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REVIEW

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Lipid rafts, caveolae, and epidermal growth factor receptor family: friends or foes?

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Abstract

Lipid rafts are dynamic microdomains enriched with cholesterol and sphingolipids that play critical roles in cellular processes by organizing and concentrating specific proteins involved in signal transduction. The interplay between lipid rafts, raft-associated caveolae and the human epidermal growth factor receptors has significant implications in cancer biology, particularly in breast and gastric cancer therapy resistance. This review examines the structural and functional characteristics of lipid rafts, their involvement in EGFR and HER2 signaling, and the impact of lipid rafts/CXCL12/CXCR4/HER2 axis on bone metastasis. We also discuss the potential of targeting lipid rafts and caveolin-1 to enhance therapeutic strategies against HER2-positive cancers and the impact of co-localization of trastuzumab or antibody drug conjugates with caveolin-1 on therapy response. Emerging evidence suggests that disrupting lipid raft integrity or silencing caveolin-1, through several strategies including cholesterol-lowering molecules, can influence HER2 availability and internalization, enhancing anti-HER2 targeted therapy and offering a novel approach to counteract drug resistance and improve treatment efficacy.

Keywords Lipid rafts, Caveolin-1, Human epidermal growth factor receptors, Target therapy resistance, Bone metastasis

Introduction

Lipid rafts are sphingolipid- and cholesterol-enriched microdomains within the cell membrane involved in many physiological and pathological processes [1]. Rafts have been reported to play a pivotal role in signal transduction in cancer, promoting receptor homo- and heterodimerization, shielding proteins from enzymatic degradation, or acting as scaffolds to enhance intracellular signaling pathways. Due to their ability to include or exclude proteins, with a strong affinity for glycosylphosphatidylinositol (GPI)-anchored proteins, Src-family kinases, palmitoylated type-I transmembrane proteins (i.e., CD44) and receptor tyrosine kinases, lipid rafts are reported to be involved in tumor initiation and progression of both carcinomas and sarcomas [2–4].

Overexpression, gene amplification, or mutation of human epidermal growth factor receptors (ErbB-HER), especially EGFR and HER2, lead to the development and support of several cancer types, such as non-small cell lung cancer (NSCLC), head and neck, breast and gastric cancers [5].

The advent of targeted therapies, the development of monoclonal antibodies and antibody-drug conjugates

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(ADCs) against EGFR (e.g. cetuximab, panitumumab) and HER2 (e.g. trastuzumab, trastuzumab-emtansine (T-DM1), trastuzumab-deruxtecan) have revolutionized the treatment of patients significantly improving their response rate and survival [6, 7]. Unfortunately, a variable proportion of patients present intrinsic or acquired resistance to these treatments, eventually leading to disease progression [8–11]. Mechanisms behind therapy resistance are diverse, and strategies to overcome resistance are currently a hot topic in cancer research [10–12].

In this review we analyze the intricate interplay between lipid rafts and the ErbB receptors family in cell membrane, with a focus on HER2, looking at how rafts and rafts-associated proteins can influence HER2 activation, signaling and response to target therapy.

Lipid raft structure: a brief overview

The Singer and Nicolson model, proposed in 1972, revolutionized the understanding of the cell membrane's structure and function by introducing the concept of the membrane as a fluid mosaic, composed of a dynamic lipid bilayer spotted with proteins [13]. According to this model, the lipid bilayer provides a flexible base through which proteins, involved in various functions, can float freely or be anchored. The membrane's fluidity is the key aspect of Singer and Nicolson's model who proposed that lipids and proteins can do lateral movements within the membrane. This fluidity allows for the dynamic reorganization of membrane components and facilitates various cellular processes, such as membrane fusion, endocytosis, and cell-cell communication [13, 14].

Later studies, initially based on solubility in detergent assays and then on advanced microscopy techniques [15, 16], have shown how cell membranes are structurally heterogeneous and contain several subdomains with different physical and biological properties, presenting liquid-disordered and liquid-ordered phases in the same lipid bilayer [17, 18].

The concept of lipid heterogeneity and its significance was brought to attention with the introduction of the "raft hypothesis," stemming from findings documented by Simons and van Meer in 1988 [19]. They proposed that the segregation of lipids into distinct domains is an initial step in the sorting process within the plasma membrane of epithelial cells. Over time, this hypothesis evolved to suggest the presence of microdomains, referred to as "rafts," which are characterized by high concentrations of glycosphingolipids, cholesterol, and phospholipids acylated with saturated fatty acids. Thus, in 1997 these rafts were theorized to be functionally linked to specific proteins involved in intracellular lipid trafficking and cell signaling [20]. Since that moment, the structure and role of lipid rafts have been further investigated by several research groups [14], culminating in a consensus definition in 2006, when rafts were described as small (10–200 nm) cholesterol and sphingolipid-enriched membrane nanodomain. Rafts can also form platforms (>300 nm) through protein-protein and protein-lipid interactions [21].

As better discussed in other reviews [17, 18], the interactions between sterols and sphingolipids in lipid rafts make these microdomains resistant to solubilization by specific non-ionic detergents. This resistance is key to isolating rafts using density gradient centrifugation. Cholesterol is a major regulator of lipids in plasma membranes, it enhances lipid order while maintaining membrane fluidity and diffusion rates, and regulates membrane permeability, ensuring mechanical stability and low leakiness. Indeed, cholesterol's role in lipid raft formation is crucial, promoting the clustering and packing of sphingolipids and cholesterol into dynamic platforms [17, 18, 22].

Lipid rafts are held together by specific proteins that anchor the inner and outer layers of the membrane. Inside the cell, scaffolding proteins such as flotillin, caveolins, and annexins help anchor the inner leaflet of the lipid rafts. These proteins stabilize the rafts by binding to the inner part of the cell membrane. On the outer side of the membrane, other proteins like glycosylphosphatidylinositol-linked proteins connect the rafts. These lipid rafts create organized platforms that facilitate signaling processes by clustering with proteins or other lipid rafts [17, 18].

Several proteins with a pivotal role in cancer, including dually acylated proteins (i.e., Src-family protein tyrosine kinases), palmitoylated type-I transmembrane proteins (i.e., CD44) or receptor tyrosine kinases with two transmembrane subunits (i.e., Insulin-like growth factors (IGFs) receptors and epidermal growth factor receptor (EGFR)), show reversible association to rafts in response to appropriate signals (Fig. 1) [1].

As further discussed below, this means that the rafts can bring together various molecules needed for transmitting signals inside the cell, ensuring that these processes happen co-ordinately, efficiently and accurately [23].

The intricate relationship between lipid rafts and caveolae

Lipid rafts can be classified into the flat type and the invaginated type. Flat lipid rafts maintain a smaller, flat, and orderly structure. The flotillin protein is essential for the structure and function of these rafts [24]. In contrast, invaginated lipid rafts, called caveolae, present a concave configuration and are rich in caveolins (Fig. 1) [1].

