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Review

Membrane rafts as a novel target in cancer therapy

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ABSTRACT

Membrane rafts are distinct plasma membrane microdomains that are enriched in sphingolipids and cholesterol. They organize receptors and their downstream molecules and regulate a number of intracellular signaling pathways. This review presents information on the dependence of several growth factor receptor signaling pathways on membrane rafts. It also discusses the involvement of rafts in the regulation of differentiation, apoptosis and cell migration connected with invasiveness and metastasis. Examples of known synthetic and naturally occurring substances that are known to affect lateral membrane organization in tumor cell growth are discussed as potential or actual therapeutics.

Contents

Abbreviations: ADAM, (a disintegrin and metalloprotease) metallopeptidase; ALPs, alkyl-lysophospholipids; CD24, signal transducer CD24 (glycosylphosphatidylinositol-anchored receptor); CD44, CD44 antigen (receptor for hyaluronic acid); CXCR4, chemokine receptor type 4; DISC, death-inducing signaling complex; DR4, death receptor 4; DRM, detergent resistant membrane; ECM, extracellular matrix; EGCG, epigallocatechin gallate; EGFR, epidermal growth factor receptor; Erk1/2, extracellular signal-regulated kinases 1 or 2; Akt/PKB, protein kinase B; FAC, focal adhesion complex; FADD, Fas-Associated protein with Death Domain; FAK, focal adhesion kinase; GTP, guanosine-5′-triphosphate; HNSCC, head and neck squamous cell carcinoma; HPCD, 2-hydroxyprophyl-beta-cyclodextrin; IGF-1, insulin-like growth factor 1; IGF-1R, type-I insulin-like growth factor receptor; IRS-1, insulin receptor substrate-1; LMP-1, latent membrane protein 1; 67LR, non-integrin laminin receptor; MβCD, methyl-β-cyclodextrin; MAP kinase, mitogen-activated protein kinase; MPP14, membrane type 1 matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OPCs, oligodendrocyte progenitor cells; PI3K, phosphoinositide 3-kinase; (n-3) PUFA, polyunsaturated fatty acids; SCID, severe combined immunodeficiency; SCLC, small cell lung cancer; Src, non-receptor tyrosine kinase; SDF-1, stromal-cell-derived factor 1; TGFα, transforming growth factor alpha; TKI, tyrosine kinase inhibitor; TRAIL, TNF-related apoptosis-inducing ligand; uPAR, urokinase-type plasminogen activator receptor

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1. Introduction

Alterations in the cell cycle, cell adhesion and migration, and programmed cell death play central roles in the initiation and progression of many types of tumor. These are complex processes that are regulated by multiple factors. Recent evidence suggests that specialized membrane domains termed membrane or lipid rafts play an active role in each of these cell processes.

Membrane rafts are distinct plasma membrane microdomains that are enriched in sphingolipids and cholesterol. They organize receptors and their downstream molecules and regulate a number of intracellular signaling pathways (for reviews see [1–3]). These membrane domains exist in a tightly packed, liquid-ordered (Lo) state. Lateral interactions of cholesterol with raft lipids [4] are essential for maintaining this raft structure [5,6]. The depletion of cholesterol from the plasma membrane disrupts rafts, leading to inappropriate cellular signaling events, and thus deregulating cellular functions [7]. Important post-translational modifications of proteins, such as the addition of a GPI anchor or sterols and palmitoylation, regulate raft affinity for the majority of proteins. Not all aspects of membrane raft organization have been resolved; in particular resting state raft formation is unknown [5,8,9]. Membrane rafts have been implicated in the regulation of cell proliferation, differentiation, apoptosis and migration, suggesting that the alteration of these domains could be involved in malignant transformation, invasiveness and metastasis, and making research on their structure and assembly/disassembly mechanisms very attractive.

Due to the various experimental approaches in research on these structures, several terms for them appear in the literature. Detergent resistant membrane (DRM) is the most commonly used term. Although DRM is not exactly equivalent to membrane raft, many researchers use this term because their results from assessments of membrane lateral heterogeneity in fact concern this membrane fraction [10]. In this paper, we use the term after the given authors for the sake of precision in the discussion of their results.

Membrane rafts have been described in numerous malignant tumor models, such as breast, colon, lung and prostate cancers, but their structure, function, and associated complex signaling pathways are still the subject of extensive studies. A better understanding of these aspects of raft biology could serve as the basis for therapeutic strategies. The regulation of membrane raft proteins and subsequent effects on cell signaling and tumor progression need to be addressed.

This article attempts to summarize the role of membrane rafts in neoplastic/tumor cell growth and invasiveness. It also considers potential novel therapeutics that focus on the membrane raft as a pharmacological target in tumor treatment.

2. Involvement of membrane rafts in the surface receptor signaling pathways in tumor/neoplastic cells

2.1. Raft-dependent EGF receptor signaling pathways

In many types of tumor, abnormal signaling by growth factor receptor(s) can facilitate cell proliferation and growth. Membrane rafts are the place where many signaling proteins, such as growth factor receptors, have been shown to localize and act as membrane signaling platforms [2,3]. Disrupting membrane rafts may be a means to change the signaling pathways (Fig. 1).

There is a certain amount of disagreement in the literature about whether membrane rafts play an inhibitory or activatory role in EGFR signaling. This indicates that depending on the cell system and pathway, membrane raft disruption by cholesterol-depleting agents could have both positive and negative effects on growth factor receptor-mediated signaling. The involvement of membrane rafts in EGFR signaling was confirmed by Zhuang et al. [11], whose results clearly suggest that these domains could play a role in the activation of these pathways. In that study, the caveolin-negative prostate cancer cell line LNCaP was treated with a raft-disrupting agent, filipin, which is a polyene macrolide that binds cholesterol, to form a complex in situ. Filipin treatment prevents the interaction of cholesterol with sphingolipids, thereby decreasing the stability of membrane rafts. Filipin has been shown to suppress ligand-dependent EGFR phosphorylation in the detergent-insoluble fraction of the membrane (DRM). After reconstitution of the raft domains with cholesterol, EGFR phosphorylation recovered to the levels observed in the control cells (without filipin treatment), suggesting that EGFR signaling might be mediated by membrane rafts [11].

