BH4, and ESCRT-II membrane repair systems, play a role in inhibiting lipid peroxidation. Additionally, TYRO3 can enhance the resistance of tumor cells to PD-1/PD-L1 therapy by inhibiting ferroptosis and supporting a pro-tumor TME. DDR2 activates YAP/TAZ-mediated expression through *SRC*, promoting ferroptosis in relapsed tumor cells. In breast cancer, FGFR4 is upregulated to confer resistance to HER2-targeted therapies by alleviating ferroptosis, and the combined inhibition of FGFR4 and HER2 may elicit a synergistic therapeutic effect by inducing ferroptosis. Furthermore, Sirtuin 3 positively regulates autophagy via the phosphorylation of AMPK. The combination of erastin and TGF-β1 therapy can reduce the expression of EMT-related markers and inhibit the invasion and metastasis of breast cancer. (Red: Tyrosine kinases; Yellow: Serine-threonine kinases.)

progression of human cancers. Recently developed small-molecule kinase inhibitors have demonstrated clinical success in treating various cancer types. Since the introduction of the first protein kinase inhibitor in the early 1980s, the FDA has approved 37 kinase inhibitors for the treatment of malignancies, including breast and lung cancer. Furthermore, approximately 150 kinase-targeted drugs are currently undergoing clinical trials, with many kinase-specific inhibitors still in the preclinical development phase. Despite these advancements, tumor recurrence continues to pose a significant challenge in the management of breast cancer. The factors contributing to tumor recurrence are multifaceted, including the presence of breast cancer stem cell-like cells (BCSCs) within primary tumors and metastatic sites. CSCs represent a subset of tumor cells that possess self-renewal capabilities and the potential to drive tumorigenesis. BCSCs are identified by the expression of specific cell surface markers such as EpCAM, CD24, and CD44 [192]. Molecular analyses of the EpCAM/CD24/CD44 and ALDH + ve CSCs indicate that the former subgroup exhibits a quiescent EMT phenotype, whereas ALDH + ve CSCs display an epithelial phenotype combined with self-renewal capability [193]. The tumor microenvironment is composed of cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), mesenchymal

stem cells (MSCs), and various immune and vascular cells, all of which contribute to the maintenance of BCSCs. RTK signaling in both tumor and stromal cells plays a vital role in regulating the phenotypes of CD24, CD44, and ALDH-positive cancer stem cells [194, 195]. BCSCs significantly influence cancer treatment outcomes, as they demonstrate resistance to conventional chemotherapy through the expression of multidrug resistance (MDR) genes. Following neoadjuvant chemotherapy, breast cancer patients exhibit an increase in the CD44/CD24 tumor cell fractions [196]. Altered or dysregulated RTK expression has been associated with the BCSC phenotype and resistance to therapy. Some studies indicate that RTK-targeted therapies may reverse multidrug resistance in breast cancer [197–199]. nRTKs activated by various RTKs, regulate critical cancer-related cellular processes, including polarity, proliferation, differentiation, migration, invasion, and angiogenesis [200]. Notably, *SRC*, the first identified proto-oncogene tyrosine kinase, is frequently linked to tumorigenesis and metastasis across multiple cancer types, particularly in breast cancer [201]. As a result, targeted therapies against tyrosine kinases have emerged as essential strategies in the treatment of breast cancer.

Anti-breast cancer drugs that target kinases

Breast cancer is a heterogeneous disease that can be classified into five molecular subtypes based on the expression of ER, PR, and HER2. These subtypes include Luminal A (characterized by low grade, ER+/PR+, HER2-, and low Ki67), Luminal B (ER+/PR+, HER2 + or HER2-, and high Ki67), triple-negative breast cancer or basal-like breast cancer (ER-/PR- and HER2-), HER2-enriched, and normal-like breast cancer [202]. For hormone receptor-positive breast cancer (Luminal A and B), standard adjuvant treatments include hormone therapy with selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene [203]. In contrast, hormone therapy is ineffective for TNBC and HER2-enriched breast cancer, as these subtypes lack hormone receptor expression [204]. Given the significant expression of RTKs in TNBC and HER2-enriched subtypes, targeting RTK functions presents a promising therapeutic strategy for these cancers.

Various strategies have been employed to inhibit RTK-dependent signaling. Mutations or overexpression of the EGFR gene can lead to tumor progression and resistance in multiple cancers, including breast cancer. Consequently, EGFR presents as an attractive drug target in breast cancer, prompting the development of EGFR inhibitors, such as small-molecule inhibitors and monoclonal antibodies (mAbs), some of which are currently in clinical use. In breast cancer, HER2 overexpression is prevalent, leading to the development of several HER2-targeted therapies. Trastuzumab (Herceptin), a humanized monoclonal antibody, specifically targets the extracellular domain of HER2 in HER2-positive breast cancer, and has been reported to improve survival rates for both early and advanced-stage patients [205]. However, the precise mechanism of action for trastuzumab remains unclear. Some studies suggest that trastuzumab inhibits HER2-HER3 heterodimerization, a process known to occur in HER2-positive breast cancer in a ligand-independent manner. Other investigations indicat that trastuzumab may induce HER2 degradation, although the underlying mechanisms have not been thoroughly explored [206]. Despite the significant improvement in disease prognosis associated with trastuzumab, resistance remains a major challenge in the treatment of HER2-positive breast cancer. Approximately 65% of HER2-positive breast cancer patients do not respond to initial trastuzumab therapy, and most patients who initially respond well eventually experience tumor recurrence [207, 208].

In 2013, the FDA approved the antibody-drug conjugate T-DM1 (trastuzumab emtansine or ado-trastuzumab emtansine, trade name Kadcyla) for the treatment of HER2-positive metastatic breast cancer in patients who had previously received trastuzumab and taxane therapy. T-DM1 comprises trastuzumab linked to the cytotoxic agent emtansine (DM1), which induces cancer cell death by binding to tubulin [209]. A randomized trial involving 991 HER2-positive advanced breast cancer patients demonstrated that those treated with T-DM1 experienced a longer median progression-free survival compared to those receiving lapatinib plus capecitabine [210]. However, a recently completed phase III trial involving 1,095 HER2-positive advanced breast cancer patients compared the efficacy of trastuzumab plus taxane, T-DM1 plus placebo, T-DM1 alone, and T-DM1 plus pertuzumab at standard doses. The results indicated that progression-free survival was not significantly enhanced in the T-DM1 and T-DM1 plus pertuzumab groups relative to the trastuzumab plus taxane group, although the arms incorporating T-DM1 exhibited superior tolerability [211]. Pertuzumab (trade name Perjeta) is another monoclonal antibody that targets HER2 and has been approved for use in combination with trastuzumab and docetaxel for neoadjuvant or adjuvant treatment of HER2-positive advanced breast cancer. Clinical trials have demonstrated that the combination of pertuzumab, trastuzumab, and docetaxel significantly improves progression-free survival in breast cancer patients compared to control groups [212, 213] (Table 2).

Table 2 Current therapy protein kinase inhibitors

Drugs	Mechanism	target	stage	Ref
Trastuzumab	Inhibits HER2	HER2	all stage	[184]
Doxorubicin	Knockdown of AMPKα1 promotes doxorubicin-induced apoptosis of breast cancer cells	AMPK α1	all stage	[228]
Lapatinib	Inhibits EGFR, HER2	EGFR, HER2	all stage	[117]
Trastuzumab	Inhibits HFR2 and HER3 dimerization, induces ADCC	HER2	all stage	[229]
Afatinib	Inhibits EGFR and HER2 signalling irreversibly	HER2/EGFR	LUX Breast1/3	[230]
Dacomitinib	Inhibits HER2, EGFR, HER4, Akt and ERK phosphorylation	EGFR, HER2, ErbB4	Phase 1B	[231]
Neratinib	Irreversibly blocks EGFR and HER2 pathway	EGFR, RET VEGFR2-3	Phase III NALA Trial	[232]
Bosutinib	inhibition of *SRC*-mediated signaling pathways	*SRC*, ABL	Phase II	[217]
Dasatinib	*SRC* inhibition affects P-cadherin downstream signaling	*SRC*	Phase I, II	[233]
Palbociclib	HR positive and EGFR negative	CDK4/6	Phase 1B	[234]
Everolimus	HR positive	mTOR	Drug resistant after metastatic	[212]
Alpelisib	HR positive	PI3K	Late stage	[235]
Olaparib	BRCA mutation	PARP	All stage	[236]

Potential use of kinase inhibitors in breast cancer treatment

Given the significant and pervasive role of *SRC* kinase in breast tumorigenesis and metastasis, *SRC* kinase inhibitors present a promising the rapeutic avenue for BC. Several *SRC* kinase inhibitors, including bosutinib, dasatinib, and saracatinib, have been developed by pharmaceutical companies and subsequently approved by the FDA for the treatment of hematologic malignancies [214]. Currently, these agents are also being rigorously investigated in clinical trials for their efficacy in treating BC. Bosutinib, a multi-kinase inhibitor, is effective against all SFKs as well as ABL. Preclinical studies have shown that bosutinib can inhibit the growth, invasion, and metastasis of BC cells both in vitro and in vivo [215, 216]. Furthermore, oral administration of bosutinib in the MMTV-PyVmT transgenic mouse model has demonstrated the ability to inhibit tumorigenesis and growth in older animals with pre-existing tumors [217]. A phase II clinical trial involving patients with metastatic BC revealed that bosutinib monotherapy exhibited tolerable safety and moderate antitumor activity in certain hormone receptor-positive BC patients [218]. Additionally, a phase I study found that the combination of bosutinib and capecitabine was safe; however, the efficacy observed in cases of locally advanced or metastatic BC was limited [219]. Consequently, after the implementation of appropriate patient selection methods, further investigation into the combination of bosutinib with other therapeutic agents is warranted (Table 2).

