Lipid rafts as signaling hubs in cancer cell survival/death and invasion: implications in tumor progression and therapy

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Abstract

Cholesterol/sphingolipid-rich membrane domains, known as lipid rafts or membrane rafts, play a critical role in the compartmentalization of signaling pathways. Physical segregation of proteins in lipid rafts may modulate the accessibility of proteins to regulatory or effector molecules. Thus, lipid rafts serve as sorting platforms and hubs for signal transduction proteins. Cancer cells contain higher levels of intracellular cholesterol and lipid rafts than their normal non-tumorigenic counterparts. Many signal transduction processes involved in cancer development (insulin-like growth factor system and phosphatidylinositol 3-kinase-AKT) and metastasis [cluster of differentiation (CD)44] are dependent on or modulated by lipid rafts. Additional proteins playing an important role in several malignant cancers (e.g., transmembrane glycoprotein mucin 1) are also being detected in association with lipid rafts, suggesting a major role of lipid rafts in tumor progression. Conversely, lipid rafts also serve as scaffolds for the recruitment and clustering of Fas/CD95 death receptors and downstream signaling molecules leading to cell death-promoting raft platforms. The partition of death receptors and downstream signaling molecules in aggregated lipid rafts has led to the formation of the so-called cluster of apoptotic signaling molecule-enriched rafts, or CASMER, which leads to apoptosis amplification and can be pharmacologically modulated. These death-promoting rafts can be viewed as a linchpin from which apoptotic signals are launched. In this review, we discuss the involvement of lipid rafts in major signaling processes in cancer cells, including cell survival, cell death, and metastasis, and we consider the potential of lipid raft modulation as a promising target in cancer therapy. — Mollinedo, F., and C. Gajate. Lipid rafts as signaling hubs in cancer cell survival/death and invasion: implications in tumor progression and therapy. J. Lipid Res. 2020. 61: 611–635.

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Abbreviations: ADAM10, a disintegrin and metalloproteinase 10; CASMER, cluster of apoptotic signaling molecule-enriched rafts; CD, cluster of differentiation; DD, death domain; DED, death effector domain; DISC, death-inducing signaling complex; DR4, death receptor 4; DR5, death receptor 5; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FADD, Fas-associated protein with a death domain; GPI, glycosylphosphatidylinositol; HA, hyaluronic acid; Hsp90, heat shock protein 90; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor type 1 receptor; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; MCL, mantle cell lymphoma; mTORC, mammalian target of rapamycin complex; MUC1, mucin 1; PDK1, phosphatidylinositol-dependent protein kinase 1; PH, pleckstrin homology; PEST, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog; TNFRSF, TNF receptor superfamily; TRAIL, TNF-related apoptosis-inducing ligand.

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Lipids are the major cell membrane components essential for maintenance of cell integrity and for numerous biological functions, including cell growth and division, and they act as critical signaling molecules and energy sources in normal and malignant tissues. Altered lipid metabolism is a newly recognized feature of malignancy. For example, increased lipid uptake, storage, and metabolism occur in a variety of cancers and contribute to tumor cell growth (1, 2). Cholesterol is a major component of mammalian cell membranes, comprising about 30% of the lipid bilayer, and it plays a crucial role in cell function and viability. Thus, cholesterol is an essential building block of the plasma membrane (Fig. 1), playing pivotal roles in maintaining the structural integrity and regulating the permeability and fluidity of cell membranes as well as processes involved in signaling initiation and cell adhesion to the extracellular matrix (ECM) (3, 4).

In addition to the central role of cholesterol in membrane biogenesis and cellular organization, it serves as an important precursor for different molecules that play a major role in physiology and cancer, including steroid hormones, oxysterols, and vitamin D, and can act as a ligand for estrogen-related receptor α (5), with different and even opposite actions on cancer cells and tumor progression (Fig. 1). For example, oxysterols, including 27-hydroxycholesterol, the most abundant oxysterol in plasma, are oxygenated derivatives of cholesterol formed by enzymatic or radical oxidation, and can affect distinct tumors in a different and complex way (6). Oxysterols have been reported to promote cancer cell proliferation (7), recruit pro-tumor neutrophils (8, 9), which seem to play an important role in cancer progression (10), and facilitate metastasis (10, 11). However, oxysterols can also suppress cancer cell proliferation acting as ligands of LXRs (12, 13). Another important steroid, vitamin D, has been shown to exert a chemoprotective action in several tumors, such as colorectal and prostate cancers (14, 15).