Localization of EGFR to the membrane raft is an important phenomenon that leads to the resistance of the triple-negative breast cancer cell lines SUM159 and SUM149 to the growth inhibition caused by EGFR tyrosine kinase inhibitor. Disrupting the membrane raft structure with lovastatin and/or atorvastatin, which are drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, decreases this resistance in breast cancer cell lines [12]. Furthermore, localization of

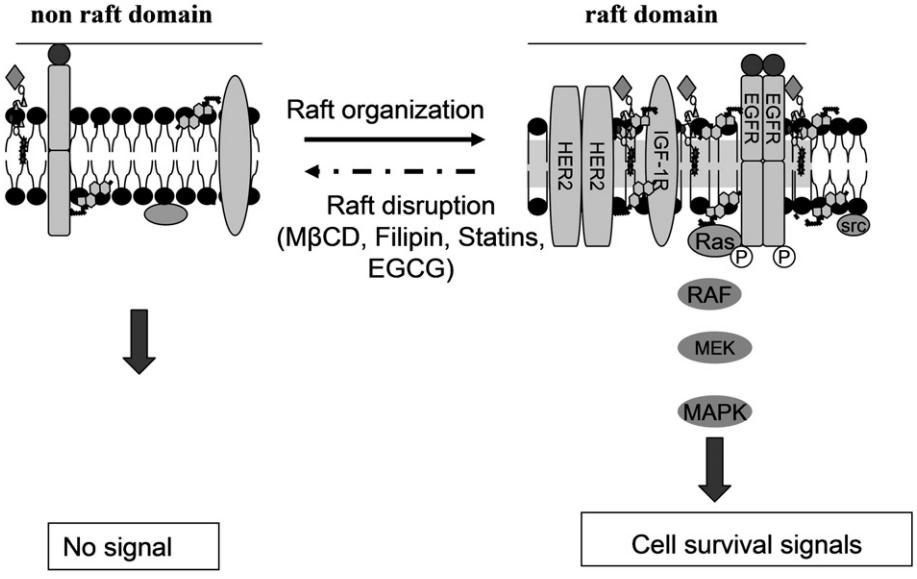

Fig. 1. The impact of raft-modulating agents on cell proliferation. One of the EGFR signaling cascades that are transduced through rafts is the Ras-Raf-MEK-MAPK pathway, which is thought to control cell growth, differentiation and survival. Raft disruption using MβCD, filipin, statins and EGCG led to a decrease in cell growth of cancer cells.

EGFR to membrane rafts provides a platform for the activation of Akt (protein kinase B) cell survival signaling pathway(s) in breast cancer cells in a manner that is independent of EGFR kinase activity.

Studies by Liu et al. [13] provided evidence that disrupting rafts inhibits EGF-induced phenomena in MDA-MB-231 cells. Methyl-beta cyclodextrin (MβCD) is a cyclic oligosaccharide that absorbs cholesterol from the cell membrane. It was found to impair directional migration of cells, EGF-induced cell adhesion, actin polymerization, Akt phosphorylation and protein kinase Cζ translocation, indicating a significant role for rafts in cancer growth and metastasis [13].

In the raft domains of breast cancer cells, the extranuclear estrogen receptor (ER) colocalizes with epidermal growth factor receptors (HER1 and HER2) where both modulate downstream events leading to MAP kinase-dependent ER phosphorylation and cell proliferation, which appears to play a critical role in resistance to endocrine therapy [14].

2.2. IGF-1 receptor signaling pathway

Membrane rafts provide a platform for activation that is dependent on insulin-like growth factor 1 (IGF-1) of two distinct PI3-K/Akt and MAPK signaling pathways that are prominent in cancer development and progression. IGF-1 signals primarily through the IGF type-1 receptor (IGF-1R) and stimulates phosphorylation of Akt and survival of oligodendrocyte progenitor cells (OPCs). IGF-1R, insulin receptor substrate 1 (IRS-1), the PI3K regulatory subunit p85, and Akt are sequestered within the cholesterol-enriched fractions in OPCs. IGF stimulation of OPCs results in increased P-IGF-IR and P-Akt in detergent-resistant membrane (DRM) fractions [15]. Cholesterol depletion by MβCD alters OPC membrane morphology and blocks IGF-1-mediated Akt phosphorylation. Furthermore, long-term inhibition of cholesterol biosynthesis with 25-hydroxycholesterol blocks IGF-1-stimulated Akt phosphorylation and cell survival [15]. Disruption of membrane rafts by depletion of cellular cholesterol with MβCD or filipin blocks the IGF-1R downstream signaling in 3T3-L1 pre-adipocytes, leading to cellular mitogenesis and adipocyte differentiation [16]. In human multiple myeloma cells, IGF-1R and β1 integrin colocalize to membrane rafts on the plasma membrane after stimulation with IGF-1 and activate the focal adhesion pathway, which is essential for optimal cell migration. After raft disruption by MβCD, IGF-1-induced adhesion was completely abolished. Conversely, replenishment with cholesterol completely restores IGF-1 enhanced adhesion. This indicates that β1 integrin-mediated adhesion induced by IGF-1 depends on intact membrane rafts [17].

2.3. Sigma receptors and the growth factor receptor c-kit

Sigma (σ) receptors are a novel family of drug-binding receptors that are expressed at high densities on a variety of tumor cell lines. Sigma receptor drugs inhibit proliferation in human carcinoma and melanoma cell lines, reduce cellular adhesion in mammary carcinoma cell lines and induce apoptosis in colon and breast adenocarcinoma cell lines [18]. The presence of σ1 receptors in both raft and non-raft fractions was recently shown, and it was postulated that σ1 receptors cause a remodeling of membrane rafts via a cholesterol-binding motif by increasing the level of membrane raft-associated cholesterol. Interestingly, the levels of both σ1 receptors and membrane rafts are elevated in cancer cell lines [18]. Sigma 1 receptor knockdown in human breast cancer cells MDA-MB-231 resulted in a reduction in the levels of cholesterol in membrane rafts (DRM). Additionally, treatment of cells with the prototypical drug SKF10047 blocked the σ1 receptor by reducing the level of receptor localized to membrane rafts, indicating that this receptor can regulate the cholesterol content in the lipid surrounding this receptor and probably that of associated molecules, such as integrin β1. MDA-MB-231 cells treated with SKF10047 or those with σ1 receptor knockdown are characterized by decreased cell growth and reduced cellular adhesion on collagen, fibronectin and laminin [19]. These studies suggest that σ1 receptor drugs may be used as a therapeutic treatment to decrease the "σ receptor membrane raft fraction" in cancer cells, thus inhibiting an oncogenic pathway and resulting in an increased sensitivity to pro-apoptotic drugs.

Membrane rafts also play an important role in polypeptide growth factor receptor c-kit signaling [20]. In small cell lung cancer (SCLC) cells, c-kit receptor associates with membrane rafts and activates the phosphoinositide 3-kinase (PI3K) pathway by facilitating the interaction of Src with specific PI3K isoforms. Inhibition of Src kinases or membrane raft disruption by MβCD or filipin impaired SCLC growth and induced apoptosis [20].

2.4. Membrane raft-affiliated receptors CD44 and CD24 and tumor progression

The process of metastasis involves cell invasion through connective tissue and transmigration through the endothelium. The focal adhesions are the sites where integrins and other proteins link the extracellular matrix (ECM) to the intracellular actin cytoskeleton [21]. The expressions of the glycosylphosphatidylinositol-anchored receptor CD24 and the hyaluronic acid (HA) receptor CD44 are increased in several tumor types, and this state is consistently associated with increased tumor invasion and metastasis in patients [22].