Caveolin is a cholesterol-binding protein that is concentrated in caveolae due to its affinity for cholesterol [25, 26]. The caveolin family includes three genes:

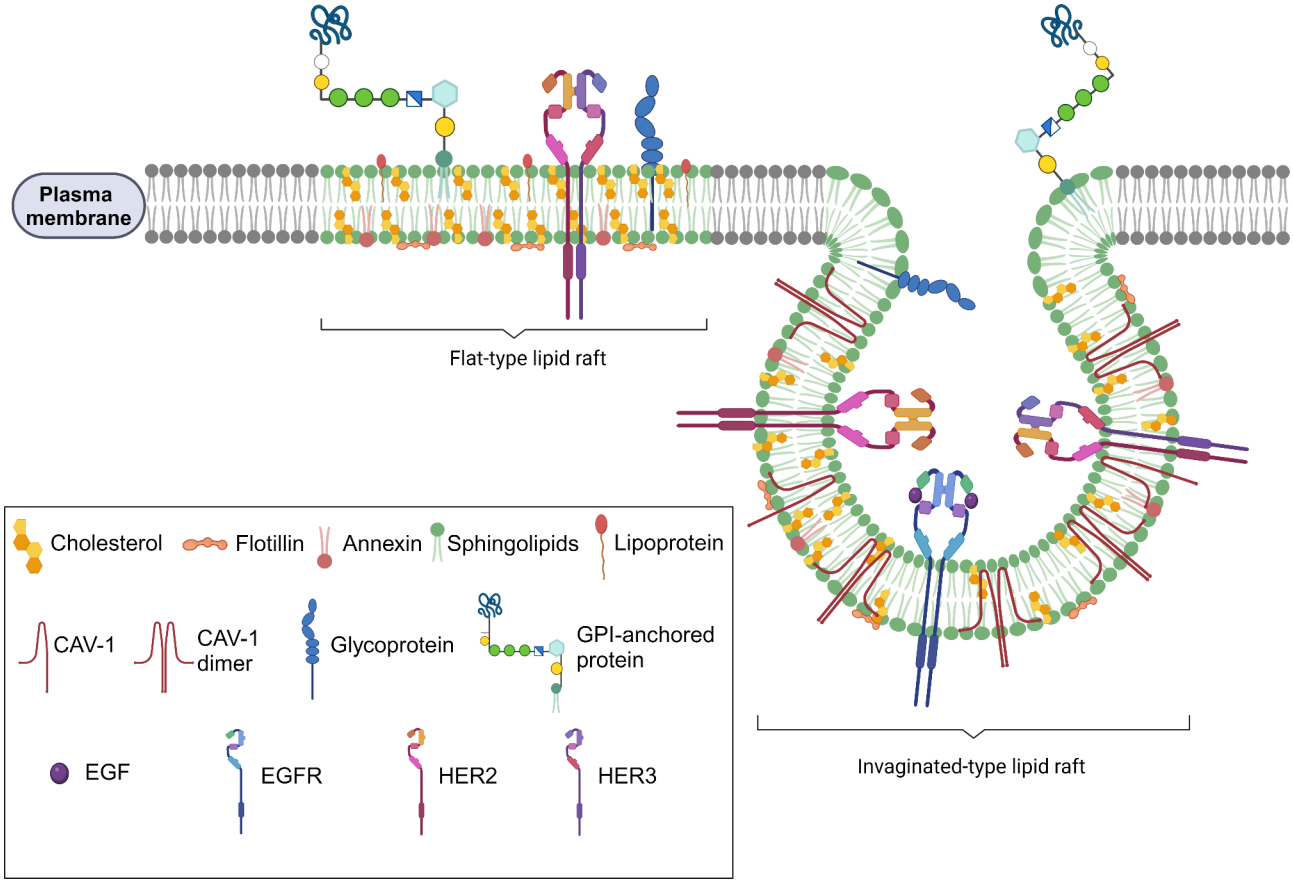

Fig. 1 Plasma membrane organization of flat and invaginated lipid rafts Lipid rafts are specialized membrane microdomains enriched with sphingolipids and cholesterol. Flat lipid rafts are small and ordered structures rich in flotillin. Invaginated lipid rafts present an invaginated configuration, called caveolae, rich in caveolins. Several proteins and receptors, such as tyrosine kinases, are associated with rafts (created with BioRender.com)

caveolin-1 (CAV-1), caveolin-2 (CAV-2), and caveolin-3 (CAV-3). Cav-1 and Cav-2 are close to human chromosome 7q31.1, whereas Cav-3 is located on a different chromosome (3p25) [27]. While caveolin-1 and caveolin-2 are colocalized and coexpressed in epithelial and mesenchymal cells including osteoblasts [28–30], caveolin-3 is predominantly found in striated and smooth muscle cells [30].

Caveolae are small (50–100 nm), flask-shaped invaginations of the plasma membrane. As reported by Simons and Toomre, the challenge of isolating pure caveolae has led to confusion about their relationship with lipid rafts [2]. Early methods, such as Triton X-100 extraction at 4 °C, included both caveolae and detergent-resistant membranes (DRMs) from all cellular membranes without distinguishing between the two substructures. Although density gradient centrifugation was commonly used, it cannot isolate pure caveolae. Immuno-isolation has produced mixed results, making double-label immunoelectron microscopy the most reliable method for identifying caveolar proteins. As proposed by Simons and Toomre, to resolve the confusion, it is important to distinguish between lipid rafts, detergent-resistant membranes, and caveolae [2]. The term "caveolae" should be reserved for the morphologically defined cell surface invaginations containing caveolin, as originally described in the 1950s [31]. These structures form when caveolin-1 integrates into lipid rafts, leading to the invagination of these microdomains and the formation of flask-shaped caveolae near the plasma membrane. Caveolae can detach to form plasmalemmal vesicles, with caveolins acting as scaffolding proteins that organize and concentrate specific lipids and lipid-modified signaling molecules [32, 33]. Indeed, lipid rafts are mainly located in the plasma membrane but can also form within internal membrane compartments like the Golgi apparatus. The partitioning of caveolins into these liquid-ordered domains may begin at the Golgi apparatus, initiating the caveolae biogenesis. Thus, it was hypothesized that lipid rafts could be precursors of caveolae, facilitating the cholesterol-dependent insertion of caveolins into membranes [33, 34].

Several studies reported that caveolins, in particular caveolin-1, can also influence various signaling proteins, including several oncogenes (i.e. Src-family tyrosine

kinases, Ha-Ras, and epidermal growth factor (EGF) receptor among others), underscoring their importance in cellular and cancer processes, such as cell growth and proliferation [33, 35–38]. Moreover, caveolin-1 can act both as tumor suppressor or promoter depending on the cancer type and stage, playing a pivotal role also in metastasis formation [39]. For example, in sarcomas caveolin-1 has been indicated as a tumor suppressor hampering metastatic dissemination through inhibition of c-Src and Met signaling [3, 39, 40]. Conversely, caveolin-1 can also act as a tumor promoter in breast cancer, playing a critical role in cancer progression, migration and metastasis [41].