Cholesterol also has a number of other functions. It is a precursor of estrogens, and high levels of estrogen are associated with an increased risk of breast cancer (16). As stated above, cholesterol can also act as a functional and endogenous agonist of estrogen-related receptor α, which has important roles in cancer, fatty acid metabolism, and skeletal homeostasis as well as mitochondrial biogenesis and function (17, 18). In addition to the above processes, cholesterol is a major regulator of lipid organization, being responsible for the close packing of acyl chains of phospholipids, promoting phase separation, and stabilizing the formation of cholesterol- and sphingolipid-rich membrane microdomains known as lipid rafts or membrane rafts (19, 20), which constitute centers of organization for signal transduction and trafficking in normal and cancer cells (21–23). On the other hand, cholesterol is the precursor to androgens, essential for prostate cancer development and...
growth (24), and preclinical studies in mouse models of prostate cancer showed an increased risk of tumor development and enhanced tumor growth in mice fed a cholesterol-enriched diet (25). Thus, cholesterol can affect tumor cells in varying ways, as it plays a structural role for the generation of membranes required in proliferating cells and is a precursor of a wide array of molecules with diverse pro- and anti-tumor functions (Fig. 1). Importantly, a major function of cholesterol in normal and cancer cells is its critical role in the formation of the so-called lipid raft membrane domains, involved in cell signaling and in the modulation of cell function and fate.

**CHOLESTEROL AND CANCER**

Cholesterol is either obtained from the diet or synthesized de novo in the cell. Thus, the cholesterol content is narrowly balanced between metabolic processes intrinsic to the cell and the regulation of cholesterol distribution in the organism, and this complex homeostatic mechanism seems to be disrupted in cancer cells. In the early 1900s, a number of reports (26–30) showed that cholesterol levels were increased in cancer cells and in the surrounding tissues as compared with the normal tissues from which they were derived. It is now generally accepted that intracellular cholesterol levels tend to be higher in several cancer cell types (31–33) (Fig. 1). Nonetheless, it remains unclear whether such changes play a causal role in disease progression or are rather a consequence of other metabolic changes occurring during disease development (34).

Increased serum cholesterol levels have also been reported to be associated with a higher risk of developing cancer, such as colon, breast, prostate, and testicular cancer (24, 35–39). On the other hand, obesity has been associated with increased risk of several different cancer types; and hypercholesterolemia, an obesity-associated comorbidity, seems to promote tumor proliferation and inflammation (40, 41). However, the association between serum cholesterol and increased risk of cancer is not clear. Different epidemiological studies have provided inconclusive and even contradictory results, suggesting either a positive association between elevated serum cholesterol level and risk for certain cancer types or no association between cholesterol and cancer (33). On the other hand, low plasma levels of LDLS, which transport cholesterol to most surrounding tissues through receptor-mediated mechanisms, have been associated with an increased risk of cancer, but genetically reduced LDL cholesterol was not, thus suggesting that low LDL cholesterol levels per se do not cause cancer (42). Hypocholesterolemia has been found in solid tumors and some hematological malignancies (43), and may be due to the high LDL-receptor activity leading to increased LDL clearance and cholesterol utilization by cancer cells (42). This inverse association between blood cholesterol level and the risk of cancer was already observed in the 1980s (44). On these grounds, the decrease in plasma cholesterol content in cancer patients might be due to the increased utilization of lipids by neoplastic cells for membrane biogenesis. While the relationship between serum cholesterol and increased risk of cancer remains unclear (32, 45), a more consistent positive link has been found between accumulation of intracellular cholesterol and increased risk of tumorigenesis (46).

A number of epidemiological studies have revealed that prolonged use of cholesterol-lowering drugs (HMG-CoA-reductase inhibitors or statins) is associated with reduced cancer-related mortality (24, 47–53). This suggests that cholesterol accumulation could contribute to the appearance of cancer. In contrast, other epidemiological studies did not find the above association (54, 55). Overall, the epidemiologic results have been mixed and largely dependent on both the type of tumor in question and the particular statin that was used (56). Statins inhibit the rate-limiting enzyme that converts HMG-CoA into mevalonate, which in turn serves as a precursor for the synthesis of a number of downstream products, including cholesterol, ubiquinone, dolichol, and the isoprenoids geranylgeranyl pyrophosphate and farnesyl pyrophosphate that bind to several small GTP-binding proteins, such as RAS and RHO, facilitating protein translocation from the cytosol to the plasma membrane (57, 58). Thus, the inhibition of the mevalonate pathway by statins provokes pleiotropic antitumor effects, although statin sensitivity of cancer cell lines varies, and ambiguous results have been obtained in clinical trials (59).

Unlike epidemiologic studies that have provided inconclusive results on a relationship between abnormal plasma cholesterol levels and cancer risk, more compelling evidence has been obtained in laboratory studies. These latter studies indicate that cholesterol is capable of regulating proliferation, migration, and signaling pathways in mammalian cancers. In vivo studies have also indicated that plasma cholesterol levels can regulate tumor growth in mouse models of breast cancer (45).