Multiple functions and cellular responses have been attributed to the activation of membrane raft-affiliated CD44, including the induction of cell motility, activation of cell survival responses, and promotion of cell adhesion [23]. CD44 has been described to play a role in tumor progression and in the promotion of metastases in various cancers, including breast, prostate [24] and head and neck squamous cell carcinoma (HNSC) [25], and it is usually associated with an unfavorable prognosis in this disease. CD44 has been associated with aggressive histological features in breast cancer, and the association of CD44 with matrix metallopeptidase 9 (MMP9) in membrane rafts in breast tumor cells promotes cell migration and invasion [26]. Intracellular signaling via Rac-GTPase, elicited by CD44 engagement, leads to ADAM2-mediated CD44 shedding and tumor cell migration [27]. It was recently demonstrated that membrane rafts play a crucial role in the localization and functionality of CD44. The model described by Donatello et al. for CD44-dependent breast cancer cell migration assumes that rafts regulate interactions between CD44 and its binding partner ezrin in the migrating MDA-MB-231 cell line. After the induction of migration, the affiliation of CD44 with membrane rafts decreased (possibly via depalmitoylation) as indicated by increased co-precipitation of CD44 with active (threonine-phosphorylated) ezrin-radixin-moesin (ERM) proteins. Also, an increased colocalization of CD44 with the non-raft protein, transferrin receptor (marker of non-raft compartments) was observed. The above-mentioned data point to rafts as pharmacological targets to downregulate cancer cell migration [28].

Treatment of human glioma cells with the membrane raft-disrupting agent MβCD resulted in an increase in CD44 shedding mediated by disintegrin and metalloproteinase 10 (ADAM10) [23]. CD44 shedding induced by cholesterol depletion is not limited to glioma but extends to other tumor cell lines, such as the pancreatic cancer cell lines MIA PaCa-2 and PANC-1 [23]. Cells that shed CD44 under physiological stimulation may also be induced to shed CD44 by cholesterol depletion. Similar patterns were observed when human neuronal glioblastoma U-251 MG cells were treated with filipin. Since these observations indicate that membrane raft disruption may cause shedding of CD44, the effect of long-term cholesterol depletion was evaluated using the cholesterol level-lowering drug simvastatin. This statin enhances CD44 shedding and suppresses tumor cell migration on a hyaluronan-coated substrate [24].

CD24 is a glycosylphosphatidylinositol-anchored cell adhesion receptor on platelets and endothelial cells and is identified as an alternative ligand for P-selectin [29]. The interaction of CD24 and P-selectin facilitates the passage of tumor cells from the bloodstream during

metastasis. Due to its localization in membrane rafts, CD24 exerts its functions by regulating the association of different proteins with membrane rafts that may form signaling platforms.

In pre-B lymphocytes and in breast cancer cell lines, the presence of CD24 regulated the activity of stromal-cell-derived factor 1 (SDF-1) by depleting the CXC chemokine receptor type 4 (CXCR4) from membrane rafts and blocking transmission signals in response to chemokine stimulation [30]. This prevents Erk phosphorylation, blocks cell motility and attenuates tumor growth. Therefore, CXCR4 metastatic potential may be modulated through alteration of its affiliation with rafts independently of its expression level.

The expression of CD24 is increased in several tumor types and is consistently associated with increased metastasis in patients. Furthermore, the localization of β1-integrins in membrane rafts is correlated with CD24. Transfection of CD24 into CD24-negative cells induces recruitment of β1 integrin into these domains. As CD24 is a major glycosylphosphatidylinositol-anchored and membrane raft-associated protein in many cell types, it might therefore act as a general gatekeeper to membrane rafts [31]. The binding of MDA-MB-231 cells to fibronectin induced the translocation of β1 integrin into the membrane raft fraction (DRM). Treatment of these cells with MβCD inhibited both the translocation of β1 integrin to DRM and the adhesion to fibronectin [31]. Binding of MDA-MB-231 cells to fibronectin seems to be membrane raft dependent, as MβCD treatment inhibited adhesion of these cells to fibronectin [31].

In summary, there is substantial evidence supporting the role of membrane rafts in the regulation of signaling from growth factor receptors that promote the growth and proliferation of cancer cells as well as from adhesion receptors that are related to cancer cell metastasis. Membrane raft disruption suppresses the activation of several growth factor receptors and thus the proliferation and progression of particular types of cancer.

3. Invasive and metastatic potential of tumor cells depends on membrane rafts

The high mortality rates associated with cancer are caused by the metastatic spread of tumor cells from the site of origin. Cell adhesion is a key factor associated with the metastatic spread of cancer cells, and inhibition of this process is considered a logical strategy in the treatment of neoplastic diseases. A variety of transmembrane proteins are involved in cell adhesion and spreading, including integrins, cadherins, selectins and cell–cell adhesion molecules of the Ig family [32]. Of these adhesion molecules, the integrins and their downstream signaling pathways have been extensively studied. Integrins are α/β heterodimeric transmembrane proteins that promote the attachment of cells to the components of ECM. Importantly, integrins have been recently found to be membrane raft associated [33]. The attachment of cells to certain ECM proteins, such as fibronectin, collagen and laminin, leads to the clustering of integrins and subsequent formation of focal adhesions [32]. Several cytoplasmic proteins are recruited into focal adhesions, such as focal adhesion kinase (FAK), c-Src, paxillin and vinculin. Specifically, FAK is a key regulator of cell adhesion and migration. It is reported that the recruitment of FAK to activated integrins is an early consequence of integrin–ligand interaction followed by rapid autophosphorylation of FAK-Y397 [34].

Tumor cell invasion relies on ECM degradation and requires matrix-degrading protrusions called invadopodia. The invasive potential of breast [35] and prostate cancer cells [36] has been linked to the raft-affiliated membrane type 1 matrix metalloproteinase (MPP14). Yamaguchi et al. [37] showed a reduction in the matrix degradation activity of MPP14 in MDA-MB-231 cells following the disruption of membrane rafts by cholesterol depletion. Two other members of the matrix metalloproteinase family, MMP2 and MMP9, are also correlated to high grades of breast cancer and are involved in the metastasis-related function of invadopodia, which are enriched in membrane rafts. A study investigating the importance of membrane rafts in regulating receptor serine protease urokinase-type plasminogen activator (uPAR) and MMP9 functionality in breast cancer demonstrated that cholesterol depletion reduces colocalization of uPAR and MMP9 with membrane rafts and significantly decreases their total mRNA and protein levels in MDA-MB-231 and ZR 751 cell lines. MβCD treatment significantly reduced breast carcinoma cell migration and invasion [38].

Several key molecules implicated in cancer cell migration and metastasis are regulated by membrane rafts via association of key signaling proteins that reduce adhesion and promote cell migration, increasing the probability of metastatic spread. Interrupting this step is considered a logical strategy for prevention and treatment of tumor metastasis.

4. Impact of raft modulating agents on pathways related to tumor growth, survival and metastasis

4.1. EGCG

Several known anticancer agents have recently been found to inhibit tumor cell proliferation or invasiveness through the disruption of membrane rafts. One of them is epigallocatechin gallate (EGCG), a major biologically active constituent of green tea. EGCG inhibits the activation of the epidermal growth factor receptor EGFR and downstream signaling pathways in several types of human cancer cells [39,40]. Adachi et al. [41] presented a hypothetical model postulating that in colon cancer cells (HT29) grown in the absence of EGF, most of the total cellular EGFR resides in the disordered domains (ld, non-raft fraction) of the plasma membrane and that during activation by EGF or TGFα, EGFR is translocated to the ordered (membrane raft) domains. Thus, the ability of EGCG to disrupt rafts in these cells might explain the ability of EGCG to inhibit the activation of EGFR. Moreover, EGCG treatment can synergistically enhance the growth inhibitory effect of the EGFR tyrosine kinase inhibitor, erlotinib, both in vitro and in animal xenograft models in HNSCC [42].