Dasatinib is an oral small molecule drug that targets multiple SFKs, including *SRC*, *LCK*, *FYN*, and *YES* [220]. Extensive preclinical studies have demonstrated that dasatinib exhibits significant antitumor efficacy across various BC subtypes. However, clinical research has shown that its effectiveness is markedly limited when administered as a monotherapy in patients with TNBC and metastatic BC [116, 221]. Consequently, a phase II study was conducted to prospectively evaluate the clinical benefits of previously identified gene characteristics for selecting patients who are likely to benefit from dasatinib treatment [222]. Unfortunately, these gene characteristics did not effectively predict the clinical sensitivity of dasatinib as a single agent. Therefore, these findings underscore the limited monotherapy activity of dasatinib in unselected BC patients, suggesting that future research should explore the potential of combining dasatinib with other therapeutic agents for specific BC patient populations.

Saracatinib is an *SRC*-ABL kinase inhibitor that exhibits moderate and manageable adverse effects when compared to dasatinib. An early preclinical study reported that saracatinib and tamoxifen could synergistically inhibit the growth of human ER-positive BC cells [223]. Additionally, the combination of saracatinib and tamoxifen was effective in preventing in vitro hormone resistance in human BC cells [224]. Notably, in the MMTV-Neu mouse model, saracatinib significantly inhibited the formation of precancerous lesions and delayed tumor onset [225]. Recently, the *SRC* inhibitor eCF506 has been identified as having greater selectivity and specificity for SFKs than the previously mentioned *SRC* kinase inhibitors. Additionally, eCF506 has been shown to reduce the growth of TNBC cells both in vitro and in vivo, demonstrating effects similar to those of bosutinib [226]. In a mouse model of TNBC metastasis, eCF506 exhibited highly effective antitumor activity against both primary tumors and bone metastases [227]. Given these promising preclinical findings, eCF506 is anticipated to emerge as a pioneering clinical candidate for the treatment of *SRC*-related breast cancer in the future.

Conclusions and prospects

Kinases play a critical role in breast cancer by regulating cell signaling pathways that influence essential biological processes, including cell growth, proliferation, and metastasis. Aberrantly activated kinases are frequently associated with tumor initiation, progression, and metastasis in breast cancer. These kinases can enhance the proliferation, invasion, and migration of tumor cells and are linked to drug resistance, making them significant targets for targeted therapies. Therefore, in-depth research into the role of kinases in breast cancer is vital for improving treatment outcomes. A comprehensive understanding of the specific mechanisms through which kinases contribute to the occurrence, progression, and metastasis of breast cancer is necessary for the development of more effective targeted treatment strategies. Additionally, studying the interactions between kinases and other signaling pathways in breast cancer is crucial for understanding the mechanisms of drug resistance, which can inform the development of more effective treatment strategies. Future research should encompass a comprehensive examination of the mechanisms of action of kinase inhibitors, the identification of more selective and effective drugs, and the formulation of personalized treatment approaches tailored to various breast cancer subtypes, while considering tumor heterogeneity and individual patient characteristics. Additionally, exploring multi-target combination therapies may prove advantageous in tackling the complexities associated with tumors and drug resistance. The development of novel tyrosine kinase inhibitors is also a pivotal focus in breast cancer treatment, as these agents can specifically inhibit tyrosine kinases, thereby obstructing the growth, proliferation, and metastasis of cancer cells. Future studies should prioritize the creation of more selective, effective, and less toxic tyrosine kinase inhibitors to enhance survival rates and improve the quality of life for breast cancer patients. Lastly, personalized treatment strategies are increasingly recognized as a significant trend in breast cancer therapy. Customizing treatment plans based on patients' genotypes, phenotypes, and the molecular characteristics of tumors-including targeted therapies aimed at tyrosine kinases-holds promise for improving treatment efficacy and survival rates while minimizing adverse side effects. This approach may involve the combination of multiple targeted therapies, the identification of new therapeutic targets, and the application of combination therapies. Moreover, strengthening research on the mechanisms of drug resistance will be instrumental in predicting and effectively managing the emergence of resistance.

Abbreviations

AA	arachidonic acid
ACSL4	long-chain acyl-CoA synthetase 4
ADA	adrenic acid
AGC	A G and C kinases
APAF1	Apoptosis Protease Activating Factor-1
BCL2	B-cell lymphoma 2
BCSCs	Breast cancer stem cell-like cells
Bif-1	Bax-interacting factor 1
CDIO 1	Cysteine Dioxygenase 1
CLK	Cdc2-like kinases
CAFs	Cancer-associated fibroblasts
CAMK	Ca^{2+}/calmodulin-dependent kinases
CK1	Casein kinase 1
CMGC	Cyclin-dependent kinases
CSC	Cancer stem cell
C-SH2	C-terminal SH2 domain
DDR2	Discoidin domain receptor tyrosine kinase 2
DISC	Death-inducing signaling complex
DRs	Death receptors
DMT1	Divalent metal transporter 1
DISC	Death-induced signal transduction
DAPKs	Death-associated protein kinases
EGFR	Epidermal growth factor receptor
EMP	Epithelial-mesenchymal plasticity
EMT	Epithelial-mesenchymal transition
ERK1	Extracellular signal-regulated kinase 1
FTH1	Ferritin heavy chain 1
FTL	Ferritin light chain
FADD	Fas-associated death domain protein
GZM	Granzyme
GPX4	Glutathione peroxidase 4
GSK-3	Glycogen synthase kinase-3
HME2	Human mammary epithelial cell
IGFIR	The type I insulin-like growth factor receptor
JAK2	Janus kinase 2
LRRK2	Leucine-rich repeat kinase 2
LIP	Labile iron pool
LPCAT3	Lysophosphatidylcholine acyltransferase 3
MSCs	Mesenchymal stem cells
MOMP	Mitochondrial outer membrane permeabilization
MAPK	Mitogen-activated protein kinase
MDR	Multidrug resistance
MELK	Maternal embryonic leucine zipper kinase
MISC	Membrane immunosuppressive compartment
MLKL	Mixed lineage kinase domain-like
MSCs	Mesenchymal stem cells
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
nRTKs	Non-receptor tyrosine kinases
NCOA4	Nuclear receptor coactivator 4
OMM	Outer mitochondrial membrane
PFD	N-terminal pore-forming domain
PUFA-PL	PUFA-containing phospholipids
PEBP1	Phosphatidylethanolamine binding protein
PDT	Photodynamic therapy
PI3K	Phosphoinositide 3-kinase PI3K
PI3Kα	Phosphoinositide 3-kinaseα
PKC	Protein kinase C
PKs	Human protein kinases
PLK1	Polo-like kinase 1
PPI	Protein-protein interactions
PTP	Protein tyrosine phosphatase
PUFAs	Polyunsaturated fatty acids
RIPK1	Receptor-interacting protein kinase 1
RGC	Receptor guanylate cyclases
RIP1	Receptor-interacting protein 1
RTKs	Receptor tyrosine kinases
STE	Homologs of yeast sterile 7, 11, and 20 kinases
SFKs	SRC family kinases
STEAP3	Sextant transmembrane epithelial antigen 3
SLC11A2	Solute carrier family 11 member 2
SLC40A1	Carrier family 40 member 1
Stearoyl-AA-PE	Stearoyl-arachidonoyl-phosphatidylethanolamine
SERMs	Selective estrogen receptor modulators
STAT3	Signal Transducer and Activator of Transcription 3