Neoplastic diseases have been associated with several changes in lipid metabolism, particularly in cholesterol metabolism (60). Because cholesterol is critical for the synthesis of cell membranes, it follows that rapidly proliferating tumor cells require more cholesterol than normal cells. Alterations in cholesterol regulation have been detected in a wide number of solid and hematological cancers. Overall, current evidence suggests an increase in intracellular cholesterol and cholesteryl esters in cancer cells that has been suggested to be due to: 1) enhanced endogenous cholesterol biosynthesis by increased HMG-CoA-reductase activity leading to cholesterol de novo synthesis; 2) increased uptake of exogenous cholesterol by overexpression of the LDL-receptor, LDLS being the major bloodstream transporters of cholesterol; 3) increased cholesterol esterification mediated by cholesterol acyltransferase enzyme activity leading to cholesterol ester storage in lipid droplets (61); and 4) lower cholesterol efflux (60) (Fig. 1).

**LIPID RAFTS AND CANCER**

The dynamic nature of cell membranes together with an uneven distribution of lipids leads to the formation of specialized membrane domains, where proteins are selectively
included or excluded. Thus, cell membranes are structurally heterogeneous and contain discrete domains with unique physical and biological properties, leading to the coexistence of liquid-disordered and liquid-ordered phases in the same lipid bilayer. In 1997, Simons and Ikonen (19) proposed the lipid raft hypothesis, where lipid rafts were defined as dynamic membrane microdomains of sphingolipids and cholesterol that act as lipid-ordered platforms, which move within a fluid bilayer. These specialized membrane subdomains are enriched in glycosphingolipids and cholesterol as well as phospholipids acylated with saturated fatty acids. Lipid rafts are dynamic assemblies of proteins and lipids that can harbor receptors and regulatory molecules, and so act as platforms for signal transduction (21). Lipid rafts float freely within the liquid-disordered bilayer of cellular membranes, have different sizes depending on the membrane composition, and can be clustered to form larger and stabilized raft platforms. A consensus definition of a lipid raft emerged at the 2006 Keystone Symposium on Lipid Rafts and Cell Function held in Steamboat Springs, CO, as follows: “membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions” (Ref. 62; p. 1597). The tight interactions between the sterols and the sphingolipids in rafts lead to their characteristic resistance to solubilization by certain non-ionic detergents, which constitutes the basis for their isolation through density gradient centrifugation (63).

Cholesterol (or other higher sterols such as ergosterol and phytosterols) is universally present in large amounts (20–40 mol%) in eukaryotic plasma membranes, whereas it is absent in the membranes of prokaryotes (64). Cholesterol has very special features that make this molecule crucial for the correct functioning of mammalian cells, such as its ability to increase lipid order in fluid membranes while maintaining fluidity and diffusion rates and regulate passive membrane permeability, assuring high mechanical coherence and low leakiness of membranes (64). On these grounds, cholesterol’s role in the formation of the so-called rafts is critical where there is a dynamic and preferential clustering and packing of sphingolipids and cholesterol into moving platforms. Thus, cholesterol is a major regulator of lipid organization, promoting phase separation and stabilizing the formation of lipid rafts with closely packed acyl chains of phospholipids. Cholesterol is thought to serve as a spacer between the hydrocarbon chains of the sphingolipids and to function as a dynamic glue that keeps the raft assembly together (21, 65).

A major feature of lipid rafts lies in their ability to dynamically recruit or exclude signaling proteins to enhance or dampen signal transduction, thus leading to the notion of lipid rafts as a lipid-based structure able to modulate cell signaling and function (21, 66). A number of proteins show reversible association to rafts in response to appropriate signals (67). The physical segregation of proteins into lipid rafts may modulate the accessibility of those proteins to regulatory or effector molecules (67). In this regard, lipid rafts can constitute concentrating platforms for individual receptors, activated by ligand binding or other means, thus preserving and protecting the ensuing generation of signaling complexes from the action of non-raft inhibitory proteins, which otherwise could affect the signaling process. Raft binding leads to the recruitment of proteins to a new micro-environment, thus providing a membrane niche where proteins that turn on and off certain signal transduction pathways can be segregated from each other, which results in facilitated downstream signaling (68).