To investigate the physiological and pathological roles of lipid rafts and caveolae, several knockout/silencing and pharmacological strategies were assessed. As previously mentioned, cholesterol is essential for the formation and integrity of both lipid rafts and caveolae. Moreover, caveolins are raft-associated proteins and they tightly bind cholesterol and sphingolipids. Thus, one of the most common pharmacological approaches used exploits this close link between lipid rafts, caveolae and cholesterols. In fact, the depletion of cholesterol from membranes through methyl-β cyclodextrin (MβCD) or cholesterol-lowering drugs, like statins, has as a consequence the disruption of both lipid raft and caveolae structures [22, 38, 42, 43].

Lipid rafts, caveolin-1, and local density of epidermal growth factor receptors

The human epidermal growth factor receptor (ErbB-HER) family consists of four tyrosine-kinase receptors: HER1 (EGFR or ErbB1), HER2 (ErbB2 or *Neu*), HER3 (ErbB3) and HER4 (ErbB4) [5, 44]. ErbB receptors structure includes an extracellular domain (ECD), a lipophilic transmembrane region, an intracellular domain containing tyrosine kinase, and a carboxy-terminal region [44] and are ubiquitously localized throughout the cell membrane of epithelial, mesenchymal, neuronal, and in their progenitor cells [45]. Except for HER2, an orphan receptor with no known ligand, ErbB receptors acquire an open conformation after binding with their ligands (e.g. EGF, neuregulin, transforming growth factor α) which allows the dimerization with identical receptors (homodimerization) or with other ErbB family members (heterodimerization), leading to the activation of pathways that control proliferation and survival in several cancers [5, 45–47].

Epidermal growth factor receptor (EGFR or HER1)

Both EGFR and HER2 have been found associated with lipid rafts, especially in their activated form [48–51].

In the A431 cell line, a paradigm of EGFR-overexpressing human carcinoma, EGFR has been reported to be localized mainly all along the plasma membrane with approximately 40% within rafts and only a small amount (7%) in caveolae. Immuno-electron microscopy (EM) revealed that treatment with cholesterol-lowering molecules caused an increase in EGFR tyrosine phosphorylation both in the presence and absence of EGF stimulation, since rafts are cholesterol-rich areas. Cholesterol depletion enhanced EGFR dimerization by changing its distribution on the plasma membrane and increasing membrane fluidity, allowing for greater lateral movement of EGFR [49, 52]. Moreover, EGF binding did not affect EGFR localization or caveolae mobilization in A431 and HEp-2 cells [49, 53].

The lateral movement of EGFR in the cell membrane and the consequent modulation of its activation, along with other membrane receptors located in the raft-enriched areas, after cholesterol depletion indicates the involvement of lipid rafts in this process, but cannot exclude the role of other structures, like actin cytoskeleton [51].

A different relationship between EGFR, lipid rafts and caveolae was found in human glioblastoma cell lines U87MG and U87MG-EGFRvIII, expressing EGFR amplification and type III mutation (EGFRvIII) respectively [54]. Immunocytochemistry and confocal microscopy revealed that EGFR, but not EGFRvIII, colocalized with lipid rafts and caveolae. The EGF-mediated phosphorylation of the receptor in U87MG cells broke this association. Disruption of lipid rafts by MβCD in U87MG induced ligand-independent tyrosine phosphorylation of EGFR. Due to the constitutive phosphorylation of EGFRvIII in U87MG-EGFRγIII, the phosphorylation levels were not influenced by EGF or MβCD. Interestingly, the treatment with the tyrosine kinase inhibitor AG1478 in both cell lines reduced receptor phosphorylation while increasing the binding between the receptor and caveolin-1 scaffolding domain [54].

Overall, the relationship between rafts, caveolae and EGFR seems to change within tumor types showing sometimes controversial results [49, 54, 55]. However, all studies suggest that the association between these players at different levels may influence receptor dimerization, trafficking and signaling. Thus, looking further into these mechanisms could lead to increased efficacy of EGFR-targeted therapies mainly in resistant patients.

Evidence of the potential benefit of modulating raft-associated molecules such as caveolin-1 or clathrin using cholesterol-lowering drugs to enhance anti-EGFR drugs was reported by Pereira P et al. [56], who showed that silencing CAV-1 in A431 cell line increased EGFR expression in cell membrane, without altering the total amount of EGFR protein within cells. Analogous results were obtained by treating cells with statins such as lovastatin. Furthermore, statins improved cetuximab and

panitumumab binding on EGFR-expressing A431 cancer cells, both in vitro and in vivo [56].

Human epidermal growth factor receptor 2 (HER2 or Erbb2)

The lipid environment and local density of HER2 and HER3 in the plasma membrane deeply influence the dimerization and biological properties of HER2. Besides small-scale associations through receptor homo- and heterodimerization, larger clusters of ErbB2 have been identified. These clusters, containing hundreds of ErbB2 receptors, are about 0.5 μm in diameter and increase in size upon ErbB2 activation. The high local concentration of ErbB and other signaling proteins within these clusters may facilitate receptor complex formation [50, 57]. Donor/acceptor photobleaching FRET measurements using confocal microscopy identified large-scale clusters with around 1000 HER2 molecules, showing in SKBR3 (a HER2-positive human breast cancer cell line) a 2-fold higher density of HER2 within clusters as compared to outside them. Interestingly, HER2 homodimerization was similar inside and outside clusters. Pointing attention to local densities of HER2 and HER3 as possible determinants of HER2 homodimerization, the analysis showed that HER2 homodimerization positively correlated with the local density of the protein, but negatively correlated with the local density of HER3. Lipid rafts also influence the association state of HER2. When HER2 dissociated from lipid rafts following crosslinking of GM1 (a raft-associated ganglioside) by the B subunit of cholera toxin (CTXB), there was a decrease in HER2-HER3 heterodimerization and a reduced tyrosine phosphorylation of HER2 (Fig. 2). Furthermore, the internalization of HER2 mediated by 4D5 (a murine monoclonal antibody, precursor to trastuzumab [58]) was blocked in CTXB-pretreated cells, although the antiproliferative effect of 4D5 remained unaffected [50].

Later studies reported that non-phosphorylated (not activated) HER2 monomers in HER2-positive SKBR3 and BT-474 human breast cancer cell lines were located mainly in the nonlipid raft membrane microdomain. On the other hand, HER2 dimerization and phosphorylation occurred primarily in lipid rafts-CAV-1-positive-enriched areas [59].

The preferential distribution of HER2 in lipid rafts was also analyzed in mouse mammary epithelial HC11 cells, expressing both EGFR and HER2, thus confirming that the receptor localization could not be modified by EGF stimulation [60]. Their analysis confirmed that HER2 was not exclusively associated with the rafts, suggesting the presence of a dynamic equilibrium with lipid rafts in the membrane protrusions. Therefore, at any given time, only a fraction of HER2 interacted directly with raft gangliosides. This transient HER2–gangliosides/lipid rafts interaction could potentially regulate the function of HER2 colocalization areas and influence the signaling-transduction pathways by compartmentalizing the receptor in different membrane domains [60].