SYK	Spleen tyrosine kinase
TRADD	TNFR1-associated death domain protein
TLR3	Toll-like receptors 3
TLR4	Toll-like receptors 4
TF	Transferrin
TFR1	Transferrin receptor 1
TAMs	Tumor-associated macrophages
TKL	Tyrosine kinase-like proteins
TKs	Tyrosine kinases
TME	Tumor microenvironment
TNBC	Triple-negative breast cancer
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRAIL	TNF-related apoptosis-inducing ligand receptor-1
xCT	Cystine/glutamate antiporter
ZBP1	Z-DNA binding protein 1

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Declarations

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Consent for publication
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Competing interests
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References

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Sci (New York NY). 2002;298(5600):1912–34.
2. Lindberg RA, Quinn AM, Hunter T. Dual-specificity protein kinases: will any hydroxyl do? Trends in biochemical sciences 1992, 17(3):114–9.
3. Shchemelinin I, Sefc L, Necas E. Protein kinases, their function and implication in cancer and other diseases. Folia Biol. 2006;52(3):81–100.
4. Cheng HC, Qi RZ, Paudel H, Zhu HJ. Regulation and function of protein kinases and phosphatases. Enzyme Res. 2011;2011:794089.
5. Roskoski R Jr. Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. Pharmacol Res. 2015;94:9–25.
6. Kondapalli L, Soltani K, Lacouture ME. The promise of molecular targeted therapies: protein kinase inhibitors in the treatment of cutaneous malignancies. J Am Acad Dermatol. 2005;53(2):291–302.
7. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature. 2001;411(6835):355–65.
8. Butti R, Das S, Gunasekaran VP, Yadav AS, Kumar D, Kundu GC. Receptor tyrosine kinases (RTKs) in breast cancer: signaling, therapeutic implications and challenges. Mol Cancer. 2018;17(1):34.
9. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. Lancet (London England). 2021;397(10286):1750–69.
10. DeRyckere D, Huelse JM, Earp HS, Graham DK. TAM family kinases as therapeutic targets at the interface of cancer and immunity. Nat Reviews Clin Oncol. 2023;20(11):755–79.
11. Qian BZ, Zhang H, Li J, He T, Yeo EJ, Soong DY, Carragher NO, Munro A, Chang A, Bresnick AR, et al. FLT1 signaling in metastasis-associated macrophages activates an inflammatory signature that promotes breast cancer metastasis. J Exp Med. 2015;212(9):1433–48.
12. Arteaga CL, Sliwkowski MX, Osborne CK, Perez EA, Puglisi F, Gianni L. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Reviews Clin Oncol. 2011;9(1):16–32.
13. Liao M, Qin R, Huang W, Zhu HP, Peng F, Han B, Liu B. Targeting regulated cell death (RCD) with small-molecule compounds in triple-negative breast cancer: a revisited perspective from molecular mechanisms to targeted therapies. J Hematol Oncol. 2022;15(1):44.
14. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006;7(8):606–19.
15. Wise R, Zolkiewska A. Metalloprotease-dependent activation of EGFR modulates CD44(+)/CD24(-) populations in triple negative breast cancer cells through the MEK/ERK pathway. Breast Cancer Res Treat. 2017;166(2):421–33.
16. Ibrahim SA, Gadalla R, El-Ghonaimy EA, Samir O, Mohamed HT, Hassan H, Greve B, El-Shinawi M, Mohamed MM, Götte M. Syndecan-1 is a novel molecular marker for triple negative inflammatory breast cancer and modulates the cancer stem cell phenotype via the IL-6/STAT3, Notch and EGFR signaling pathways. Mol Cancer. 2017;16(1):57.
17. Lopez J, Tait SW. Mitochondrial apoptosis: killing cancer using the enemy within. Br J Cancer. 2015;112(6):957–62.
18. Jin Z, El-Deiry WS. Overview of cell death signaling pathways. Cancer Biol Ther. 2005;4(2):139–63.
19. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006;25(34):4798–811.
20. Fuchs Y, Steller H. Live to die another way: modes of programmed cell death and the signals emanating from dying cells. Nat Rev Mol Cell Biol. 2015;16(6):329–44.
21. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in Cell-free extracts: requirement for dATP and Cytochrome C. Cell. 1996;86(1):147–57.
22. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Alnemri ES. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol Cell. 1998;1(7):949–57.
23. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell. 2002;2(3):183–92.
24. Edlich F, Banerjee S, Suzuki M, Cleland MM, Arnoult D, Wang C, Neutzner A, Tjandra N, Youle RJ. Bcl-x(L) retrotranslocates Bax from the mitochondria into the cytosol. Cell. 2011;145(1):104–16.
25. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging. 2016;8(4):603–19.
26. Kischkel FC, Lawrence DA, Tinel A, LeBlanc H, Virmani A, Schow P, Gazdar A, Blenis J, Arnott D, Ashkenazi A. Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. J Biol Chem. 2001;276(49):46639–46.
27. Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell. 2003;114(2):181–90.
28. Bozec D, Iuga AC, Roda G, Dahan S, Yeretssian G. Critical function of the necroptosis adaptor RIPK3 in protecting from intestinal tumorigenesis. Oncotarget. 2016;7(29):46384–400.
29. de Melo Gagliato D, Jardim DL, Marchesi MS, Hortobagyi GN. Mechanisms of resistance and sensitivity to anti-HER2 therapies in HER2 + breast cancer. Oncotarget. 2016;7(39):64431–46.
30. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen PL, Bono P, Kataja V, Piccart MJ, Joensuu H, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J Natl Cancer Inst. 2013;105(13):960–7.