The precise mechanisms by which proteins sort into lipid rafts are not fully understood. The lipid shell model suggests that individual membrane proteins can be surrounded by a dynamic shell of raft-lipids (69). These shells are thermodynamically stable mobile entities in the plane of the membrane that are able to target the protein they encase to preexisting rafts (69, 70). Some structural properties of the proteins and posttranslational lipid modifications can account for the localization of proteins in rafts, including: 1) hydrophobic membrane-spanning sequences or transmembrane domains; 2) hydrophobic tails, such as glycosylphosphatidylinositol (GPI) anchors, N-myristoylation, or S-palmitoylation; and 3) protein-protein and protein-lipid interactions (67). Proteins with raft affinity include dually acylated proteins [e.g., Src-family protein tyrosine kinases (71), palmitoylated type-I transmembrane proteins [e.g., cluster of differentiation (CD) 44] (72), transmembrane proteins, such as flotillin (73), and receptor tyrosine kinases with two transmembrane subunits [e.g., insulin receptor (74) and epidermal growth factor (EGF) receptor (EGFR) (75)]. GPI-anchored proteins have a preference for lipid rafts because phosphoinositide anchors typically have saturated chains. Protein palmitoylation represents one of the major raft-targeting signals because the saturated nature of the fatty acyl chain coupled with its chain length enables palmitate to serve as a major driving force for proteins to associate with lipid rafts (76).

Interestingly, cancer cells have been reported to show elevated levels of membrane lipid rafts and cholesterol (77), as assessed by filipin staining (a naturally fluorescent polyene antibiotic that binds to cholesterol but not to esterified sterols) and binding of Alexa 568-conjugated cholera toxin-B subunit (which binds to ganglioside GM1, a raft component) to detect plasma membrane cholesterol and lipid rafts, respectively, followed by confocal microscopy analysis (77). A number of human prostate and breast cancer cell lines (PC-3, LNCaP, MCF-7, and MDA-MB-231) show stronger cholesterol and GM1 staining compared with their non-tumorigenic cell line counterparts (PZ-HPV7 and MCF-10A) (77). Furthermore, 1.5- to 2-fold higher cholesterol and raft levels in tumorigenic versus non-tumorigenic melanoma cells have also been reported (78), as assessed by using filipin and the fluorescent di-4-ANEPPDHQ probe, used to measure membrane lipid order (79), followed by flow cytometry analysis. The apparent higher presence of rafts in cancer cells is of importance because lipid rafts can serve as concentrating platforms to sort different signaling processes, and they harbor many cancer-related signaling and adhesion molecules (21). Lipid rafts are also involved...
in major processes related to cancer progression and therapy (Fig. 2), most of them linked to the hallmarks of cancer (80).

SPATIAL COMPARTMENTALIZATION OF SIGNALING PATHWAYS IN LIPID RAFTS

A critical and key feature of lipid rafts is their ability to compartmentalize different signaling pathways (21, 23, 81–88), thus acting as sorting platforms where signaling routes can be harbored to launch potent signals delivered from the membrane to other cellular sites in an efficient and rapid manner. Proteins involved in different signaling cascades could be either recruited or excluded from rafts through mechanisms not yet well understood, which could lead to the interaction or segregation of different signaling cascades. The presence of different proteins within a limited cellular region facilitates protein-protein interactions. Clustering of lipid rafts to form large raft platforms provides a mechanism by which an increasing number of proteins can be recruited to exert their functions in a safe and favorable way. These raft platforms provide the appropriate scaffolds for the recruitment of receptors, adaptor proteins, and other signaling molecules to generate dynamic headquarters to control what gets in and what stays out, thus constituting signaling units from which signals can be delivered to exert and modulate various cell functions (21, 23, 82, 84, 85). A key hallmark of membrane rafts lies in the physical segregation of proteins in lipid rafts that may modulate the accessibility of proteins to regulatory or effector molecules (67). This raft-mediated sorting of proteins creates a situation that limits and favors the interaction of raft-located proteins to other molecules present in the same membrane domain. Thus, a protein in membrane rafts might interact with other proteins already recruited in rafts, an event that otherwise would have never taken place (89). In this context, the final functional role of a protein in rafts would depend on its inherent activity as well as on the environment and proteins located in the same membrane raft with which it interacts. In this regard, it might be envisaged that the functional role of raft-located chaperones, kinases, phosphatases, or other proteins would rather depend on the client or substrate proteins physically available in the same membrane domain. On these grounds, sorting of proteins in rafts may restrict or potentiate signaling cross-talk. Membrane rafts are assumed to be transient and dynamic structures (90, 91), and advanced imaging techniques have revealed that several signaling molecules are transiently recruited to membrane domains with the aid of protein-protein, protein-lipid, and/or lipid-lipid interactions (23, 92–95). This transient and dynamic spatial compartmentalization of signaling pathways in lipid rafts provides a high flexibility and plasticity in signal transduction. In some way, membrane rafts can be viewed as signaling rafts that allow different signaling pathways to operate more efficiently and prevent or modulate cross-talk with other signaling cascades. Recruitment or exclusion of proteins in membrane rafts would highly depend on protein modifications, protein-protein interactions, and raft lipid composition. Given the relevant role of lipid rafts in signal transduction, membrane rafts and modulation of raft organization have become appealing and promising targets for therapeutic intervention in cancer chemotherapy (82, 96–98). Several examples of spatial compartmentalization of distinct signaling pathways in relation to cancer (survival, cell death, metastasis) are described in the following sections and are briefly summarized in Fig. 3.