It is known that in over 90% of head and neck squamous cell carcinomas, both EGFR and one of its ligands, TGF-α, are overexpressed, which is associated with a poor prognosis for patients. The same group of researchers extensively studied the impact of EGCG on EGFR-dependent pathway activation in this type of cancer and presented a similar model which showed an inhibitory effect of EGCG on EGFR activation through changes in membrane raft organization and promotion of internalization of the non-activated EGFR monomer into the cytoplasm through phosphorylation of EGFR at serine 1046/1047 by p38MAPK. Ultimately, treatment with EGCG causes a marked reduction in phosphorylated (activated) EGFRs that are otherwise preferentially present in membrane rafts, and thereby inhibits EGFR signaling that is prominent in HNSCC [43].

Another important target molecule for EGCG is a non-integrin laminin receptor (67LR) localized in membrane rafts, the expression of which is significantly increased in tumor cells and which has been directly correlated to the metastatic phenotype [44]. This receptor has been implicated in laminin-induced tumor cell attachment and migration and in tumor angiogenesis and metastasis. The specific binding of EGCG to 67LR in the plasma membrane of human myelogenous leukemia cell line KU812 results in the ability of EGCG to downregulate high-affinity IgE receptor I (FcεRI) expression, exerting an inhibitory effect on Erk1/2 phosphorylation and consequently inhibiting cancer cell growth [45]. Additionally, EGCG seems to play a role in modulating multidrug resistance. Trastuzumab is a monoclonal antibody that interferes with the HER2/neu receptor. In trastuzumab-resistant BT474 human breast cancer cells, high-concentration EGCG treatment caused a dose-dependent decrease in growth and cellular ATP production, and induced apoptosis. EGCG suppressed Akt activity, leading to the induction of the forkhead box O transcription factor (FOXO3a) and cyclin-dependent kinase (CDK) inhibitor ($p27^{Kip1}$) [46]. Tamoxifen is an antagonist of the estrogen receptor in breast tissue. In tamoxifen-

resistant breast carcinoma cells, EGCG treatment caused cell growth inhibition and dose-dependent apoptosis [47]. Using EGCG in combination with trastuzumab or tamoxifen may be a novel strategy for the treatment of breast cancer.

4.2. PUFA

There is substantial experimental and clinical evidence suggesting that consumption of n-3 polyunsaturated fatty acids (PUFA), including docosahexaenoic acid (DHA, $22{:}6^{\Delta 4,7,10,13,16,19}$) and eicosapentaenoic acid (EPA, $20{:}5^{\Delta 5,8,11,14,17}$) is protective against colon tumorigenesis [48,49]. DHA can influence cellular membrane composition, thus changing plasma membrane properties, including membrane fluidity, phase behavior, permeability, fusion, flip-flop and protein function, following incorporation into membrane phospholipids [50]. Studies on the effect of DHA supplementation on breast, lung and colon carcinomas in vitro, and on an in vivo model of lung adenocarcinoma used to focus on EGFR protein localization, activity and downstream signaling showed diminished EGFR levels in membrane rafts, but increased EGFR phosphorylation, specifically at the Y1068 residue. In addition, Ras activation and Ras/Sos1 association with membrane rafts were significantly decreased [51]. Similar results were obtained for the MDA-MB-231 breast cancer cell line, where lipid alterations by n-3 PUFA reduced the levels of EGFR in rafts and increased whole cell levels of phosphorylated EGFR, which was associated with apoptotic signaling and growth inhibition, rather than growth promotion [52]. It was also recently shown that DHA-induced alteration in both the lateral and subcellular localization of EGFR in immortalized colonocytes suppresses EGFR downstream signal transduction, which has implications for the molecular basis of colon cancer prevention by DHA [53].

4.3. Emodin inhibits tumor progress by raft disruption

Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) is one of the main active components found in the roots and rhizomes of *Rheum palmatum* L. It inhibits human cancer cell migration by suppressing the PI3K-Cdc42/Rac1 signaling pathway [54] and limits the invasiveness of human cancer cells by suppressing MMP-9 expression through the inhibition of the activator protein 1 (AP-1) and nuclear factor kappa B (NF-κB) signaling pathways [55]. Confocal microscopy analysis of MDA-MB-231 showed that emodin markedly suppressed integrin β1 clustering and its colocalization with rafts. Another interesting finding is that emodin inhibits membrane raft clustering similarly to MβCD [56]. Emodin and MβCD markedly suppress translocation of integrin β1, FAK, paxillin and vinculin from the soluble cytosol to the insoluble (DRM) fraction after exposure to fibronectin. Further, mass spectrometry analysis of the effect of emodin on the cholesterol level and lipid profile showed that the cholesterol content in the DRM fraction decreased by ~16% in the emodin-treated cells compared to the control group, which is similar to the effect of MβCD. A similar effect was observed for various major sphingolipids: their DRM fraction content remarkably decreased after treatment with emodin. Moreover, cholesterol replenishment totally abolished the inhibitory effect of emodin as well as that of MβCD on the translocation of integrin β1 and focal adhesion complex (FAC) molecules to the membrane raft fraction. This suggests that emodin is likely to disrupt the integrin signaling pathway by reduction of membrane cholesterol. Thus, the inhibition of membrane raft clustering by emodin might be the underlying mechanism leading to the suppression of integrin clustering and FAC formation, meaning emodin is a promising candidate for the development of a novel therapeutic agent to prevent cancer metastasis [56]. The overall impact of raft disrupting agents on the process of metastasis is shown in Fig. 2.

4.4. Role of statins in the prevention and treatment of cancer

Statins are inhibitors of the first committed enzyme of the mevalonate pathway, 3-hydroxy-methylglutaryl (HMG) CoA reductase. As structural analogs of HMG-CoA reductase, statins block the conversion of HMG-CoA to mevalonic acid. In addition, since statins act at an early step in the synthesis of cholesterol, they also inhibit the production of isoprenoids such as geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate [57]. These isoprenoids are involved in cellular signal transduction by activating farnesylated proteins (for example, Ras proteins) and geranylgeranylated proteins (for example, RhoA and Rac1). The statin family consists of several drugs. Because of their cholesterol-lowering properties, statins are widely prescribed for the treatment of cardiovascular diseases. Furthermore, statins inhibit cell signaling pathways associated with the invasive and metastatic properties of various cancers, most probably through disintegration of the membrane rafts resulting from the reduced cholesterol content [58]. Several studies have investigated the possible therapeutic value of statins in prostate cancer treatment. Many scientists assume that the effect of statins on prostate cancer results from their effects on cholesterol synthesis and point to the accumulation of cholesterol in solid tumors, and to studies linking the increased risk of aggressive prostate cancer to elevated cholesterol levels [59]. However, *in vitro* studies showed that statins could