31. Ruiz-Saenz A, Dreyer C, Campbell MR, Steri V, Gulizia N, Moasser MM. HER2 amplification in tumors activates PI3K/Akt signaling independent of HER3. Cancer Res. 2018;78(13):3645–58.
32. Martínez-Sáez O, Chic N, Pascual T, Adamo B, Vidal M, González-Farré B, Sanfeliu E, Schettini F, Conte B, Brasó-Maristany F, et al. Frequency and spectrum of PIK3CA somatic mutations in breast cancer. Breast cancer Research: BCR. 2020;22(1):45.
33. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer. 2008;8(12):915–28.
34. Saldana SM, Lee HH, Lowery FJ, Khotskaya YB, Xia W, Zhang C, Chang SS, Chou CK, Steeg PS, Yu D, et al. Inhibition of type I insulin-like growth factor receptor signaling attenuates the development of breast cancer brain metastasis. PLoS ONE. 2013;8(9):e73406.
35. Esparís-Ogando A, Ocaña A, Rodríguez-Barrueco R, Ferreira L, Borges J, Pandiella A. Synergic antitumoral effect of an IGF-IR inhibitor and trastuzumab on HER2-overexpressing breast cancer cells. Annals Oncology: Official J Eur Soc Med Oncol. 2008;19(11):1860–9.
36. Caputo E, Manco G, Mandrich L, Guardiola J. A novel aspartyl proteinase from apocrine epithelia and breast tumors. J Biol Chem. 2000;275(11):7935–41.
37. Basmaciogullari S, Autiero M, Culerrier R, Mani JC, Gaubin M, Mishal Z, Guardiola J, Granier C, Piatier-Tonneau D. Mapping the CD4 binding domain of gp17, a glycoprotein secreted from seminal vesicles and breast carcinomas. Biochemistry. 2000;39(18):5332–40.
38. Yoder BJ, Tso E, Skacel M, Pettay J, Tarr S, Budd T, Tubbs RR, Adams JC, Hicks DG. The expression of fascin, an actin-bundling motility protein, correlates with hormone receptor-negative breast cancer and a more aggressive clinical course. Clin cancer Research: Official J Am Association Cancer Res. 2005;11(1):186–92.
39. Carracedo A, Lorente M, Egia A, Blázquez C, García S, Giroux V, Malicet C, Villuendas R, Gironella M, González-Feria L, et al. The stress-regulated protein p8 mediates cannabinoid-induced apoptosis of tumor cells. Cancer Cell. 2006;9(4):301–12.
40. Keppler D. Towards novel anti-cancer strategies based on Cystatin function. Cancer Lett. 2006;235(2):159–76.
41. Wu C, Guan Q, Wang Y, Zhao ZJ, Zhou GW. SHP-1 suppresses cancer cell growth by promoting degradation of JAK kinases. J Cell Biochem. 2003;90(5):1026–37.
42. Bousquet C, Delesque N, Lopez F, Saint-Laurent N, Estève JP, Bedecs K, Buscail L, Vaysse N, Susini C. sst2 somatostatin receptor mediates negative regulation of insulin receptor signaling through the tyrosine phosphatase SHP-1. J Biol Chem. 1998;273(12):7099–106.
43. Reck M. Nintedanib: examining the development and mechanism of action of a novel triple angiokinase inhibitor. Expert Rev Anticancer Ther. 2015;15(5):579–94.
44. Liu CY, Huang TT, Chu PY, Huang CT, Lee CH, Wang WL, Lau KY, Tsai WC, Chao TI, Su JC, et al. The tyrosine kinase inhibitor nintedanib activates SHP-1 and induces apoptosis in triple-negative breast cancer cells. Exp Mol Med. 2017;49(8):e366.
45. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. Oncogene. 2000;19(49):5548–57.
46. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2000;103(2):211–25.
47. Balko JM, Cook RS, Vaught DB, Kuba MG, Miller TW, Bhola NE, Sanders ME, Granja-Ingram NM, Smith JJ, Meszoely IM, et al. Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. Nat Med. 2012;18(7):1052–9.
48. Li HC, Xia ZH, Chen YF, Yang F, Feng W, Cai H, Mei Y, Jiang YM, Xu K, Feng DX. Cantharidin inhibits the growth of Triple-Negative breast cancer cells by suppressing autophagy and inducing apoptosis in vitro and in vivo. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and Pharmacology 2017, 43(5):1829–40.
49. Su CC, Lee KI, Chen MK, Kuo CY, Tang CH, Liu SH. Cantharidin induced oral squamous cell carcinoma cell apoptosis via the JNK-Regulated mitochondria and Endoplasmic reticulum Stress-Related signaling pathways. PLoS ONE. 2016;11(12):e0168095.
50. Yang T, Yu R, Cheng C, Huo J, Gong Z, Cao H, Hu Y, Dai B, Zhang Y. Cantharidin induces apoptosis of human triple negative breast cancer cells through mir-607-mediated downregulation of EGFR. J Translational Med. 2023;21(1):597.
51. Spano D, Marshall JC, Marino N, De Martino D, Romano A, Scoppettuolo MN, Bello AM, Di Dato V, Navas L, De Vita G, et al. Dipyridamole prevents triple-negative breast-cancer progression. Clin Exp Metastasis. 2013;30(1):47–68.
52. Al Mamun A, Mimi AA, Aziz MA, Zaeem M, Ahmed T, Munir F, Xiao J. Role of pyroptosis in cancer and its therapeutic regulation. Eur J Pharmacol. 2021;910:174444.
53. Fang Y, Tian S, Pan Y, Li W, Wang Q, Tang Y, Yu T, Wu X, Shi Y, Ma P, et al. Pyroptosis: A new frontier in cancer. Volume 121. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie; 2020. p. 109595.
54. Song Z, Zou J, Wang M, Chen Z, Wang Q. A comparative review of pyroptosis in mammals and fish. J Inflamm Res. 2022;15:2323–31.
55. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526(7575):660–5.
56. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, Wang K, Shao F. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature. 2017;547(7661):99–103.
57. Wu D, Chen Y, Sun Y, Gao Q, Yu B, Jiang X, Guo M. Gasdermin family: a promising therapeutic target for cancers and inflammation-driven diseases. J Cell Communication Signal. 2020;14(3):293–301.
58. Liu Z, Wang C, Yang J, Zhou B, Yang R, Ramachandran R, Abbott DW, Xiao TS. Crystal structures of the Full-Length murine and human gasdermin D reveal mechanisms of autoinhibition, lipid binding, and oligomerization. Immunity. 2019;51(1):43–e4944.
59. Orning P, Weng D, Starheim K, Ratner D, Best Z, Lee B, Brooks A, Xia S, Wu H, Kelliher MA, et al. Pathogen Blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. Volume 362. New York, NY): Science; 2018. pp. 1064–9. 6418.
60. Zhang F, Liu Q, Ganesan K, Kewu Z, Shen J, Gang F, Luo X, Chen J. The Antitriple Negative Breast cancer Efficacy of Spatholobus suberectus Dunn on ROS-Induced Noncanonical Inflammasome Pyroptotic Pathway. Oxidative medicine and cellular longevity 2021, 2021:5187569.
61. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene. 2008;27(48):6194–206.
62. Rogers C, Fernandes-Alnemri T, Mayes L, Alnemri D, Cingolani G, Alnemri ES. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. Nat Commun. 2017;8:14128.
63. Hou J, Zhao R, Xia W, Chang CW, You Y, Hsu JM, Nie L, Chen Y, Wang YC, Liu C, et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. Nat Cell Biol. 2020;22(10):1264–75.
64. Chen KW, Demarco B, Heilig R, Shkarina K, Boettcher A, Farady CJ, Pelczar P, Broz P. Extrinsic and intrinsic apoptosis activate pannexin-1 to drive NLRP3 inflammasome assembly. EMBO J 2019, 38(10).
65. Sarhan J, Liu BC, Muendlein HI, Li P, Nilson R, Tang AY, Rongvaux A, Bunnell SC, Shao F, Green DR, et al. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during yersinia infection. Proc Natl Acad Sci USA. 2018;115(46):E10888–97.
66. Liu Y, Fang Y, Chen X, Wang Z, Liang X, Zhang T, Liu M, Zhou N, Lv J, Tang K et al. Gasdermin E-mediated target cell pyroptosis by CAR T cells triggers cytokine release syndrome. Sci Immunol 2020, 5(43).
67. Zhou Z, He H, Wang K, Shi X, Wang Y, Su Y, Wang Y, Li D, Liu W, Zhang Y et al. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. Science (New York, NY): 2020, 368(6494).
68. Labi V, Erlacher M. How cell death shapes cancer. Cell Death Dis. 2015;6(3):e1675.
69. Chauhan D, Vande Walle L, Lamkanfi M. Therapeutic modulation of inflammasome pathways. Immunol Rev. 2020;297(1):123–38.
70. Ershaid N, Sharon Y, Doron H, Raz Y, Shani O, Cohen N, Monteran L, Leider-Trejo L, Ben-Shmuel A, Yassin M, et al. NLRP3 inflammasome in fibroblasts links tissue damage with inflammation in breast cancer progression and metastasis. Nat Commun. 2019;10(1):4375.
71. Balko JM, Schwarz LJ, Luo N, Estrada MV, Giltnane JM, Dávila-González D, Wang K, Sánchez V, Dean PT, Combs SE, et al. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. Sci Transl Med. 2016;8(334):334ra353.
72. Duru N, Fan M, Candas D, Menaa C, Liu HC, Nantajit D, Wen Y, Xiao K, Eldridge A, Chromy BA, et al. HER2-associated radioresistance of breast cancer stem cells isolated from HER2-negative breast cancer cells. Clin cancer Research: Official J Am Association Cancer Res. 2012;18(24):6634–47.
73. Xiong H, Zhang ZG, Tian XQ, Sun DF, Liang QC, Zhang YJ, Lu R, Chen YX, Fang JY. Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. Neoplasia (New York NY). 2008;10(3):287–97.