LIPID RAFTS AND SURVIVAL SIGNALING

Shortly after the identification of lipid rafts in mammalian cell membranes, these membrane domains were rapidly associated to the proper function of several signal transduction processes involved in the immune response and cell survival (FcεRI receptor, T-cell receptor, B-cell receptor, EGFR, insulin receptor, ephrinB1 receptor, neurotrophin and GDNF signaling, Hedgehog signaling, H-RAS, integrins, eNOS, and AKT) (21).

Autocrine/paracrine growth factor receptor/ligand signaling loops, such as insulin-like growth factor (IGF) and EGF pathways, contribute to increased cell survival and unregulated proliferation of cancer cells in the tumor microenvironment. Overexpression and overactivation of a number of growth factor systems as well as of survival signaling routes are critical for tumor development, and the regulation of their respective downstream signaling pathways has been shown to be dependent on their presence in lipid rafts (23).

IGF system

IGFs are natural hormones that play crucial roles in cell growth and survival, apoptosis suppression, angiogenesis,
Fig. 3. Different types of lipid rafts harboring and modulating survival/proliferating, cell death/apoptosis and cell migration/invasion signaling pathways in cancer cells. Lipid rafts or membrane rafts are depicted in different colors (blue, green, and red) to highlight the heterogeneity of lipid rafts regarding both lipid and protein composition and their putative relationship to a cell signaling process or determined cell function. Irrespective of the color herein represented, lipid rafts are enriched in cholesterol and sphingolipids, but subtle changes in the raft lipidome are likely to occur to enable the recruitment of specific proteins that eventually trigger specific signaling events. Segregation of different signaling pathways in distinct raft platforms within the plasma membrane enhances signaling efficiency by increasing the proximity and effective concentration of components in the raft scaffold (a, b). Dynamic inclusion and exclusion of proteins into rafts modulate cell function (c). See the text for further details.
and metastasis in various tumors (99–101). IGFs include insulin (5.8 kDa) as well as the small proteins IGF-1 (7.6 kDa) and IGF-2 (7.5 kDa) with a similar molecular structure to insulin. The IGFs’ actions are mediated through their interaction with receptors that belong to the large class of tyrosine kinase receptors. Two homologous receptors are the homodimeric disulphide-linked (αβ), insulin receptor and the insulin-like growth factor type 1 receptor (insulin receptor and IGF-1R; ≈60% homology), made up of two β subunits (95 kDa), containing a single transmembrane domain and cytoplasmic domains exhibiting tyrosine kinase activity, and two extracellular α subunits (135 kDa), containing the ligand binding domain. These subunits are linked through disulphide bonds in a beta-alpha-alpha-beta configuration. A third unrelated receptor is the mannose 6-phosphate/IGF-2 receptor (M6P/IGF-2R). In addition, the IGF system includes a family of six IGF-binding proteins that act as IGF carriers and bind to IGFs with an equal or higher affinity than the IGF-1R (102), thus regulating IGFs’ bioavailability to receptors. IGFs and insulin are able to cross-bind to each other’s receptor, albeit with varying affinities (103).

The human type 1 IGF receptor is closely related to human insulin receptor (53% sequence identity in their ectodomains) and their respective monomers can form heterodimers. Aberrant IGF-1R signaling is involved in cancer proliferation and metastasis (104) and has been considered as an interesting anti-cancer target (105). Overexpression of IGF-1R occurs in many types of cancer, including breast cancer (100, 106), glioma (107), gastrointestinal cancer (108), pancreatic cancer (109), and multiple myeloma (101, 110). Furthermore, IGF-1R has potent anti-apoptotic and transforming activities, and thereby increased IGF-1R expression and activity is associated with tumor metastasis, poor prognosis, and treatment resistance in cancer patients (111–114). IGF-1 exerts potent mitogenic and antiapoptotic effects through its binding to IGF-1R, leading to activation of the intrinsic tyrosine kinase activity within the intracellular part of IGF-1R. Once activated, IGF-1R binds to intracellular adapter proteins, predominantly insulin receptor substrate (IRS)-1 or IRS-2, leading to their recruitment and phosphorylation (115). These adapter proteins serve as scaffolds and are necessary to transmit signals downstream in the cell through the phosphatidylinositol 3-kinase (PI3K)-AKT and RAS-RAF-MEK-MAPK/ERK signaling pathways, which promote cell survival and cell proliferation (115, 116). Lipid rafts have been shown to be required for IGF-1R downstream signaling in 3T3-L1 cells, but not for the activation of the receptor itself by its ligand (117, 118). IRS-1 has been reported to localize in membrane domains, where it is tyrosine phosphorylated in the presence of IGF-1 (119). This suggests that localization on lipid rafts enables IGF-1R to have a close contact with downstream signaling molecules recruited into lipid rafts (Fig. 5), thus transmitting survival signals (117).