Among the molecules associated with lipid rafts, in addition to caveolin-1 and GM3, MAL2 (Mal, T Cell Differentiation Protein 2) is a resident protein of lipid raft involved in apical trafficking and has also been shown to

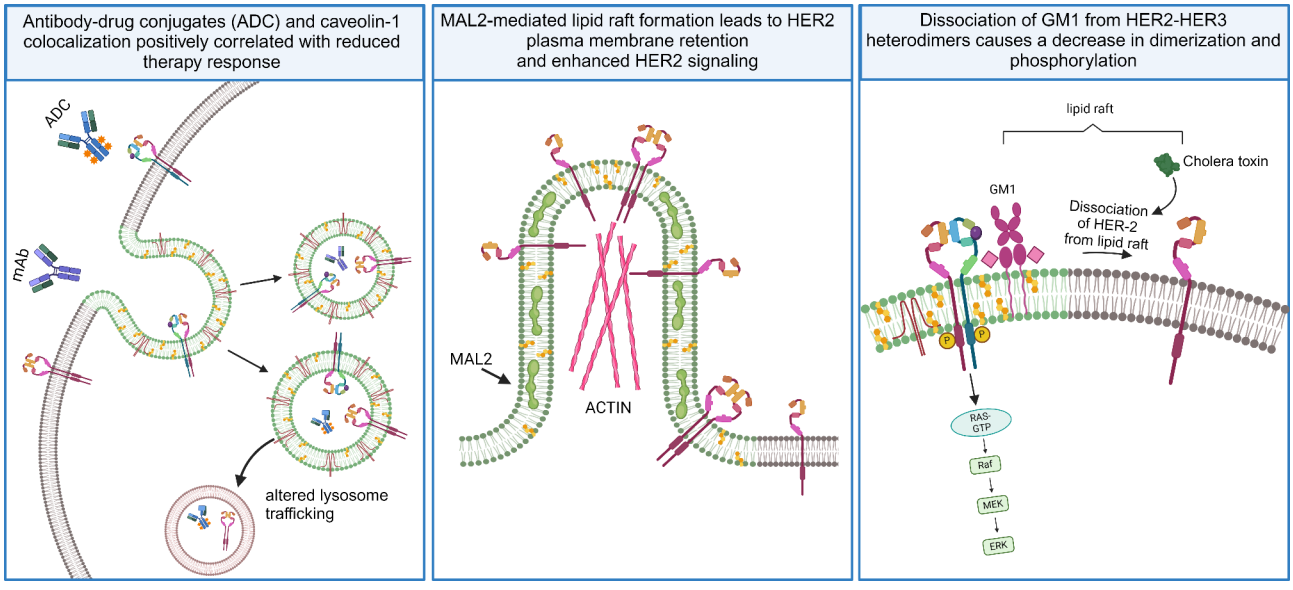

Fig. 2 Impact of lipid rafts and lipid raft-associated proteins on HER2 expression in the cell membrane. Left panel, colocalization of caveolin-1 and anti-HER2 ADCs can impair HER2 and ADC degradation by the lysosome, reducing therapy response. Middle panel, MAL2 lipid raft-associated protein can cause HER2 retention in the cell membrane. MAL2 inhibition can resensitize to target therapies HER2-positive resistant cells. Right panel, HER2 dissociation by the B subunit of cholera toxin from GM1 raft-associated ganglioside decreases HER2 dimerization and phosphorylation (created with BioRender.com)

play a role in HER2 retention on the cell membrane of breast cancer cell lines. Jeong et al. showed that MAL2 interacted closely with HER2 within lipid rafts and this interaction diminished by depleting membrane cholesterol with MβCD. Indeed, knocking down MAL2 reduced total HER2, pHER2, and pEGFR levels. It also caused abnormal internalization of HER2 together with EGFR in response to receptor activation but did not cause dissociation of the heterodimers. Moreover, MAL2 downmodulation led to a decrease of membrane protrusions, crucial for HER2 signaling in breast cancer cells and normally seen in SKBR3 cells. An increased HER2/MAL2 interaction was observed in trastuzumab-resistant cells and targeting MAL2, through its knockdown or after MβCD treatment, re-sensitized resistant cells to trastuzumab caused HER2 internalization (Fig. 2) [61].

Caveolin-1 and HER2 availability in cell membrane

Several HER2-positive cancer cell lines representative of different cancer types, such as UMUC14 bladder, NCI-N87 gastric, BT-474 and SKBR3 breast cancer cell lines, have protein expression of HER2 which inversely correlated with that of CAV-1 [38, 62].

Caveolae-mediated endocytosis was investigated as a novel resistance mechanism to trastuzumab emtansine (T-DM1). NCI-N87 cells resistant to T-DM1 (N87-TM) were obtained by continuous in vitro exposure to the ADC. N87-TM presented more intracellular CAV-1 than the parental NCI-N78 cell line. Indeed, N87-TM cells internalize ADCs into intracellular CAV-1 and alter their trafficking to the lysosome compared with N87 cells. However, CAV-1 knockdown was not sufficient to re-sensitize N87-TM cells to T-DM1. The analysis of a panel of HER2-positive cell lines showed no positive correlation between CAV-1 protein levels and decreased T-DM1 sensitivity. Given that high levels of CAV-1 protein alone do not necessarily induce the formation of caveolar endocytic compartments, the study further assessed the propensity for caveolae-mediated T-DM1 internalization across this cell line panel. Interestingly, some CAV-1-high cell lines (e.g., JIMT1, N87-TM) displayed colocalization of T-DM1 and CAV-1, whereas other CAV-1-high cell lines (e.g., SKOV3, HCC1954) did not. Intriguingly, the amount of ADC colocalization with CAV-1 positively correlated with a reduced response to T-DM1 [62].

Pereira and coworkers confirmed that, in cancer cells lacking CAV-1, HER2 was exclusively present at the cell membrane. On the other hand, cancer cells expressing CAV-1 exhibited reduced HER2 staining at the cell membrane. Interestingly, immunofluorescence staining of HER2-positive tumor samples of gastric cancer patients revealed the same relation between CAV-1 levels and HER2 expression in the cell membrane. Pronounced silencing of CAV-1 increased HER2 half-life at the cell membrane in NCI-N87 and UMUC14 cells, without impact on total HER2 protein levels. In contrast, induction of CAV-1 overexpression promoted loss of HER2 at the cell membrane. Downregulation of CAV-1 through siRNA, MβCD or lovastatin treatment resulted in a decreased HER2 endocytosis mediated by trastuzumab and increased stability of HER2 at cell membrane in NCI-N87 cells. Treatment with lovastatin in vivo improved the tumor avidity for trastuzumab and its therapeutic efficacy on NCI-N87 and BT-474 xenograft [38]. Caveolin-1 depletion mediated by statins like lovastatin also increased TDM1 binding, internalization and efficacy in the heterogenous gastric cancer model [63]. Moreover, lovastatin significantly enhanced the formation of HER2-HER2 homodimers and HER2-EGFR heterodimers in NCI-N87 cells, without altering HER2 phosphorylation (Fig. 3) [64].