74. Lo HW, Cao X, Zhu H, Ali-Osman F. Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin cancer Research: Official J Am Association Cancer Res. 2008;14(19):6042–54.
75. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang CH, Saso K, Butler MO, Minden MD, Hirano N. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. Nat Med. 2018;24(3):352–9.
76. Regua AT, Aguayo NR, Jalboush SA, Doheny DL, Manore SG, Zhu D, Wong GL, Arrigo A, Wagner CJ, Yu Y et al. TrkA interacts with and phosphorylates STAT3 to enhance gene transcription and promote breast cancer stem cells in Triple-Negative and HER2-Enriched breast cancers. Cancers 2021, 13(10).
77. Liu M, Li Y, Kong B, Zhang G, Zhang Q. Polydatin down-regulates the phosphorylation level of STAT3 and induces pyroptosis in triple-negative breast cancer mice with a high-fat diet. Annals Translational Med. 2022;10(4):173.
78. Volchuk A, Ye A, Chi L, Steinberg BE, Goldenberg NM. Indirect regulation of HMGB1 release by gasdermin D. Nat Commun. 2020;11(1):4561.
79. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018;281(1):8–27.
80. Wu TC, Xu K, Martinek J, Young RR, Banchereau R, George J, Turner J, Kim KI, Zurawski S, Wang X, et al. IL1 receptor antagonist controls transcriptional signature of inflammation in patients with metastatic breast cancer. Cancer Res. 2018;78(18):5243–58.
81. Ben-Sasson SZ, Hogg A, Hu-Li J, Wingfield P, Chen X, Crank M, Caucheteux S, Ratner-Hurevich M, Berzofsky JA, Nir-Paz R, et al. IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. J Exp Med. 2013;210(3):491–502.
82. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature. 1995;378(6552):88–91.
83. Fabbi M, Carbotti G, Ferrini S. Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP. J Leukoc Biol. 2015;97(4):665–75.
84. Liu X, Hu J, Li Y, Cao W, Wang Y, Ma Z, Li F. Mesenchymal stem cells expressing interleukin-18 inhibit breast cancer in a mouse model. Oncol Lett. 2018;15(5):6265–74.
85. Park IH, Yang HN, Lee KJ, Kim TS, Lee ES, Jung SY, Kwon Y, Kong SY. Tumor-derived IL-18 induces PD-1 expression on immunosuppressive NK cells in triple-negative breast cancer. Oncotarget. 2017;8(20):32722–30.
86. Zhang Z, Zhang H, Li D, Zhou X, Qin Q, Zhang Q. Caspase-3-mediated GSDME induced pyroptosis in breast cancer cells through the ROS/JNK signalling pathway. J Cell Mol Med. 2021;25(17):8159–68.
87. Bao Y, Qian C, Liu MY, Jiang F, Jiang X, Liu H, Zhang Z, Sun F, Fu N, Hou Z, et al. PRKAA/AMPKα phosphorylation switches the role of RASAL2 from a suppressor to an activator of autophagy. Autophagy. 2021;17(11):3607–21.
88. Pillai S, Mahmud I, Mahar R, Griffith C, Langsen M, Nguyen J, Wojtkowiak JW, Swietach P, Gatenby RA, Bui MM, et al. Lipogenesis mediated by OGR1 regulates metabolic adaptation to acid stress in cancer cells via autophagy. Cell Rep. 2022;39(6):110796.
89. Racanelli AC, Kikkers SA, Choi AMK, Cloonan SM. Autophagy and inflammation in chronic respiratory disease. Autophagy. 2018;14(2):221–32.
90. Tang C, Livingston MJ, Liu Z, Dong Z. Autophagy in kidney homeostasis and disease. Nat Rev Nephrol. 2020;16(9):489–508.
91. Onorati AV, Dyczynski M, Ojha R, Amaravadi RK. Targeting autophagy in cancer. Cancer. 2018;124(16):3307–18.
92. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell. 2011;147(4):728–41.
93. Li Q, Liu Y, Sun M. Autophagy and Alzheimer's disease. Cell Mol Neurobiol. 2017;37(3):377–88.
94. Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Volume 334. New York, NY): Science; 2011. pp. 678–83. 6056.
95. Jewell JL, Russell RC, Guan KL. Amino acid signalling upstream of mTOR. Nat Rev Mol Cell Biol. 2013;14(3):133–9.
96. Yadav RB, Burgos P, Parker AW, Iadevaia V, Proud CG, Allen RA, O'Connell JP, Jeshtadi A, Stubbs CD, Botchway SW. mTOR direct interactions with Rheb-GTPase and raptor: sub-cellular localization using fluorescence lifetime imaging. BMC Cell Biol. 2013;14:3.
97. Ktistakis NT, Tooze SA. Digesting the expanding mechanisms of autophagy. Trends Cell Biol. 2016;26(8):624–35.
98. Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, Kim H, Neufeld TP, Dillin A, Guan KL. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol. 2013;15(7):741–50.
99. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol Biol Cell. 2008;19(5):2092–100.
100. Kim YM, Jung CH, Seo M, Kim EK, Park JM, Bae SS, Kim DH. mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. Mol Cell. 2015;57(2):207–18.
101. Airiau K, Vacher P, Micheau O, Prouzet-Mauleon V, Kroemer G, Moosavi MA, Djavaheri-Mergny M. TRAIL triggers CRAC-Dependent calcium influx and apoptosis through the recruitment of autophagy proteins to Death-Inducing signaling complex. Cells 2021, 11(1).
102. Patra S, Praharaj PP, Klionsky DJ, Bhutia SK. Vorinostat in autophagic cell death: A critical insight into autophagy-mediated, -associated and -dependent cell death for cancer prevention. Drug Discovery Today. 2022;27(1):269–79.
103. Qiao Y, Choi JE, Tien JC, Simko SA, Rajendiran T, Vo JN, Delekta AD, Wang L, Xiao L, Hodge NB, et al. Autophagy Inhibition by targeting PIKfyve potentiates response to immune checkpoint Blockade in prostate cancer. Nat cancer. 2021;2:978–93.
104. Wen X, Klionsky DJ. At a glance: A history of autophagy and cancer. Sem Cancer Biol. 2020;66:3–11.
105. Li J, Chen X, Kang R, Zeh H, Klionsky DJ, Tang D. Regulation and function of autophagy in pancreatic cancer. Autophagy. 2021;17(11):3275–96.
106. Ariosa AR, Lahiri V, Lei Y, Yang Y, Yin Z, Zhang Z, Klionsky DJ. A perspective on the role of autophagy in cancer. Biochim Et Biophys Acta Mol Basis Disease. 2021;1867(12):166262.
107. O'Connell CE, Vassilev A. Combined Inhibition of p38MAPK and PIKfyve synergistically disrupts autophagy to selectively target cancer cells. Cancer Res. 2021;81(11):2903–17.
108. Liu J, Liu Y, Wang Y, Li C, Xie Y, Klionsky DJ, Kang R, Tang D. TMEM164 is a new determinant of autophagy-dependent ferroptosis. Autophagy. 2023;19(3):945–56.
109. Xue Q, Yan D, Chen X, Li X, Kang R, Klionsky DJ, Kroemer G, Chen X, Tang D, Liu J. Copper-dependent autophagic degradation of GPX4 drives ferroptosis. Autophagy. 2023;19(7):1982–96.
110. Chen X, Song X, Li J, Zhang R, Yu C, Zhou Z, Liu J, Liao S, Klionsky DJ, Kroemer G, et al. Identification of HPCAL1 as a specific autophagy receptor involved in ferroptosis. Autophagy. 2023;19(1):54–74.
111. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature. 1984;308(5961):693–8.
112. Basu A. Regulation of autophagy by protein kinase C-ε in breast cancer cells. Int J Mol Sci 2020, 21(12).
113. Nakano I, Masterman-Smith M, Saigusa K, Paucar AA, Horvath S, Shoemaker L, Watanabe M, Negro A, Bajpai R, Howes A, et al. Maternal embryonic leucine zipper kinase is a key regulator of the proliferation of malignant brain tumors, including brain tumor stem cells. J Neurosci Res. 2008;86(1):48–60.
114. Marie SK, Okamoto OK, Uno M, Hasegawa AP, Oba-Shinjo SM, Cohen T, Camargo AA, Kosoy A, Carlotti CG Jr., Toledo S, et al. Maternal embryonic leucine zipper kinase transcript abundance correlates with malignancy grade in human Astrocytomas. Int J Cancer. 2008;122(4):807–15.
115. Pickard MR, Green AR, Ellis IO, Caldas C, Hedge VL, Mourtada-Maarabouni M, Williams GT. Dysregulated expression of Fau and MELK is associated with poor prognosis in breast cancer. Breast cancer Research: BCR. 2009;11(4):R60.
116. Herold CI, Chadaram V, Peterson BL, Marcom PK, Hopkins J, Kimmick GG, Favaro J, Hamilton E, Welch RA, Bacus S, et al. Phase II trial of dasatinib in patients with metastatic breast cancer using real-time pharmacodynamic tissue biomarkers of Src Inhibition to escalate dosing. Clin cancer Research: Official J Am Association Cancer Res. 2011;17(18):6061–70.
117. Moreira C, Kaklamani V. Lapatinib and breast cancer: current indications and outlook for the future. Expert Rev Anticancer Ther. 2010;10(8):1171–82.
118. Ren XS, Sato Y, Harada K, Sasaki M, Furubo S, Song JY, Nakanuma Y. Activation of the PI3K/mTOR pathway is involved in cystic proliferation of cholangiocytes of the PCK rat. PLoS ONE. 2014;9(1):e87660.
119. Lin Z, Liu T, Kamp DW, Wang Y, He H, Zhou X, Li D, Yang L, Zhao B, Liu G. AKT/mTOR and c-Jun N-terminal kinase signaling pathways are required for Chrysotile asbestos-induced autophagy. Free Radic Biol Med. 2014;72:296–307.
120. Shinde A, Hardy SD, Kim D, Akhand SS, Jolly MK, Wang WH, Anderson JC, Khodadadi RB, Brown WS, George JT, et al. Spleen tyrosine Kinase-Mediated autophagy is required for Epithelial-Mesenchymal plasticity and metastasis in breast cancer. Cancer Res. 2019;79(8):1831–43.