TNF-related apoptosis-inducing ligand (TRAIL) death receptor has been shown to activate IGF-1R signaling in MGC803 and BGC823 human gastric cancer cell lines through translocation of IGF-1R, via Casitas B-lineage lymphoma b (Cbl-b), into lipid rafts, thus antagonizing TRAIL-induced apoptosis (120). This redistribution of IGF-1R in lipid rafts, leading to the activation of a survival signaling pathway, might contribute to the relative resistance of gastric cancer cells to the apoptotic effect of TRAIL (121–123). The IGF system plays a key role in the pathogenesis of most cancers, including multiple myeloma, as well as in the development of drug resistance (101). IGF-1 has been shown to trigger clustering of membrane rafts, which coalesce into large domains in the human multiple myeloma cell line OPM6 (124). IGF-1 also induces a rapid and transient translocation of IGF-1R, β1 integrin, and p85<sup>PI3K</sup> to lipid rafts in multiple myeloma OPM6 cells, suggesting a role for IGF-1 in trafficking and homing of multiple myeloma cells in the bone marrow (124), where reciprocal interactions with the bone marrow foster multiple myeloma cell survival, proliferation, and drug resistance (101). On the other hand, IGF-1 stimulation in oligodendrocyte progenitor cells led to the recruitment of IGF-1R, p85<sup>PI3K</sup>, and AKT (also known as protein kinase B) into cholesterol-enriched rafts, where AKT and IGF-1R were phosphorylated (125). Disruption of rafts by cholesterol depletion with methyl-β-cyclodextrin or through long-term inhibition of cholesterol biosynthesis with 25-hydroxycholesterol inhibited IGF-1-mediated AKT phosphorylation and cell survival (125). Interestingly, segregation of IGF-1R in and out of cholesterol-enriched lipid rafts has been suggested to dynamically regulate pro- and anti-apoptotic effects of IGF-1 on apoptosis induced by death receptors in colon carcinoma cells (126).

**PI3K/AKT signaling**

IGF-1 is the most potent natural activator of the PI3K/AKT signaling pathway, which in turn constitutes a major cell survival pathway and regulates a wide range of target proteins that control cell proliferation, survival, growth, and other processes, thus serving a key role in the occurrence and development of tumors (127, 128). As mentioned above, IGF-1R stimulation triggers autophosphorylation and subsequent phosphorylation and activation of IRS-1/2 proteins, which serve as scaffolds to promote recruitment of class I PI3K, a heterodimeric enzyme composed of a 110 kDa catalytic subunit and an 85 kDa regulatory subunit. Class I PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3) in the plasma membrane, being the major activator of AKT.

AKT (about 60 kDa) belongs to the AGC family of serine/threonine kinases and has three conserved domains, namely: pleckstrin homology (PH) domain, which binds phosphoinositides with high affinity, as well as catalytic and regulatory domains (129). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate to generate the lipid second messenger PIP3 at the cell membrane that binds to the AKT-PH domain, and this binding allosterically activates AKT by promoting high-affinity substrate binding (130). An increased local concentration of PIP3 promotes membrane recruitment of phosphatidylinositol-dependent protein.
RAS/RAF/MAPK and PI3K/AKT signaling pathways in human cancers (134, 135). AKT abnormal overexpression or activation has been reported in many human solid tumors and hematological malignancies, being associated with increased cancer cell proliferation and survival and becoming an important cancer therapeutic target (136, 137). Receptor tyrosine kinases, such as IGF-1R, EGFR, human epidermal growth factor receptor 2 (HER2/Erbb2), and platelet-derived growth factor receptor (PDGFR), are activated in many cancers, and they trigger downstream signal cascades, including the RAS/RAF/MAPK and PI3K/AKT signaling pathways (138–141). Moreover, the direct interaction of RAS with the p110 catalytic subunit of PI3K (142) shows a connection between the above two RAS/RAF/MAPK and PI3K/AKT cascades of major importance in cancer development (143, 144). The spatial compartmentalization of the PI3K/AKT signaling in lipid rafts (Fig. 3) constitutes an advantageous way to segregate this survival signaling from the action of the above negative regulators. PTEN has been localized to non-raft regions, and the genetic targeting of PTEN to membrane rafts abolished PDK1 activation, AKT membrane recruitment, and AKT activity (145).