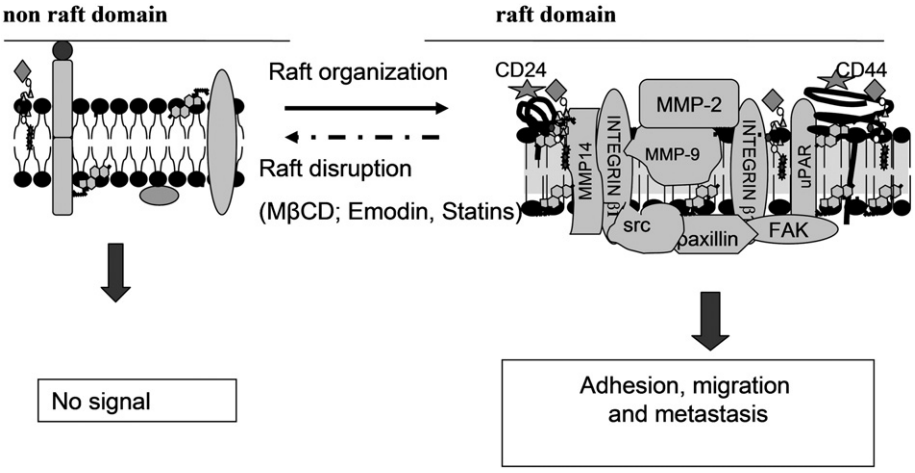

Fig. 2. Metastatic potential of tumor cells depends on membrane rafts. One of the EGFR signaling cascades that are transduced through rafts is the Ras-Raf-MEK-MAPK pathway, which is thought to control cell growth, differentiation and survival. Raft disruption using MβCD, filipin, statins and EGCG led to a decrease in cell growth of cancer cells. Membrane rafts recruit degradation enzymes, adhesion receptors and other proteins crucial for cell migration pathways. Lipid raft disruption by cholesterol depletion reduced cellular adhesion and inhibited migration and invasion of cancer cells.

also inhibit prostate cancer by preventing the activation of small GTP-binding proteins of the Ras superfamily [60], which play important roles in the development and progression of prostate cancer. Statins have a beneficial effect in the radiosensitizing of cancer cells. Cells in the G1 and G2-M phases of the cell cycle are most sensitive to ionizing radiation-induced cell death. Statins may sensitize these cells to radiation through G1 cell cycle arrest [60]. It has been shown that Ras overexpression, which promotes cell cycle progression, may confer radiation resistance, whereas a Ras-associated increase in radiation resistance can be reversed by lovastatin in osteosarcoma cells [61].

Rosuvastatin, a relatively new statin, was shown to inhibit prostate cancer cell growth and decrease tumor size in mice xenograft models. Moreover, rosuvastatin inhibits angiogenesis by reducing the number of blood vessels within tumors of statin-treated mice [62]. Studies on human U251 and U87 glioma cells suggest that the modulation of membrane rafts, Fas translocation into membrane rafts, and the activation of the PI3K/Akt/caspase-3 signaling pathway are involved in the antitumor effect of simvastatin and may have a role in cancer prevention and treatment [63]. Simvastatin profoundly impaired basal and growth factor-stimulated human small-cell lung cancer (SCLC) cell growth in vitro and induced apoptosis. SCLC cells treated with simvastatin were sensitized to the effects of the chemotherapeutic agent etoposide. Moreover, SCLC tumor growth in vivo was inhibited by simvastatin. In addition to its ability to block Ras membrane localization, the drug selectively downregulated H-Ras protein at the post-translational level [64]. EBV-associated immunoblastic lymphomas express each of the EBV nuclear antigens (EBNAs) and latent membrane proteins (LMPs) [65] that induce proliferation of B cells, so the virus is thought to be directly responsible for the pathogenesis of these tumors [66]. EBV LMP-1 localizes to membrane rafts and constitutively activates NF-κB via tumor necrosis factor receptor-associated factors [65]. Simvastatin, but not pravastatin, dissociates LMP-1 from membrane rafts and reduces activation of NF-κB, which in turn leads to apoptosis in vitro and prolongs the survival of SCID mice with EBV-induced lymphomas. Simvastatin separated LMP-1 from membrane rafts, inhibited NF-κB activation, and induced apoptosis in lymphoblastoid cell lines (LCLs) [67].

Jeon et al. performed a study on the role of membrane rafts in the interaction of components of the migration pathway and focal adhesion in non-small cell lung cancer (NSCLC) cells [68]. The results suggest that cholesterol depletion using MβCD followed by lovastatin treatment inhibited NSCLC migration via inhibition of the phosphorylation of raft-associated Src and translocation of molecules comprising focal adhesion complexes from membrane rafts.

Statins can affect several important cellular functions. Numerous studies have been performed to explore their interaction with chemotherapeutic agents, as they may influence the success of cancer treatments. Lovastatin triggers apoptosis of ovarian cancer cells as a single agent via a mevalonate-dependent mechanism. It can synergize with doxorubicin and potentiate apoptosis by blocking drug efflux through a mevalonate-independent mechanism that enables the intracellular retention and genotoxic action of doxorubicin [69]. In the treatment of non-small-cell lung carcinomas (NSCLCs) with atorvastatin and carboplatin (platinum-based chemotherapeutic agents), the growth of xenograft A549 tumors in nude mice was reduced and the survival rate enhanced compared to the results with carboplatin alone. Atorvastatin in combination with carboplatin had stronger effects on growth inhibition and apoptosis of NSCLC than either agent used separately, mainly via inhibition of Akt (PKB, a serine/threonine protein kinase) activity and resultant upregulation of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1). Additional atorvastatin administration resulted in synergistic inhibition of NSCLC and H1299 cell invasion and stimulation of TIMP-1 expression with carboplatin via stronger and persistent inhibition of Akt activity both in vivo and in vitro [70].

Involvement of lipid rafts in signaling pathways related to the growth, proliferation and motility of cancer cells indicates the possibility of treatment that targets these domains. Some naturally occurring and synthesized agents have the ability to remodel lipid rafts and thereby inhibit proliferation, alter cancer cell adhesion, and decrease motility (Fig. 2), indicating membrane rafts as promising pharmaceutical targets.

5. Sensitivity of tumor cells to apoptosis

5.1. Activation of the pro-apoptotic pathway by raft domains

There has been an extraordinary increase in research activity aimed at understanding the mechanisms and processes that underlie cell death. The main focus is impaired apoptosis in cancer cells and its relationship to the resistance of cancer cells to currently available drugs. Membrane rafts may form signaling platforms capable of activating both pro- and anti-apoptotic pathways, which may be inhibited upon membrane raft disruption [71].

The integrity of membrane rafts is required for the initiation of apoptosis, where membrane rafts provide a signaling platform for the activation of pro-apoptotic membrane receptor molecules [71]. This activation occurs via receptor oligomerization by agents that promote the integrity of the membrane rafts in the absence of receptor ligand molecules (Fig. 3A). Two major apoptotic pathways, extrinsic and intrinsic (mediated by mitochondria), may require membrane rafts for signal transduction [72]. The extrinsic pathway is initiated by the death receptors located at the cell membrane, which trigger the apoptotic signal when activated. The most characterized death receptor is Fas (CD95 Apo-1). After stimulation by its ligand FasL, Fas aggregates and recruits the adaptor molecule Fas-associated death domain-containing protein (FADD) through interaction between its death domain and the clustered receptor death domains. FADD interacts with procaspase-8 to form the death-inducing signaling complex (DISC) and causes the activation of downstream signaling and apoptosis [73]. Activation of the Fas receptor is possible upon receptor assembly into the membrane rafts (Fig. 3A). It occurs in cancer cells including leukemia Jurkat T, M624 melanoma, and breast cancer cells after irradiation or drug treatment [74]. Several anticancer drugs have been shown to suppress growth and induce the apoptosis of tumor cells through alteration of the membrane raft.