121. Bi Y, Gong L, Liu P, Xiong X, Zhao Y. Nuclear ErbB2 represses DEPTOR transcription to inhibit autophagy in breast cancer cells. Cell Death Dis. 2021;12(4):397.
122. Shornale Akter M, Uddin MH, Atikur Rahman S, Hossain MA, Ashik MAR, Zaman NN, Faruk O, Hossain MS, Parvin A, Rahman MH. Transcriptomic analysis revealed potential regulatory biomarkers and repurposable drugs for breast cancer treatment. Cancer Rep (Hoboken NJ). 2024;7(5):e2009.
123. Rout AK, Dehury B, Parida SN, Rout SS, Jena R, Kaushik N, Kaushik NK, Pradhan SK, Sahoo CR, Singh AK, et al. A review on structure-function mechanism and signaling pathway of serine/threonine protein PIM kinases as a therapeutic target. Int J Biol Macromol. 2024;270(Pt 1):132030.
124. Chia SKL, Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, von Minckwitz G, Mansi J, Barrios CH, et al. PIK3CA alterations and benefit with neratinib: analysis from the randomized, double-blind, placebo-controlled, phase III extenet trial. Breast cancer Research: BCR. 2019;21(1):39.
125. Hänggi K, Ruffell B. Cell death, therapeutics, and the immune response in cancer. Trends cancer. 2023;9(5):381–96.
126. Moujalled D, Gangatirkar P, Kauppi M, Corbin J, Lebois M, Murphy JM, Lalaoui N, Hildebrand JM, Silke J, Alexander WS, et al. The necroptotic cell death pathway operates in megakaryocytes, but not in platelet synthesis. Cell Death Dis. 2021;12(1):133.
127. Dannappel M, Vlantis K, Kumari S, Polykratis A, Kim C, Wachsmuth L, Eftychi C, Lin J, Corona T, Hermance N, et al. RIPK1 maintains epithelial homeostasis by inhibiting apoptosis and necroptosis. Nature. 2014;513(7516):90–4.
128. Qin X, Ma D, Tan YX, Wang HY, Cai Z. The role of necroptosis in cancer: A double-edged sword? Biochim Et Biophys Acta Reviews cancer. 2019;1871(2):259–66.
129. Park JE, Lee JH, Lee SY, Hong MJ, Choi JE, Park S, Jeong JY, Lee EB, Choi SH, Lee YH, et al. Expression of key regulatory genes in necroptosis and its effect on the prognosis in non-small cell lung cancer. J Cancer. 2020;11(18):5503–10.
130. He L, Peng K, Liu Y, Xiong J, Zhu FF. Low expression of mixed lineage kinase domain-like protein is associated with poor prognosis in ovarian cancer patients. OncoTargets Therapy. 2013;6:1539–43.
131. Schmidt SV, Seibert S, Walch-Rückheim B, Vicinus B, Kamionka EM, Pahne-Zeppenfeld J, Solomayer EF, Kim YJ, Bohle RM, Smola S. RIPK3 expression in cervical cancer cells is required for PolyIC-induced necroptosis, IL-1α release, and efficient paracrine dendritic cell activation. Oncotarget. 2015;6(11):8635–47.
132. Yatim N, Jusforgues-Saklani H, Orozco S, Schulz O, Barreira da Silva R, Reis e Sousa C, Green DR, Oberst A, Albert ML. RIPK1 and NF-κB signaling in dying cells determines cross-priming of CD8$^+$ T cells. Volume 350. New York, NY: Science; 2015. pp. 328–34. 6258.
133. Kang YJ, Bang BR, Han KH, Hong L, Shim EJ, Ma J, Lerner RA, Otsuka M. Regulation of NKT cell-mediated immune responses to tumours and liver inflammation by mitochondrial PGAM5-Drp1 signalling. Nat Commun. 2015;6:8371.
134. Aaes TL, Kaczmarek A, Delvaeye T, De Craene B, De Koker S, Heyndrickx L, Delrue I, Taminau J, Wiernicki B, De Groote P, et al. Vaccination with necroptotic cancer cells induces efficient Anti-tumor immunity. Cell Rep. 2016;15(2):274–87.
135. Strilic B, Yang L, Albarrán-Juárez J, Wachsmuth L, Han K, Müller UC, Pasparakis M, Offermanns S. Tumour-cell-induced endothelial cell necroptosis via death receptor 6 promotes metastasis. Nature. 2016;536(7615):215–8.
136. Seifert L, Werba G, Tiwari S, Giao Ly NN, Alothman S, Alqunaibit D, Avanzi A, Barilla R, Daley D, Greco SH, et al. The necrosome promotes pancreatic oncogenesis via CXCL1 and Mincle-induced immune suppression. Nature. 2016;532(7598):245–9.
137. Jiao D, Cai Z, Choksi S, Ma D, Choe M, Kwon HJ, Baik JY, Rowan BG, Liu C, Liu ZG. Necroptosis of tumor cells leads to tumor necrosis and promotes tumor metastasis. Cell Res. 2018;28(8):868–70.
138. Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity. 2013;38(2):209–23.
139. Zhang X, Fan C, Zhang H, Zhao Q, Liu Y, Xu C, Xie Q, Wu X, Yu X, Zhang J, et al. MLKL and FADD are critical for suppressing progressive lymphoproliferative disease and activating the NLRP3 inflammasome. Cell Rep. 2016;16(12):3247–59.
140. Dara L, Liu ZX, Kaplowitz N. Questions and controversies: the role of necroptosis in liver disease. Cell Death Discovery. 2016;2:16089.
141. Linkermann A, Green DR. Necroptosis. The New England journal of medicine 2014, 370(5):455–465.
142. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, Lewis R, Lalaoui N, Metcalf D, Webb AI, et al. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. Immunity. 2013;39(3):443–53.
143. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell. 1991;66(2):233–43.
144. Loetscher H, Pan YC, Lahm HW, Gentz R, Brockhaus M, Tabuchi H, Lesslauer W. Molecular cloning and expression of the human 55 Kd tumor necrosis factor receptor. Cell. 1990;61(2):351–9.
145. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, Dixit VM. The receptor for the cytotoxic ligand TRAIL. Volume 276. New York, NY): Science; 1997. pp. 111–3. 5309.
146. Walczak H, Degli-Esposti MA, Johnson RS, Smolak PJ, Waugh JY, Boiani N, Timour MS, Gerhart MJ, Schooley KA, Smith CA, et al. TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. EMBO J. 1997;16(17):5386–97.
147. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 2001;104(4):487–501.
148. Bodmer JL, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. Trends Biochem Sci. 2002;27(1):19–26.
149. Papoff G, Hausler P, Eramo A, Pagano MG, Di Leve G, Signore A, Ruberti G. Identification and characterization of a ligand-independent oligomerization domain in the extracellular region of the CD95 death receptor. J Biol Chem. 1999;274(53):38241–50.
150. Siegel RM, Frederiksen JK, Zacharias DA, Chan FK, Johnson M, Lynch D, Tsien RY, Lenardo MJ. Fas preassociation required for apoptosis signaling and dominant Inhibition by pathogenic mutations. Sci (New York NY). 2000;288(5475):2354–7.
151. Edmond V, Ghali B, Penna A, Taupin JL, Daburon S, Moreau JF, Legembre P. Precise mapping of the CD95 pre-ligand assembly domain. PLoS ONE. 2012;7(9):e46236.
152. Siegmund D, Lang I, Wajant H. Cell death-independent activities of the death receptors CD95, TRAILR1, and TRAILR2. FEBS J. 2017;284(8):1131–59.
153. Suda T, Hashimoto H, Tanaka M, Ochi T, Nagata S. Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing. J Exp Med. 1997;186(12):2045–50.
154. Schneider P, Holler N, Bodmer JL, Hahne M, Frei K, Fontana A, Tschopp J. Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with downregulation of its proapoptotic activity and loss of liver toxicity. J Exp Med. 1998;187(8):1205–13.
155. Tauzin S, Chaigne-Delalande B, Selva E, Khadra N, Daburon S, Contin-Bordes C, Blanco P, Le Seyec J, Ducret T, Counillon L, et al. The naturally processed CD95L elicits a c-yes/calcium/PI3K-driven cell migration pathway. PLoS Biol. 2011;9(6):e1001090.
156. Malleter M, Tauzin S, Bessede A, Castellano R, Goubard A, Godey F, Levêque J, Jézéquel P, Campion L, Campone M, et al. CD95L cell surface cleavage triggers a prometastatic signaling pathway in triple-negative breast cancer. Cancer Res. 2013;73(22):6711–21.
157. Cursi S, Rufini A, Stagni V, Condò I, Matafora V, Bachi A, Bonifazi AP, Coppola L, Superti-Furga G, Testi R, et al. Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression. EMBO J. 2006;25(9):1895–905.
158. Senft J, Helfer B, Frisch SM. Caspase-8 interacts with the p85 subunit of phosphatidylinositol 3-kinase to regulate cell adhesion and motility. Cancer Res. 2007;67(24):11505–9.
159. Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F, Biamonte F. Ferroptosis and cancer: mitochondria Meet the iron maiden cell death. Cells 2020, 9(6).
160. Agmon E, Solon J, Bassereau P, Stockwell BR. Modeling the effects of lipid peroxidation during ferroptosis on membrane properties. Sci Rep. 2018;8(1):5155.
161. Battaglia AM, Sacco A, Vecchio E, Scicchitano S, Petriaggi L, Giorgio E, Bulotta S, Levi S, Faniello CM, Biamonte F, et al. Iron affects the sphere-forming ability of ovarian cancer cells in non-adherent culture conditions. Front Cell Dev Biology. 2023;11:1272667.
162. El Hout M, Dos Santos L, Hamaï A, Mehrpour M. A promising new approach to cancer therapy: targeting iron metabolism in cancer stem cells. Sem Cancer Biol. 2018;53:125–38.
163. Richardson DR, Ponka P. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. Biochim Biophys Acta. 1997;1331(1):1–40.
164. Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. Cell Res. 2016;26(9):1021–32.
165. Yang WS, Stockwell BR. Ferroptosis: death by lipid peroxidation. Trends Cell Biol. 2016;26(3):165–76.