Autocrine IGF-1/IGF-1R signaling is responsible for constitutive PI3K/AKT activation in a number of hematological and solid tumors (146–148). Interestingly, IGF-1-mediated activation of AKT is dependent on lipid rafts (145, 149, 150) (Fig. 3). Lipid rafts seem to play a crucial role in triggering the PI3K/AKT signaling pathway by facilitating AKT recruitment and activation upon PI3 accumulation in the plasma membrane (145, 151). An endogenous raft-resident AKT has been reported in LNCaP human prostate cells that showed a different substrate affinity as compared to non-raft AKT, which suggests the presence of distinct AKT populations with differential signaling behavior (152). We have found that raft-mediated AKT signaling is critical for cell survival in mantle cell lymphoma (MCL) (68). MCL cells show constitutive activation of the PI3K/AKT pathway (153), and most of the PI3K, AKT, PDK1, and mTOR signaling molecules have been located in lipid rafts in these cells, thus favoring raft-mediated AKT phosphorylation (68) (Fig. 3). Following treatment with the alkylphospholipid edelfosine (1-Octadecyl-2-Omethyl-rac-glycero-3-phosphocholine), a raft-targeted antitumor drug (82, 97, 98, 154), AKT as well as PI3K, PDK1, and mTOR were displaced from lipid rafts, thus leading to AKT dephosphorylation and apoptosis (68). Raft disruption by cholesterol extraction or inactivation with methyl-β-cyclodextrin, filipin III, or 5-cholestene-5-β-ol has been reported to abrogate the binding of AKT and PDK1 to the membrane by PH domains and to increase sensitivity to apoptosis stimuli in normal, premalignant (HaCaT), and malignant (A431) epidermal human keratinocytes (155). Disruption of lipid rafts by methyl-β-cyclodextrin-mediated cholesterol depletion also impairs activation of the PI3K/AKT pathway in different cell types, including rat alveolar macrophage NR8383, human lung adenocarcinoma A549, and human T cell leukemia Jurkat cell lines, favoring apoptosis (156). Psychosine (galactosylsphingosine) is the glycosphingolipid intermediate in the biosynthesis of cerebrosides that accumulates in the rare infantile neurodegenerative Krabbe disease, leading to the loss of oligodendrocytes and widespread demyelination due to apoptotic processes as well as aberrant inflammatory response (157). Psychosine has been shown to preferentially accumulate in and disrupt the structure of lipid rafts (158, 159), leading to the inhibition of PI3K recruitment to lipid rafts after IGF-1R activation and, hence, inhibition of the IGF-1R-PI3K-AKT pathway in lipid rafts, increasing in this way neuronal vulnerability (160). On the other hand, activation of LXR induces transcriptional induction of inducible degrader of the LDL receptor (IDOL), an E3 ubiquitin ligase that modulates cholesterol levels by ubiquitination of the LDL receptor (LDLR) on its cytoplasmic domain, thereby targeting it for degradation (161, 162). LXR activation induces the expression of ABC transporters implicated in cholesterol efflux (163, 164). Thus, LXR activation ultimately leads to a decrease in LDL uptake and an increase in cholesterol efflux, altogether decreasing the intracellular pool of cholesterol as well as the lipid raft size and number (165). Elevation of circulating cholesterol in SCID mice promoted tumor growth and increased Akt phosphorylation as well as the cholesterol content and the extent of protein tyrosine phosphorylation in lipid rafts isolated from LNCaP (androgen-sensitive human prostate adenocarcinoma cells) xenograft tumors, and reduced apoptosis in the xenografts (166). LXR activation in LNCaP prostate cancer cells led to: 1) a decrease in tumor cholesterol content due to an increase in cholesterol efflux by ABCG1-stimulated reverse cholesterol transport; 2) smaller and thinner lipid rafts; 3) downregulation of AKT survival signaling in lipid rafts; and 4) apoptosis of LNCaP cells in both xenografted nude mice and cell culture (167).

Taken together, these findings indicate how cholesterol-rich lipid rafts can segregate, potentiate, and preserve a particular signaling route, such as the above PI3K-AKT signal transduction pathway, providing a scaffold for the recruitment of specific proteins in a favorable environment, and highlight the importance of cholesterol-rich rafts in the regulation of cell survival in cancer cells.

**Estrogen receptor**

Approximately 70% of human breast cancers are hormone-dependent and estrogen receptor (ER)-positive (168). In mammals there are two ERs, ERα (595 amino acids; 67 kDa) and ERβ (530 amino acids; 59 kDa), both members of the nuclear receptor superfamily of hormone-inducible...
transcription factors (169, 170). Although most ERs localize in tumor cell nuclei, where they are translocated as dimers to induce transcriptional changes in estrogen-responsive genes, a significant pool of ERs occurs in extranuclear sites in tumor cells (171). A fraction of the ERs is localized to the plasma membrane region of 17β-estradiol target cells (172) through palmitoylation, a dynamic enzymatic modification mediated by palmitoyl-acyltransferases (172, 173). This subset of ERs located in lipid rafts has been found to exert rapid cell responses to 17β-estradiol, the main circulating estrogen hormone, providing cell survival signals through the activation of signaling cascades such as the MAPK/ERK and PI3K-AKT pathways (174, 175). Raft-located ERs may interact with transmembrane growth factor receptors such as EGFR, HER2, IGF-1R, and other signaling molecules, including components of the RAS/MAPK and PI3K/AKT pathways (176).