5.2. Agents that promote the integrity of the membrane rafts

Edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH3)) is a synthetic lipid in the class of alkyl-lysophospholipids (ALPs) which is used as an antitumor lipid drug. Edelfosine accumulates in membrane rafts and alters their lipid and protein composition in leukemic cells, with limited effect on normal cells. Edelfosine induces coclustering of FADD and procaspase-8 in membrane rafts in a ligand-independent manner, and thus gives rise to the formation of DISC [75]. Further studies have shown that edelfosine targets two different subcellular structures in a cell type-dependent manner, namely plasma membrane rafts in leukemic cells [75] and the endoplasmic reticulum in solid tumor cells [76]. Recent evidence shows that the novel antitumor drugs, marine-derived cyclodepsipetide, Aplidin^{R} and perifosine, which is octadecyl-(1,1-dimethyl-piperidinio-4-yl)-phosphate (D-21266), also induce translocation of Fas/CD95 and downstream signaling molecules into membrane rafts in leukemic cells. Disruption of membrane rafts abolishes the induction of apoptosis by these drugs, which demonstrates the important role of these domains in the apoptosis induced by these drugs [75,77].

This is also the case with avicin D, a plant triterpenoid that selectively inhibits the growth of tumor cells via activation of the apoptotic caspase pathway independently of the association between Fas ligands and Fas. By using a series of human leukemic cell lines deficient in cell death receptors, it was demonstrated that upon avicin D treatment, Fas translocates into membrane rafts and interacts with FADD and procaspase-8 to form DISC and thus mediates cell apoptosis. MβCD interference with

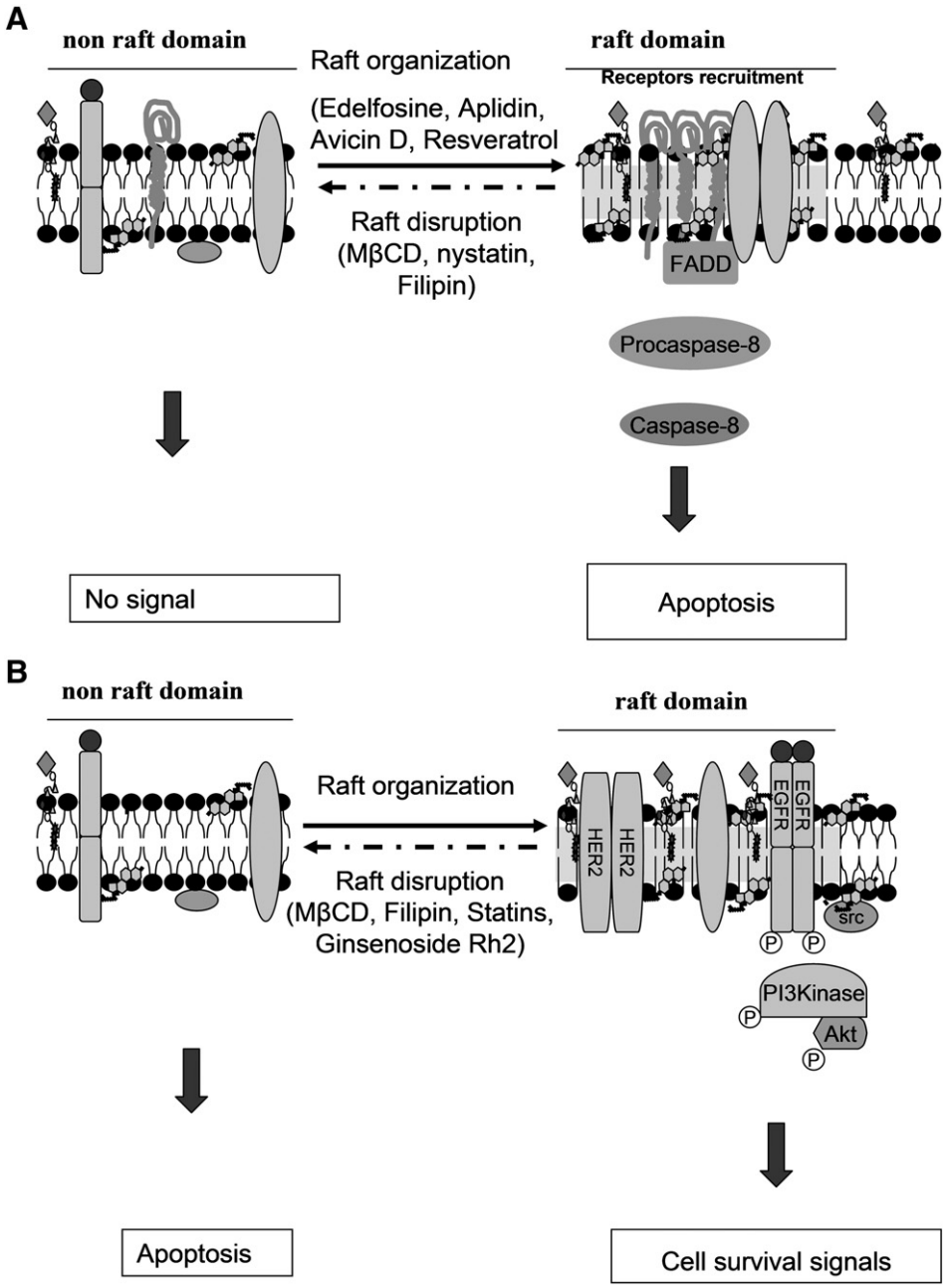

Fig. 3. Pro- and anti-apoptotic effects of lipid raft disruption. Apoptotic pathways mediated by lipid rafts involving apoptotic membrane receptor recruitment into membrane rafts. The group of anticancer agents acting at the level of the cell membrane mediates the activation of death receptors independently of its ligand through its co-clustering with membrane rafts and promotion of the apoptotic signal (A). Raft-dependent EGFR-mediated activation of the PI3K/Akt signaling pathway promotes cell survival. Disrupting the cholesterol-rich rafts interferes with EGFR signaling and inhibits a critical cell survival pathway (B).

membrane raft organization not only prevents the clustering of Fas and its DISC complex but also reduces the sensitivity of the cells to avicin D [78].

It was demonstrated that the natural polyphenol, resveratrol (3,5,4′-trihydroxystilbene) exhibits antitumor activities [79]. Resveratrol-induced apoptosis of a colon cancer cell line involves ligand-independent redistribution of Fas (CD95) into membrane rafts. Not all colon cancer cells are equally sensitive to resveratrol-induced cell death: SW620 cells appear the most sensitive and HT29 is the most resistant. In cells that resist resveratrol-mediated apoptosis, this polyphenol still induces redistribution of Fas and other death receptors (DR4 and DR5) into membrane rafts. Although this redistribution is not sufficient to trigger cell death, pretreatment with resveratrol dramatically enhanced cell apoptosis upon stimulation of the death receptor agonists with recombinant TNFα, anti-CD95(Fas) antibodies and TRAIL. In this synergistic effect with resveratrol, TRAIL appeared to be the most potent ligand. Similar observations were made for HCT116 cells. The cholesterol-sequestering molecule nystatin suppressed resveratrol-induced changes in membrane raft components, including cholesterol and sphingomyelin, and in the DISC components, consequently sensitizing colon cancer HT29 cells to death receptor stimulation [79].