166. Zhang Y, Lima CF, Rodrigues LR. Anticancer effects of lactoferrin: underlying mechanisms and future trends in cancer therapy. Nutr Rev. 2014;72(12):763–73.
167. Seibt TM, Proneth B, Conrad M. Role of GPX4 in ferroptosis and its Pharmacological implication. Free Radic Biol Med. 2019;133:144–52.
168. Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ 3rd, Kang R, Tang D. Autophagy promotes ferroptosis by degradation of ferritin. Autophagy. 2016;12(8):1425–8.
169. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. Nat Chem Biol. 2017;13(1):91–8.
170. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017;482(3):419–25.
171. Brown JE, Krodel M, Pazos M, Lai C, Prieto AL. Cross-phosphorylation, signaling and proliferative functions of the Tyro3 and Axl receptors in Rat2 cells. PLoS ONE. 2012;7(5):e36800.
172. Jiang Z, Lim SO, Yan M, Hsu JL, Yao J, Wei Y, Chang SS, Yamaguchi H, Lee HH, Ke B et al. TYRO3 induces anti-PD-1/PD-L1 therapy resistance by limiting innate immunity and tumoral ferroptosis. J Clin Investig 2021, 131(8).
173. Anastasiou D. Tumour microenvironment factors shaping the cancer metabolism landscape. Br J Cancer. 2017;116(3):277–86.
174. Kimbung S, Loman N, Hedenfalk I. Clinical and molecular complexity of breast cancer metastases. Sem Cancer Biol. 2015;35:85–95.
175. Lin CC, Mabe NW, Lin YT, Yang WH, Tang X, Hong L, Sun T, Force J, Marks JR, Yao TP, et al. RIPK3 upregulation confers robust proliferation and collateral cystine-dependence on breast cancer recurrence. Cell Death Differ. 2020;27(7):2234–47.
176. Mabe NW, Fox DB, Lupo R, Decker AE, Phelps SN, Thompson JW, Alvarez JV. Epigenetic Silencing of tumor suppressor Par-4 promotes chemoresistance in recurrent breast cancer. J Clin Investig. 2018;128(10):4413–28.
177. Fox DB, Garcia NMG, McKinney BJ, Lupo R, Noteware LC, Newcomb R, Liu J, Locasale JW, Hirschey MD, Alvarez JV. NRF2 activation promotes the recurrence of dormant tumour cells through regulation of redox and nucleotide metabolism. Nat Metabolism. 2020;2(4):318–34.
178. Tsai JH, Yang J. Epithelial-mesenchymal plasticity in carcinoma metastasis. Genes Dev. 2013;27(20):2192–206.
179. Tang X, Ding CK, Wu J, Sjol J, Wardell S, Spasojevic I, George D, McDonnell DP, Hsu DS, Chang JT, et al. Cystine addiction of triple-negative breast cancer associated with EMT augmented death signaling. Oncogene. 2017;36(30):4235–42.
180. Lin CC, Yang WH, Lin YT, Tang X, Chen PH, Ding CC, Qu DC, Alvarez JV, Chi JT. DDR2 upregulation confers ferroptosis susceptibility of recurrent breast tumors through the Hippo pathway. Oncogene. 2021;40(11):2018–34.
181. Nagy P, Friedländer E, Tanner M, Kapanen AI, Carraway KL, Isola J, Jovin TM. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. Cancer Res. 2005;65(2):473–82.
182. Zhang L, Li Y, Wang Q, Chen Z, Li X, Wu Z, Hu C, Liao D, Zhang W, Chen ZS. The PI3K subunits, P110α and P110β are potential targets for overcoming P-gp and BCRP-mediated MDR in cancer. Mol Cancer. 2020;19(1):10.
183. Zou Y, Zheng S, Xie X, Ye F, Hu X, Tian Z, Yan SM, Yang L, Kong Y, Tang Y, et al. N6-methyladenosine regulated FGFR4 attenuates ferroptotic cell death in recalcitrant HER2-positive breast cancer. Nat Commun. 2022;13(1):2672.
184. Wang S, Wang Y, Li Q, Li X, Feng X. A novel circular RNA confers trastuzumab resistance in human epidermal growth factor receptor 2-positive breast cancer through regulating ferroptosis. Environ Toxicol. 2022;37(7):1597–607.
185. Ma S, Henson ES, Chen Y, Gibson SB. Ferroptosis is induced following Siramesine and lapatinib treatment of breast cancer cells. Cell Death Dis. 2016;7(7):e2307.
186. Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, Superti-Furga G, Stockwell BR. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. ACS Chem Biol. 2015;10(7):1604–9.
187. Cocucci E, Meldolesi J. Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol. 2015;25(6):364–72.
188. Yu DD, Wu Y, Shen HY, Lv MM, Chen WX, Zhang XH, Zhong SL, Tang JH, Zhao JH. Exosomes in development, metastasis and drug resistance of breast cancer. Cancer Sci. 2015;106(8):959–64.
189. Yang SJ, Wang DD, Zhong SL, Chen WQ, Wang FL, Zhang J, Xu WX, Xu D, Zhang Q, Li J, et al. Tumor-derived Exosomal circPSMA1 facilitates the tumorigenesis, metastasis, and migration in triple-negative breast cancer (TNBC) through miR-637/Akt1/β-catenin (cyclin D1) axis. Volume 12. Cell death & disease; 2021. p. 420. 5.
190. Yang C, Zhang J, Liao M, Yang Y, Wang Y, Yuan Y, Ouyang L. Folate-mediated one-carbon metabolism: a targeting strategy in cancer therapy. Drug Discovery Today. 2021;26(3):817–25.
191. Yu M, Gai C, Li Z, Ding D, Zheng J, Zhang W, Lv S, Li W. Targeted exosome-encapsulated Erastin induced ferroptosis in triple negative breast cancer cells. Cancer Sci. 2019;110(10):3173–82.
192. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003;100(7):3983–8.
193. Liu S, Cong Y, Wang D, Sun Y, Deng L, Liu Y, Martin-Trevino R, Shang L, McDermott SP, Landis MD, et al. Breast cancer stem cells transition between epithelial and mesenchymal States reflective of their normal counterparts. Stem Cell Rep. 2014;2(1):78–91.
194. Bussard KM, Mutkus L, Stumpf K, Gomez-Manzano C, Marini FC. Tumor-associated stromal cells as key contributors to the tumor microenvironment. Breast cancer Research: BCR. 2016;18(1):84.
195. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for Anti- and Pro-Angiogenic therapies. Genes cancer. 2011;2(12):1097–105.
196. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007;131(6):1109–23.
197. Nakayama A, Takagi S, Yusa T, Yaguchi M, Hayashi A, Tamura T, Kawakita Y, Ishikawa T, Ohta Y. Antitumor activity of TAK-285, an investigational, Non-Pgp substrate HER2/EGFR kinase inhibitor, in cultured tumor cells, mouse and rat xenograft tumors, and in an HER2-Positive brain metastasis model. J Cancer. 2013;4(7):557–65.
198. Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and Mdr gene expression in colorectal cancer. Cancer. 1993;71(8):2454–60.
199. Jin Y, Zhang W, Xu J, Wang H, Zhang Z, Chu C, Liu X, Zou Q. UCH-L1 involved in regulating the degradation of EGFR and promoting malignant properties in drug-resistant breast cancer. Int J Clin Exp Pathol. 2015;8(10):12500–8.
200. Angelucci A. Targeting tyrosine kinases in cancer: lessons for an effective targeted therapy in the clinic. Cancers 2019, 11(4).
201. Elsberger B. Translational evidence on the role of Src kinase and activated Src kinase in invasive breast cancer. Crit Rev Oncol/Hematol. 2014;89(3):343–51.
202. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, Shi B. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J cancer Res. 2015;5(10):2929–43.
203. Mirkin S, Pickar JH. Selective Estrogen receptor modulators (SERMs): a review of clinical data. Maturitas. 2015;80(1):52–7.
204. Rimawi MF, Shetty PB, Weiss HL, Schiff R, Osborne CK, Chamness GC, Elledge RM. Epidermal growth factor receptor expression in breast cancer association with biologic phenotype and clinical outcomes. Cancer. 2010;116(5):1234–42.
205. Hudis CA. Trastuzumab–mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1):39–51.
206. Emde A, Köstler WJ, Yarden Y. Therapeutic strategies and mechanisms of tumorigenesis of HER2-overexpressing breast cancer. Crit Rev Oncol/Hematol. 2012;84 Suppl 1(Suppl 1):e49–57.
207. Ahmad S, Gupta S, Kumar R, Varshney GC, Raghava GP. Herceptin resistance database for Understanding mechanism of resistance in breast cancer patients. Sci Rep. 2014;4:4483.
208. Vu T, Sliwkowski MX, Claret FX. Personalized drug combinations to overcome trastuzumab resistance in HER2-positive breast cancer. Biochim Biophys Acta. 2014;1846(2):353–65.
209. Teicher BA, Doroshow JH. The promise of antibody-drug conjugates. N Engl J Med. 2012;367(19):1847–8.
210. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh DY, Diéras V, Guardino E, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med. 2012;367(19):1783–91.
211. Perez EA, Barrios C, Eiermann W, Toi M, Im YH, Conte P, Martin M, Pienkowski T, Pivot X, Burris H 3, et al. Trastuzumab emtansine with or without Pertuzumab versus trastuzumab plus taxane for human epidermal growth factor receptor 2-Positive, advanced breast cancer: primary results from the phase III MARIANNE study. J Clin Oncology: Official J Am Soc Clin Oncol. 2017;35(2):141–8.
212. Baselga J, Campone M, Piccart M, Burris HA 3rd, Rugo HS, Sahmoud T, Noguchi S, Gnant M, Pritchard KI, Lebrun F, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 2012;366(6):520–9.