Caveolin-1 modulation through cholesterol-lowering molecules enhanced anti-HER2 radioligand efficacy

Immuno-positron emission tomography (immuno-PET) is an advanced molecular imaging technique combining monoclonal antibody specificity labelled with a positron-emitting radionuclide, such as Zirconium-89 (^{89}Zr), with PET imaging sensitivity [65, 66]. Immuno-PET can help in HER2-positive tumor diagnosis, therapy planning by quantifying HER2 expression and monitoring treatment response, allowing for therapy adjustments.

As discussed above, cholesterol-lowering molecules and drugs can increase anti-HER2 target therapy efficacy by altering lipid rafts and/or caveolin-1 expression. Statins showed promising results also in enhancing HER2-targeting radioligand therapy in esophagogastric-resistant cancers through similar mechanisms [67].

Recent studies have explored the modulation of HER2 endocytosis to increase pertuzumab uptake in HER2-positive gastric cancers (NCI-N87 cancer xenograft) allowing a pre-targeted imaging approach. In lovastatin-pretreated mice, tumor uptake of the radiolabel analog of pertuzumab 4 h post-injection was higher than in control mice, suggesting the potential temporal lovastatin treatment to enhance the avidity of pertuzumab in HER2-positive tumors [64].

These findings have led to identify CAV-1 as a possible predictive biomarker of response to HER2-targeted therapies in gastric cancer. A prototype of ^{89}Zr-labeled anti-CAV-1 antibody showed a high affinity for HER2-positive/CAV-1-high NCI-N87 gastric cells injected subcutaneously or intragastrically in mice through immuno-PET. Biodistribution studies of the radioimmunoconjugate confirmed in vivo imaging results suggesting the potential use of CAV-1-PET and optical imaging for detecting gastric tumors and their therapy response [68].

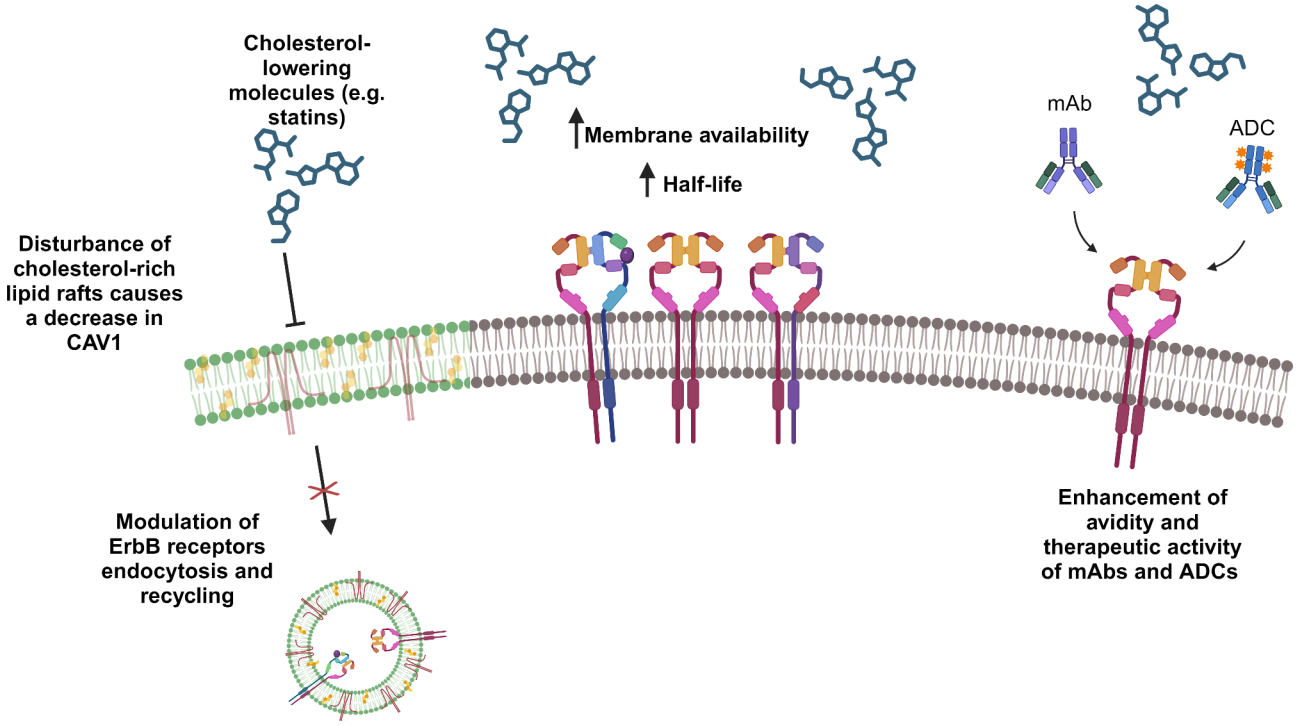

Fig. 3 Effect of cholesterol-lowering molecules on lipid raft,caveolin-1 and HER2. Destruction of lipid-raft and inhibition of raft-associated proteins such as caveolin-1 can reduce ErbB receptors recycling, increase their availability in cell membranes, and enhance anti-HER2 mAb and ADCs avidity and efficacy (created by BioRender.com)

Lipid rafts /CXCL12/CXCR4/HER2 axis in bone metastasis

Metastatic spread is a highly inefficient biological process, nonetheless it is the leading cause of cancer-related deaths [69]. Lipid rafts can play pivotal roles in cancer cell metastatization through various mechanisms, which include angiogenesis, epithelial-to-mesenchymal transition (EMT), migration and cancer cell adhesion because of their interaction with molecules such as VEGF, TGFβ and CD44 [1, 4].

The interplay between lipid rafts and epidermal growth factor receptors, in particular HER2, was also found to be associated with bone metastasis in prostate cancer cells through CXCL12/CXCR4 axis transactivation of the HER2 receptor in lipid rafts [70]. Chinni et al. reported that HER2 and CXCR4, the receptor of CXCL12 chemokine, co-localize within lipid rafts on the plasma membrane of PC-3 and C4-2B prostate cancer cells. The study confirmed the presence of CXCR4 predominantly in lipid rafts, whereas HER2 was present both in lipid rafts and non-raft membrane regions as reported in other cell lines cancer models previously discussed. Interestingly, the CXCL12/CXCR4 interaction specifically enhanced HER2 activation within lipid rafts without significantly altering HER2 phosphorylation status outside these subdomains. The CXCL12/CXCR4/HER2 axis activated downstream signaling pathways involving Src, Akt and matrix metalloproteinase-9 (MMP-9), which promoted migration and metastasis, particularly to the bone. PC3 cells injected into human fetal femur fragments previously implanted into severe combined immunodeficient mice confirmed that overexpression of CXCR4 in prostate cancer cells significantly enhances bone tumor growth and osteolysis. The bone microenvironment, rich in CXCL12, provides a conducive setting for CXCR4-expressing cancer cells to grow and expand within this niche. Lipid rafts on the cell membrane empower CXCL12/CXCR4-induced HER2 receptor transactivation supporting invasion and metastatic growth in the bone microenvironment. Given these findings, the authors proposed several potential therapeutic strategies to counteract this metastatic process. One approach involves disrupting lipid rafts using MβCD, which inhibits the localization of CXCR4 and HER2 in these microdomains. This disruption impaired the CXCL12/CXCR4-induced transactivation of HER2, resulting in reduced downstream signaling and the invasive capabilities of cancer cells [70].