The majority of established human NSCLC cell lines are either partially or completely resistant to TRAIL despite similar levels of expression of the receptors DR4 and DR5 in TRAIL-sensitive cell lines. The TRAIL-induced redistribution of DR4 and DR5 into membrane rafts contributes to the sensitivity to TRAIL in the sensitive NSCLC H460 cell line, which was also confirmed in intervention tests using the cholesterol-sequestering agent nystatin [80]. Other studies demonstrated that TRAIL does not induce effective membrane raft aggregation

capable of inducing apoptosis in gastric cancer cells. This implies that membrane raft dysfunction is a potential reason for the resistance of gastric cancer cells to TRAIL. Treatment of the MGC803 cell line with the anthracycline epirubicin at least partially enhances TRAIL-induced apoptosis through death receptor (DR4 and DR5) redistribution into membrane rafts. Pretreatment with 50 μg/ml nystatin partially prevented epirubicin-induced membrane raft aggregation and DR4 and DR5 clustering [81].

These examples demonstrate the importance of membrane rafts in clustering apoptotic death receptors, which is crucial to trigger apoptotic signal transduction. Current treatments targeting lipid rafts focus mainly on the activation of these apoptotic pathways by agents causing ligand-independent redistribution of death receptors into membrane rafts (Fig. 3A). It points to the necessity to test the effect of anti-raft agents/drugs on particular cancer cell lines, whether the raft-disruptive effect prevents proliferation or apoptosis.

5.3. Membrane rafts promoting anti-apoptotic signaling pathways and the potential of raft-modulating agents in cancer therapy

In some types of cancer, membrane rafts are capable of promoting anti-apoptotic signaling pathways, for example via activation of Akt signaling. Such pathways play an important role in the regulation of cancer cell survival. An increased activity of Akt protects cancer cells from apoptotic death and is correlated with the progression of several human cancers. Akt activation promotes cell survival via the phosphorylation and inactivation of pro-apoptotic proteins, including caspase-9 and Bad, and via activation of NFκB, and upregulation of the expression of anti-apoptotic genes such as *Bcl-xL* and *FLIP* [82,83]. The interaction of stimulated membrane receptors including the ErbB family of surface receptors, IGFRs, or signaling proteins such as Ras with Akt takes place in membrane rafts [84] and stimulates Akt kinase activity via PI3K (phosphoinositide 3-kinase) [85].

Membrane rafts were also found to mediate c-Src-dependent activation of PI3K/Akt signaling in the SUM159 breast cancer cell line [86]. In this case, the raft structure disruption could activate the apoptosis pathway or sensitize tumor cells to various cytotoxic drugs. EGF-induced Akt1 phosphorylation was substantially reduced in a dose- and time-dependent manner in the caveolin-negative human prostate cancer cell line LnCaP, when treated with the raft-disrupting agent filipin. The reconstitution of rafts with cholesterol induced cytoprotection from a PI3K-dependent apoptotic signal by restoring constitutive and EGF-induced levels of phosphorylated Akt1 [11].

In LNCaP cells, membrane raft disruption by 2-hydroxyprophyl-beta-cyclodextrin (HPCD) induced apoptosis via downregulation of EGFR/Akt and EGFR/Erk signaling pathways [87]. These data indicate a potential use of raft-modulating agents in cancer therapy (Fig. 3B).

Li et al. [86] also showed that membrane rafts are critical for the function of the PI3-K/Akt molecules involved in cell survival and proliferation. Cholesterol depletion using MβCD or simvastatin in human epidermoid carcinoma cell line A431 results in apoptosis involving Bcl-xL downregulation, caspase-3 activation, and Akt inactivation regardless of EGFR activation, and this occurs in parallel with reduced raft formation [88]. Reconstitution of membrane rafts through cholesterol addition reactivated Akt and restored cell viability. EGF treatment could not reverse the MβCD effect on cell survival, which indicates that the integrity of membrane rafts is critical for both basal Akt activity and EGF-induced Akt activation for cell survival. It was reported that various solid tumors have elevated levels of cholesterol, which correlates with an enhanced content of the membrane raft fraction in their membranes. In addition, cholesterol metabolism is dysregulated in many malignancies, including myeloid leukemia and lung and breast cancers [89,90]. MβCD inhibited proliferation in prostate (LNCaP, PC-3) and breast (MCF-7, MDA-MB-231) cancer cell lines in a dose-dependent manner, whereas their normal counterparts (PZ-HPV7, MCF-10A) showed resistance to MβCD-induced cell death [88].

Therefore, it seems that the use of statins as cholesterol-lowering agents and raft-disrupting agents might be crucial in the therapy of some types of tumor. Simvastatin stimulates cholangiocarcinoma apoptosis and this activity was closely associated with a decreased total cellular cholesterol level. Treatment with simvastatin triggers disruption of the colocalization of Rac1 within membrane raft structures in Mz-ChA-1 cells and downregulation of Rac1 activity [90]. These findings indicate that raft-targeted therapy using statins has a role to play in cancer chemotherapy.

It was previously demonstrated that membrane rafts are involved in the control of keratinocyte proliferation and metabolic activity. Disruption of membrane rafts using MβCD invariably causes apoptotic cell death in a concentration- and time-dependent manner. In addition to MβCD, other cholesterol-targeting and -depleting substances, such as filipin III, mevastatin, and cholesterol oxidase, also induced cell death in cultured HaCaT keratinocytes [91]. Cholesterol perturbation by different agents resulted in downregulation of Akt activity and downstream signal transduction to mTOR/$p70^{S6K}$ and FoxO3a, which is a dominant survival pathway, the blockage of which leads to apoptotic and autophagic cell death. Furthermore, Akt inhibition by cholesterol depletion sensitizes cells to the death ligand, TRAIL and the chemotherapeutic agents, etoposide and dixorubicin. Thus, raft disruption may be a promising method for increasing the chemosensitivity of malignant tumors to cystostatic treatment [92].

Recent studies by Pommier et al. on prostate cancer cell (LNCaP) culture and on xenografted nude mice showed that liver X receptors (LXRs) change the structural integrity of membrane rafts upon stimulation with synthetic receptor agonist T0901317 in a similar way to cholesterol-depleting agents. This downregulates the Akt survival pathway and thus induces apoptosis. Upon replenishment of cell membranes with exogenous cholesterol, the opposite effect to the situation in LXR stimulated cells was observed [93].

Interestingly, the cholesterol analogue ginsenoside (Rh2), which is a saponin derived from ginseng, was shown to reduce membrane rafts in the plasma membrane of human epidermoid carcinoma cells A431. Rh2 treatment of A431 cells resulted in the internalization and thus redistribution of rafts from the plasma membrane to the cytoplasm, leading to Akt inactivation and subsequently to apoptosis. In addition, Rh2 treatment blocked EGF-stimulated Akt activation but did not affect EGF-mediated EGFR phosphorylation and Erk1/2 activation, indicating a selective effect of Rh2 treatment on Akt [94]. In HeLa cells, Rh2 induced apoptosis via membrane raft disruption and ligand-independent Fas oligomerization [95].