213. Swain SM, Baselga J, Kim SB, Ro J, Semiglazov V, Campone M, Ciruelos E, Ferrero JM, Schneeweiss A, Heeson S, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med. 2015;372(8):724–34.
214. Fraser C, Dawson JC, Dowling R, Houston DR, Weiss JT, Munro AF, Muir M, Harrington L, Webster SP, Frame MC, et al. Rapid discovery and Structure-Activity relationships of pyrazolopyrimidines that potently suppress breast cancer cell growth via SRC kinase Inhibition with exceptional selectivity over ABL kinase. J Med Chem. 2016;59(10):4697–710.
215. Jallal H, Valentino ML, Chen G, Boschelli F, Ali S, Rabbani SA. A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo. Cancer Res. 2007;67(4):1580–8.
216. Hebbard L, Cecena G, Golas J, Sawada J, Ellies LG, Charbono A, Williams R, Jimenez RE, Wankell M, Arndt KT, et al. Control of mammary tumor differentiation by SKI-606 (bosutinib). Oncogene. 2011;30(3):301–12.
217. Campone M, Bondarenko I, Brincat S, Hotko Y, Munster PN, Chmielowska E, Fumoleau P, Ward R, Bardy-Bouxin N, Leip E, et al. Phase II study of single-agent bosutinib, a Src/Abl tyrosine kinase inhibitor, in patients with locally advanced or metastatic breast cancer pretreated with chemotherapy. Annals Oncology: Official J Eur Soc Med Oncol. 2012;23(3):610–7.
218. Isakoff SJ, Wang D, Campone M, Calles A, Leip E, Turnbull K, Bardy-Bouxin N, Duvillié L, Calvo E. Bosutinib plus capecitabine for selected advanced solid tumours: results of a phase 1 dose-escalation study. Br J Cancer. 2014;111(11):2058–66.
219. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Cornelius LA, Das J, Doweyko AM, et al. Discovery of N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem. 2004;47(27):6658–61.
220. Finn RS, Bengala C, Ibrahim N, Roché H, Sparano J, Strauss LC, Fairchild J, Sy O, Goldstein LJ. Dasatinib as a single agent in triple-negative breast cancer: results of an open-label phase 2 study. Clin cancer Research: Official J Am Association Cancer Res. 2011;17(21):6905–13.
221. Schott AF, Barlow WE, Van Poznak CH, Hayes DF, Moinpour CM, Lew DL, Dy PA, Keller ET, Keller JM, Hortobagyi GN. Phase II studies of two different schedules of dasatinib in bone metastasis predominant metastatic breast cancer: SWOG S0622. Breast Cancer Res Treat. 2016;159(1):87–95.
222. Pusztai L, Moulder S, Altan M, Kwiatkowski D, Valero V, Ueno NT, Esteva FJ, Avritscher R, Qi Y, Strauss L, et al. Gene signature-guided dasatinib therapy in metastatic breast cancer. Clin cancer Research: Official J Am Association Cancer Res. 2014;20(20):5265–71.
223. Herynk MH, Beyer AR, Cui Y, Weiss H, Anderson E, Green TP, Fuqua SA. Cooperative action of Tamoxifen and c-Src Inhibition in preventing the growth of Estrogen receptor-positive human breast cancer cells. Mol Cancer Ther. 2006;5(12):3023–31.
224. Hiscox S, Jordan NJ, Smith C, James M, Morgan L, Taylor KM, Green TP, Nicholson RI. Dual targeting of Src and ER prevents acquired antihormone resistance in breast cancer cells. Breast Cancer Res Treat. 2009;115(1):57–67.
225. Jain S, Wang X, Chang CC, Ibarra-Drendall C, Wang H, Zhang Q, Brady SW, Li P, Zhao H, Dobbs J, et al. Src Inhibition blocks c-Myc translation and glucose metabolism to prevent the development of breast cancer. Cancer Res. 2015;75(22):4863–75.
226. Beetham H, Griffith BGC, Murina O, Loftus AEP, Parry DA, Temps C, Culley J, Muir M, Unciti-Broceta A, Sims AH, et al. Loss of Integrin-Linked kinase sensitizes breast cancer to SRC inhibitors. Cancer Res. 2022;82(4):632–47.
227. Temps C, Lietha D, Webb ER, Li XF, Dawson JC, Muir M, Macleod KG, Valero T, Munro AF, Contreras-Montoya R, et al. A conformation selective mode of inhibiting SRC improves drug efficacy and tolerability. Cancer Res. 2021;81(21):5438–50.
228. Tran QH, Hoang DH, Song M, Choe W, Kang I, Kim SS, Ha J. Melatonin and doxorubicin synergistically enhance apoptosis via autophagy-dependent reduction of AMPKα1 transcription in human breast cancer cells. Exp Mol Med. 2021;53(9):1413–22.
229. Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E, Castro G Jr., Untch M, Smith I, Gianni L, et al. 11 Years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin adjuvant (HERA) trial. Lancet (London England). 2017;389(10075):1195–205.
230. Harbeck N, Huang CS, Hurvitz S, Yeh DC, Shao Z, Im SA, Jung KH, Shen K, Ro J, Jassem J, et al. Afatinib plus Vinorelbine versus trastuzumab plus Vinorelbine in patients with HER2-overexpressing metastatic breast cancer who had progressed on one previous trastuzumab treatment (LUX-Breast 1): an open-label, randomised, phase 3 trial. Lancet Oncol. 2016;17(3):357–66.
231. Roskoski R Jr. Orally effective FDA-approved protein kinase targeted covalent inhibitors (TCIs). Pharmacol Res. 2021;165:105422.
232. Oh DY, Bang YJ. HER2-targeted therapies - a role beyond breast cancer. Nat Reviews Clin Oncol. 2020;17(1):33–48.
233. Fornier MN, Morris PG, Abbruzzi A, D'Andrea G, Gilewski T, Bromberg J, Dang C, Dickler M, Modi S, Seidman AD, et al. A phase I study of dasatinib and weekly Paclitaxel for metastatic breast cancer. Annals Oncology: Official J Eur Soc Med Oncol. 2011;22(12):2575–81.
234. Gnant M, Dueck AC, Frantal S, Martin M, Burstein HJ, Greil R, Fox P, Wolff AC, Chan A, Winer EP, et al. Adjuvant Palbociclib for early breast cancer: the PALLAS trial results (ABCSG-42/AFT-05/BIG-14-03). J Clin Oncology: Official J Am Soc Clin Oncol. 2022;40(3):282–93.
235. Copur MS, Jonglertham P. Alpelisib for PIK3CA-Mutated advanced breast cancer. N Engl J Med. 2019;381(7):686–7.
236. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, Gelber RD, de Azambuja E, Fielding A, Balmaña J, et al. Adjuvant Olaparib for patients with BRCA1- or BRCA2-Mutated breast cancer. N Engl J Med. 2021;384(25):2394–405.

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