Conclusions

Lipid rafts are involved into the regulation of EGFR and HER2 signaling and their associated pathways in cancer cells. Their ability to compartmentalize and modulate receptor activity makes them a critical factor in cancer initiation and progression. Among other raft-associated

proteins, caveolin-1 seems to modulate receptor localization, thereby affecting the sensitivity of cancer cells to targeted therapies. Preclinical data revealed that targeting lipid rafts and caveolin-1, through for example cholesterol-lowering drugs, might represent a promising therapeutic strategy to enhance the efficacy of HER2-targeted treatments. Indeed, co-localization of trastuzumab or ADCs with caveolin-1 could be a possible marker of predictive response to treatments.

Abbreviations

^{89}Zr	Zirconium-89
ADC	Antibody drug coniugate
CAV-1	Caveolin-1
CAV-2	Caveolin-2
CAV-3	Caveolin-3
CTXB	B subunit of cholera toxin
CXCL12	CXC motif chemokine 12
CXCR4	CXC chemokine receptor type 4
DRM	Detergent-resistant membrane
ECD	Extracellular domain
EGF	Epidermal growth factor
EGFR, HER1	Epidermal growth factor receptor 1
EMT	Epithelial-to-mesenchymal transition
ErbB, HER	Human epidermal growth factor receptor
GM1	Monosialotetrahexosylganglioside 1
GM3	Monosialotetrahexosylganglioside 3
GPI	Glycosylphosphatidylinositol
HER2, ErbB2, Neu	Epidermal growth factor receptor 2
HER3, ErbB3	Epidermal growth factor receptor 3
HER4, ErbB4	Epidermal growth factor receptor 4
IGF	Insulin-like growth factor
MAL2	Mal, T Cell Differentiation Protein 2
MMP-9	metalloproteinase-9
MβCD	Methyl-β-cyclodextrin
PET	Positron emission tomography
siRNA	Short interfering RNA
T-DM1	Trastuzumab emtansine
TGFβ	Transforming growth factor-β
VEGF	Vascular endothelial growth factor

Author contributions

F.R. and P.-L.L. conceived and designed the outline of the article; F.R. and P.-L.L. prepared the original draft; F.R. and C.C. prepared the figures; F.R., M.S.S., L.S., L.L., A.P., S.A., C.C., O.M.P., P.N. and P.-L.L. contributed to the writing and editing of the manuscript and figures; P.-L.L supervised the work. All authors have read and agreed to the published version of the manuscript.

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Data availability

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Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

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References

1. Li B, Qin Y, Yu X, Xu X, Yu W. Lipid raft involvement in signal transduction in cancer cell survival, cell death and metastasis. Cell Prolif. 2022;55:e13167. https://doi.org/10.1111/cpr.13167.
2. Simons K, Toomre D. Lipid rafts and signal transduction, Nature reviews. Mol cell Biology. 2000;1:31–9. https://doi.org/10.1038/35036052.
3. Wiechen K, Sers C, Agoulnik A, Arlt K, Dietel M, Schlag PM, Schneider U. Down-regulation of caveolin-1, a candidate tumor suppressor gene, in sarcomas. Am J Pathol. 2001;158:833–9. https://doi.org/10.1016/S0002-9440(10)64031-X.
4. Greenlee JD, Subramanian T, Liu K, King MR. Rafting down the Metastatic Cascade: the role of lipid rafts in Cancer Metastasis, Cell Death, and clinical outcomes. Cancer Res. 2021;81:5–17. https://doi.org/10.1158/0008-5472.CAN-20-2199.
5. Roskoski R. The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacol Res. 2014;79:34–74. https://doi.org/10.1016/j.phrs.2013.11.002.
6. Ayati A, Moghimi S, Salarinejad S, Safavi M, Pouramiri B, Foroumadi A. A review on progression of epidermal growth factor receptor (EGFR) inhibitors as an efficient approach in cancer targeted therapy. Bioorg Chem. 2020;99:103811. https://doi.org/10.1016/j.bioorg.2020.103811.
7. Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions, Nature reviews. Drug Discovery. 2023;22:101–26. https://doi.org/10.1038/s41573-022-00579-0.
8. Rimawi MF, de Angelis C, Schiff R. Resistance to Anti-HER2 Therapies in Breast Cancer, American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting (2015) e157-64. https://doi.org/10.14694/EdBook_AM.2015.35.e157
9. Chong CR, Jänne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. Nat Med. 2013;19:1389–400. https://doi.org/10.1038/nm.3388.
10. Chhouri H, Alexandre D, Grumolato L. Mechanisms of Acquired Resistance and Tolerance to EGFR targeted therapy in Non-small Cell Lung Cancer. Cancers. 2023;15. https://doi.org/10.3390/cancers15020504.
11. Guidi L, Pellizzari G, Tarantino P, Valenza C, Curigliano G. Resistance to antibody-drug Conjugates Targeting HER2 in breast Cancer: Molecular Landscape and Future challenges. Cancers. 2023;15. https://doi.org/10.3390/cancers15041130.
12. Wu S-G, Shih J-Y. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. Mol Cancer. 2018;17:38. https://doi.org/10.1186/s12943-018-0777-1.
13. Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Sci (New York N Y). 1972;175:720–31. https://doi.org/10.1126/science.175.4023.720.
14. Bagatolli LA, Mouritsen OG. Is the fluid mosaic (and the accompanying raft hypothesis) a suitable model to describe fundamental features of biological membranes? What may be missing? Front Plant Sci. 2013;4:457. https://doi.org/10.3389/fpls.2013.00457.
15. Yu J, Fischman DA, Steck TL. Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. J Supramolecular Struct. 1973;1:233–48. https://doi.org/10.1002/jss.400010308.
16. Sezgin E, Schwille P. Fluorescence techniques to study lipid dynamics, Cold Spring Harbor perspectives in biology 3 (2011) a009803. https://doi.org/10.1101/cshperspect.a009803
17. Sezgin E, Levental I, Mayor S, Eggeling C. The mystery of membrane organization: composition, regulation and roles of lipid rafts, Nature reviews. Mol cell Biology. 2017;18:361–74. https://doi.org/10.1038/nrm.2017.16.
18. Mollinedo F, Gajate C. Lipid rafts as signaling hubs in cancer cell survival/death and invasion: implications in tumor progression and therapy: Thematic Review Series: Biology of lipid rafts. J Lipid Res. 2020;61:611–35. https://doi.org/10.1194/jlr.TR119000439.
19. Simons K, van Meer G. Lipid sorting in epithelial cells. Biochemistry. 1988;27:6197–202. https://doi.org/10.1021/bi00417a001.
20. Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997;387:569–72. https://doi.org/10.1038/42408.
21. Pike LJ. Rafts defined: a report on the Keystone Symposium on lipid rafts and cell function. J Lipid Res. 2006;47:1597–8. https://doi.org/10.1194/jlr.E600002-JLR200.
22. Simons K, Ehehalt R. Cholesterol, lipid rafts, and disease. J Clin Investig. 2002;110:597–603. https://doi.org/10.1172/JCI16390.
23. Reeves VL, Thomas CM, Smart EJ. Lipid rafts, caveolae and GPI-linked proteins, advances in experimental medicine and biology 729 (2012) 3–13. https://doi.org/10.1007/978-1-4614-1222-9_1