New discoveries elucidate the complex role that the cell membrane and in particular, rafts and their associated molecules, play in the tightly coordinated processes of programmed cell death and cell survival. The results of these studies lead to the conclusion that the integrity of membrane raft domains plays a significant role in regulation of cell survival through increased activation of Akt, which protects cells from apoptotic death. Several raft-disrupting agents, such as MβCD and cholesterol-depleting simvastatin and filipin, impaired Akt phosphorylation, diminished Akt activity, and increased apoptosis, which suggest that there is potential for membrane rafts in anticancer therapy. This should however be experimentally verified in the case of particular cancer cell types.

6. Conclusion and perspectives

The growing interest in membrane microdomains (rafts) as targets for cancer treatment is related to their role in the regulation of multiple stages of malignant transformation of cells, such as growth, susceptibility to apoptosis, invasiveness and metastatic ability. Membrane rafts provide a signaling platform for several growth factor receptors, including the family of the receptor tyrosine kinases (EGFR, also known as ErbB-1 or HER1), ErbB-2 (HER2), ErbB-3 (HER3), and ErbB-4 (HER4). Most of these, in particular EGFR and HER2, are overexpressed in many

Table 1
Anticancer therapeutics that target membrane rafts.

Therapeutics	Cancer cell type	References
Growth and metabolism of cancer cells		
Methyl-β-cyclodextrin (MβCD), filipin	Prostate cancer cell line LNCa	[11]
	Human breast cancer cell line MDA-MB-231	[13]
	Small cell lung cancer (SCLC)	[20]
Lovastatin	EGFR TKIs resistant human breast cancer cell line SUM 159	[12]
n-3 polyunsaturated fatty acids (PUFA)	Human lung adenocarcinoma epithelial cell line A549; colon adenocarcinoma cell line WiDr and human breast carcinoma cell line MDA-MB-231	[51,52]
Sigma 1 receptor and prototypical σ1 receptor drug (SKF10047)	Human breast carcinoma cell line MDA-MB-231	[19]
Epigallocatechin gallate (EGCG)	Head and neck squamous cell carcinoma (HNSCC)	[39,42]
	Human colorectal cancer cell lines (Caco2, HCT116, HT29, SW480, and SW837)	[40,41]
	MMTV-Her-2/neu mammary gland breast tumor NF639 cells	[96]
	Human prostate cancer cell line (LNCaP)	[97]
Simvastatin	Human small-cell lung cancer (SCLC) cells	[64]
Adhesion and metastatic potential of cancer cells		
Emodin (3-methyl-1,6,8-trihydroxyanthraquinone	Human breast carcinoma cell line MDA-MB-231; human cervix epitheloid carcinoma cell line HeLa; human hepatocarcinoma cell line HepG2	[56]
MβCD and/or nystatin	Human multiple myeloma (MM) cells	[17]
	Human prostate cancer cells PC-3	[36]
	Human breast cancer cell lines MDA-MB-231 and ZR 751	[37,38]
MβCD, filipin, simvastatin	Human glioblastoma cell line U-251 MG and U87	[23,63]
MβCD, lovastatin	Human non-small cell lung cancer (NSCLC)	[68]
Apoptosis sensitivity in cancer cells		
Edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH3) and perifosine	Human multiple myeloma	[75]
Aplidine	Human multiple myeloma	[77]
Avicin D	Human leukemia cell lines, Daudi, NB4, and parental Jurkat cells	[78]
Resveratrol (3,5,4′-trihydroxystilbene)	Human colon carcinoma cell lines: HT29, HCT116, SW480, and SW620	[79]
TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and epirubicin	Non-small cell lung carcinomas (NSCLC): A549, H460	[80]
	Human gastric cancer cells MGC-803	[81]
2-Hydroxyprophyl-beta-cyclodextrin (HPCD); MβCD, simvastatin	Human prostate cancer cell line LNCaP	[87]
	Human epidermoid carcinoma cell line A431; human breast cancer cell lines MCF-7 and MDA-MB-231; human prostate cancer cell lines PC-3 and LNCaP	[88]
	Human cholangiocarcinoma cell lines (Mz-ChA-1, HuH-28, TFK-1, SG231, and HuCCT1)	[90]
	Human glioblastoma cell line U-251 MG and U87	[63]
	EBV-associated lymphomas	[67]
MβCD, filipin III, mevastatin,	HaCaT keratinocytes	[91]
	Small cell lung cancer (SCLC)	[20]
Synthetic liver X receptor agonist T0901317	Prostate cancer cell (LNCaP)	[93]
Ginsenoside Rh2	Human epithelial carcinoma cell line (HeLa)	[95]

types of tumor, including prostate, head and neck and breast tumors. When stimulated by binding to their ligands, these receptors are potent stimulators of tumor growth and survival. Therefore, raft-disrupting agents could become important anticancer therapeutics in such cases (Table 1).

Defects in the apoptosis pathway allow cells to grow in an uncontrolled manner and make them resistant to apoptosis-inducing agents, thus reducing the effectiveness of currently used anticancer therapy. Many apoptotic death receptors are located in rafts, which is crucial to trigger apoptotic signal transduction. In cells derived from blood malignancies, the raft-dependent group of synthetic ether-linked analogues of phosphatidylcholine and lysophosphatidylcholine, collectively named antitumor ether lipids (e.g. edelfosine and perifosine), cause ligand-independent intracellular activation of the death receptor Fas/CD95 by its recruitment together with downstream signaling molecules into clusters of membrane rafts. This illustrates the pro-apoptotic role of rafts. In solid tumors, mainly prostate and breast tumors, raft EGFR-mediated signaling also activates the PI3K-Akt pathway, which contributes to the anti-apoptotic effects of EGFR activation. Akt activation is closely related to cancer cell growth, because it affects cancer cell survival and proliferation. Raft-disrupting agents, such as MβCD and filipin and the naturally occurring EGCG and Rh2, have been shown to change the structural integrity of membrane rafts, preventing the activation of Akt and inducing apoptosis.

Statins decrease low-density lipoprotein (LDL) cholesterol levels and thus disrupt raft integrity. Several randomized clinical trials have shown that statins exhibit antitumor effects against various leukemia and solid tumor cells. Disruption of membrane rafts by statins results in the suppression of proliferation and induction of apoptosis. The synergistic effect of statins and currently used chemotherapeutics is of great importance as it may significantly enhance the effectiveness of cancer treatments. Considering the extensive experience on the safety of statins in humans, the investigation of utilization of statins as therapy alone or in combination with traditional chemotherapeutics for cancer may result in reasonable progress. Overall, these examples demonstrate the importance of membrane rafts as a target for improved strategy for the treatment of certain types of cancer and suggest a potential use of raft-modulating agents as anticancer drugs.

The discovery of the role of membrane rafts in important cancer cell processes leading to tumor growth and metastasis should promote further research in this area with an emphasis on naturally occurring factors that modulate or disrupt membrane rafts as potential potent drugs in the treatment of cancer.

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