24. Rajendran L, Le Lay S, Illges H. Raft association and lipid droplet targeting of flotillins are independent of caveolin. Biol Chem. 2007;388:307–14. https://doi.org/10.1515/BC.2007.034.
25. Murata M, Peränen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol-binding protein. Proc Natl Acad Sci USA. 1995;92:10339–43. https://doi.org/10.1073/pnas.92.22.10339.
26. Ikonen E, Parton RG. Caveolins and cellular cholesterol balance, traffic (Copenhagen, Denmark) 1 (2000) 212–7. https://doi.org/10.1034/j.1600-0854.2000.010303.x
27. Williams TM, Lisanti MP. The caveolin proteins, genome biology 5 (2004) 214. https://doi.org/10.1186/gb-2004-5-3-214
28. Solomon KR, Danciu TE, Adolphson LD, Hecht LE, Hauschka PV. Caveolin-enriched membrane signaling complexes in human and murine osteoblasts. J bone Mineral Research: Official J Am Soc Bone Mineral Res. 2000;15:2380–90. https://doi.org/10.1359/jbmr.2000.15.12.2380.
29. Solomon KR, Adolphson LD, Wank DA, McHugh KP, Hauschka PV. Caveolae in human and murine osteoblasts. J bone Mineral Research: Official J Am Soc Bone Mineral Res. 2000;15:2391–401. https://doi.org/10.1359/jbmr.2000.15.12.2391.
30. Quest AFG, Leyton L, Párraga M. Caveolins, caveolae, and lipid rafts in cellular transport, signaling, and disease. Biochem cell Biology = Biochimie et Biol cellulaire. 2004;82:129–44. https://doi.org/10.1139/o03-071.
31. YAMADA E. The fine structure of the gall bladder epithelium of the mouse. J Biophys Biochem Cytol. 1955;1:445–58. https://doi.org/10.1083/jcb.1.5.445.
32. Anderson RG. The caveolae membrane system, Annual review of biochemistry 67 (1998) 199–225. https://doi.org/10.1146/annurev.biochem.67.1.199
33. Galbiati F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. Cell. 2001;106:403–11. https://doi.org/10.1016/S0092-8674(01)00472-X.
34. Gkantiragas I, Brügger B, Stüven E, Kaloyanova D, Li XY, Löhr K, Lottspeich F, Wieland FT, Helms JB. Sphingomyelin-enriched microdomains at the Golgi complex. Mol Biol Cell. 2001;12:1819–33. https://doi.org/10.1091/mbc.12.6.1819.
35. Lucero HA, Robbins PW. Lipid rafts-protein association and the regulation of protein activity, archives of biochemistry and biophysics 426 (2004) 208–24. https://doi.org/10.1016/j.abb.2004.03.020
36. Pike LJ. Growth factor receptors, lipid rafts and caveolae: an evolving story. Biochim Biophys Acta. 2005;1746:260–73. https://doi.org/10.1016/j.bbamcr.2005.05.005.
37. Williams TM, Lisanti MP. Caveolin-1 in oncogenic transformation, cancer, and metastasis, American journal of physiology. Cell Physiol. 2005;288:C494–506. https://doi.org/10.1152/ajpcell.00458.2004.
38. Pereira PMR, Sharma SK, Carter LM, Edwards KJ, Pourat J, Ragupathi A, Janjigian YY, Durack JC, Lewis JS. Caveolin-1 mediates cellular distribution of HER2 and affects trastuzumab binding and therapeutic efficacy. Nat Commun. 2018;9:5137. https://doi.org/10.1038/s41467-018-07608-w.
39. Quest AFG, Gutierrez-Pajares JL, Torres VA. Caveolin-1: an ambiguous partner in cell signalling and cancer. J Cell Mol Med. 2008;12:1130–50. https://doi.org/10.1111/j.1582-4934.2008.00331.x.
40. Cantiani L, Manara MC, Zucchini C, de Sanctis P, Zuntini M, Valvassori L, Serra M, Olivero M, Di Renzo MF, Colombo MP, Picci P, Scotlandi K. Caveolin-1 reduces osteosarcoma metastases by inhibiting c-Src activity and met signaling. Cancer Res. 2007;67:7675–85. https://doi.org/10.1158/0008-5472.CAN-06-4697.
41. Qian X-L, Pan Y-H, Huang Q-Y, Shi Y-B, Huang Q-Y, Hu Z-Z, Xiong L-X. Caveolin-1: a multifaceted driver of breast cancer progression and its application in clinical treatment. OncoTargets Therapy. 2019;12:1539–52. https://doi.org/10.2147/OTT.S191317.
42. Patel HH, Insel PA. Lipid rafts and caveolae and their role in compartmentation of redox signaling. Antioxid Redox Signal. 2009;11:1357–72. https://doi.org/10.1089/ars.2008.2365.
43. Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Caveolae and signalling in cancer, Nature reviews. Cancer. 2015;15:225–37. https://doi.org/10.1038/nrc3915.
44. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network, Nature reviews. Mol cell Biology. 2001;2:127–37. https://doi.org/10.1038/35052073.
45. Yarden Y. Biology of HER2 and its importance in breast Cancer. Oncology. 2001;61:1–13. https://doi.org/10.1159/000055396.
46. Burgess AW, Cho H-S, Eigenbrot C, Ferguson KM, Garrett TPJ, Leahy DJ, Lemmon MA, Sliwkowski MX, Ward CW, Yokoyama S. An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors, molecular cell 12 (2003) 541–52. https://doi.org/10.1016/s1097-2765(03)00350-2
47. Garrett TPJ, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Kofler M, Jorissen RN, Nice EC, Burgess AW, Ward CW. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors, molecular cell 11 (2003) 495–505. https://doi.org/10.1016/s1097-2765(03)00048-0
48. Roepstorff K, Thomsen P, Sandvig K, van Deurs B. Sequestration of epidermal growth factor receptors in non-caveolar lipid rafts inhibits ligand binding. J Biol Chem. 2002;277:18954–60. https://doi.org/10.1074/jbc.M201422200.
49. Ringerike T, Blystad FD, Levy FO, Madshus IH, Stang E. Cholesterol is important in control of EGF receptor kinase activity but EGF receptors are not concentrated in caveolae. J Cell Sci. 2002;115:1331–40. https://doi.org/10.1242/jcs.115.6.1331.
50. Nagy P, Vereb G, Sebestyén Z, Horváth G, Lockett SJ, Damjanovich S, Park JW, Jovin TM, Szöllosi J. Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2. J Cell Sci. 2002;115:4251–62. https://doi.org/10.1242/jcs.00118.
51. Orr G, Hu D, Ozçelik S, Opresko LK, Wiley HS, Colson SD. Cholesterol dictates the freedom of EGF receptors and HER2 in the plane of the membrane. Biophys J. 2005;89:1362–73. https://doi.org/10.1529/biophysj.104.056192.
52. Campbell MR, Ruiz-Saenz A, Zhang Y, Peterson E, Steri V, Oeffinger J, Sampang M, Jura N, Moasser MM. Extensive conformational and physical plasticity protects HER2-HER3 tumorigenic signaling. Cell Rep. 2022;38:110285. https://doi.org/10.1016/j.celrep.2021.110285.
53. Kazazic M, Roepstorff K, Johannessen LE, Pedersen NM, van Deurs B, Stang E, Madshus IH. EGF-induced activation of the EGF receptor does not trigger mobilization of caveolae. Traffic. 2006;7:1518–27. https://doi.org/10.1111/j.1600-0854.2006.00487.x.
54. Abulrob A, Giuseppin S, Andrade MF, McDermid A, Moreno M, Stanimirovic D. Interactions of EGFR and caveolin-1 in human glioblastoma cells: evidence that tyrosine phosphorylation regulates EGFR association with caveolae. Oncogene. 2004;23:6967–79. https://doi.org/10.1038/sj.onc.1207911.
55. Couet J, Sargiacomo M, Lisanti MP. Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. J Biol Chem. 1997;272:30429–38. https://doi.org/10.1074/jbc.272.48.30429.
56. Pereira PMR, Mandleywala K, Ragupathi A, Lewis JS. Acute Statin Treatment improves antibody Accumulation in EGFR- and PSMA-Expressing tumors, clinical cancer research. Official J Am Association Cancer Res. 2020;26:6215–29. https://doi.org/10.1158/1078-0432.CCR-20-1960.
57. Nagy P, Jenei A, Kirsch AK, Szöllosi J, Damjanovich S, Jovin TM. Activation-dependent clustering of the erbB2 receptor tyrosine kinase detected by scanning near-field optical microscopy. J Cell Sci. 1999;112(11):1733–41. https://doi.org/10.1242/jcs.112.11.1733.
58. Albanell J, Baselga J. Trastuzumab, a humanized anti-HER2 monoclonal antibody, for the treatment of breast cancer. Drugs Today (Barcelona Spain: 1998). 1999;35:931–46.
59. Alawin OA, Ahmed RA, Ibrahim BA, Briski KP, Sylvester PW. Antiproliferative effects of γ-tocotrienol are associated with lipid raft disruption in HER2-positive human breast cancer cells. J Nutr Biochem. 2016;27:266–77. https://doi.org/10.1016/j.jnutbio.2015.09.018.
60. Sottocornola E, Misasi R, Mattei V, Ciarlo L, Gradini R, Garofalo T, Berra B, Colombo I, Sorice M. Role of gangliosides in the association of ErbB2 with lipid rafts in mammary epithelial HC11 cells. FEBS J. 2006;273:1821–30. https://doi.org/10.1111/j.1742-4658.2006.05203.x.
61. Jeong J, Shin JH, Li W, Hong JY, Lim J, Hwang JY, Chung J-J, Yan Q, Liu Y, Choi J, Wysolmerski J. MAL2 mediates the formation of stable HER2 signaling complexes within lipid raft-rich membrane protrusions in breast cancer cells. Cell Rep. 2021;37:110160. https://doi.org/10.1016/j.celrep.2021.110160.
62. Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, Tylaska L, King L, Zhou D, Dushin R, Myers JS, Rosfjord E, Lucas J, Gerber H-P, Loganzo F. Caveolae-Mediated Endocytosis as a Novel mechanism of resistance to Trastuzumab Emtansine (T-DM1), molecular cancer therapeutics 17 (2018) 243–53. https://doi.org/10.1158/1535-7163.MCT-17-0403
63. Pereira PMR, Mandleywala K, Monette S, Lumish M, Tully KM, Panikar SS, Cornejo M, Mauguen A, Ragupathi A, Keltee NC, Mattar M, Janjigian YY, Lewis JS. Caveolin-1 temporal modulation enhances antibody drug efficacy in heterogeneous gastric cancer. Nat Commun. 2022;13:2526. https://doi.org/10.1038/s41467-022-30142-9.
64. Pereira PMR, Mandleywala K, Ragupathi A, Carter LM, Goos JACM, Janjigian YY, Lewis JS. Temporal modulation of HER2 membrane availability increases Pertuzumab Uptake and Pretargeted Molecular Imaging of Gastric Tumors, Journal of nuclear medicine: official publication. Soc Nuclear Med. 2019;60:1569–78. https://doi.org/10.2967/jnumed.119.225813.

65. Manafi-Farid R, Ataeinia B, Ranjbar S, Jamshidi Araghi Z, Moradi MM, Pirich C, Beheshti M. ImmunoPET: antibody-based PET imaging in solid tumors. Front Med. 2022;9:916693. https://doi.org/10.3389/fmed.2022.916693.
66. Brown EL, Shmuel S, Mandleywala K, Panikar SS, Berry N-K, Rao Y, Zidel A, Lewis JS, Pereira PMR. Immuno-PET detects antibody-drug potency on Coadministration with statins, Journal of nuclear medicine: official publication. Soc Nuclear Med. 2023;64:1638–46. https://doi.org/10.2967/jnumed.122.265172.
67. Rao Y, Samuels Z, Carter LM, Monette S, Panikar SS, Pereira PMR, Lewis JS. Statins enhance the efficacy of HER2-targeting radioligand therapy in drug-resistant gastric cancers. Proc Natl Acad Sci USA. 2023;120:e2220413120. https://doi.org/10.1073/pnas.2220413120.
68. Surendra Panikar S, Shmuel S, Lewis JS, Pereira PMR. PET and optical imaging of Caveolin-1 in gastric tumors. ACS Omega. 2023;8:35884–92. https://doi.org/10.1021/acsomega.3c03614.
69. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147:275–92. https://doi.org/10.1016/j.cell.2011.09.024.
70. Chinni SR, Yamamoto H, Dong Z, Sabbota A, Bonfil RD, Cher ML. CXCL12/CXCR4 transactivates HER2 in lipid rafts of prostate cancer cells and promotes growth of metastatic deposits in bone. Mol cancer Research: MCR. 2008;6:446–57. https://doi.org/10.1158/1541-7786.MCR-07-0117.

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