On the other hand, cholesterol-rich lipid rafts have been found to act as sites of intersection of androgen receptor (110 kDa) and AKT signaling in prostate cancer cells (177). Androgen receptor plays a major role in the pathogenesis of prostate cancer (178), and a functional interaction between transient receptor potential melastatin 8 (TRPM8) and androgen receptor in lipid rafts has been shown to promote cancer cell migration (179).

**LIPID RAFTS AND CELL DEATH SIGNALING**

An outstanding and rather peculiar feature of mammalian cells lies in the presence of death receptors at their cell surface that provide these cells with the ability to commit suicide via apoptosis and regulate their own fate. Death receptor ligands characteristically initiate signaling via receptor oligomerization, which in turn results in the recruitment of specialized adaptor proteins and activation of caspase cascades. These death receptors belong to the TNF receptor superfamily (TNFRSF) and are highly conserved and found in almost all mammalian cells (180). The most extensively studied death receptors are Fas (CD95, APO-1, TNFRSF6), TNF-receptor 1 (TNF-R1, CD120a, TNFRSF1A), TRAIL-receptor 1 [TRAIL-R1, death receptor 4 (DR4), TNFRSF10A], and TRAIL-receptor 2 [TRAIL-R2, death receptor 5 (DR5), APO-2, KILLER, TNFRSF10B] (180, 181). These death receptors are type-1 transmembrane proteins with a C-terminal intracellular tail, characterized by an ~80 amino acid cytoplasmic sequence termed the death domain (DD), which is essential for apoptosis induction, a membrane-spanning region, and an extracellular ligand-binding N-terminal domain, characterized by the presence of up to six cysteine-rich domains, which defines their ligand specificity (181). Activation of Fas/CD95, TRAIL-R1, and TRAIL-R2 is often associated with cell death; however, TNF-R1 stimulation usually induces cytokine generation, inflammation, and cell survival, unless the TNF-R1 cytotoxic potential is unmasked by inhibiting these survival pathways (181).

When it comes to death receptors and their ability to promote apoptosis, Fas/CD95 is the prototype of this receptor family, transmitting apoptotic signals through the presence of its cytoplasmic DD (182, 183), and it is often taken as the epitome of death receptors as a whole. Human mature Fas/CD95 is a 45–48 kDa (319 amino acids) single spanning transmembrane protein with a 157-aminoc acid N-terminal extracellular region, comprising three cysteine-rich domains that bind to its cognate ligand FasL/CD95L, a 17-amino acid transmembrane domain, and a 145-amino acid C-terminal cytoplasmic domain that is relatively abundant in charged amino acids and harbors a DD domain of 88 amino acids, which is homologous to other death receptors and plays a critical role in transmitting the death signal from the cell surface to intracellular pathways (84, 184). Binding of Fas/CD95 to its cognate ligand FasL/CD95L leads to receptor oligomerization and aggregation, as well as to the recruitment of the adaptor protein, Fas-associated protein with a DD (FADD), through homotypic interaction between the DDs of both Fas/CD95 and FADD. Then, subsequent binding of procaspase-8 or -10 to FADD, through their respective and analogous death effector domains (DEDs) (185), leads to the formation of the so-called “death-inducing signaling complex” (DISC), made up of Fas/CD95, FADD, and procaspase-8 (186), which launches an apoptotic signaling cascade. Procaspase-8 has a low basal level of enzymatic activity, but the recruitment and concentration of procaspase-8 molecules in close proximity to each other within the DISC drives its activation through self-cleavage, triggering a cascade of downstream effector caspsases that eventually lead to apoptosis (187). Quantitative mass spectrometry and Western blots as well as mathematical modeling show that up to 94% of more procaspase-8 than FADD is present in the DISC (188, 189). Because procaspase-8-10 contain two DED motifs in tandem, each procaspase-8-10 molecule can interact with FADD through one of its DED motifs and with another procaspase-8-10 molecule through the other DED motif, as well as with two procaspase-8-10 molecules through the two tandemly arranged DED motifs. Thus, once recruited to FADD, multiple procaspase-8-10 molecules bind each other via their tandem DEDs to form a DISC chain-based procaspase-8-10 activation platform (188, 189), thereby facilitating both proximity-induced dimerization and proteolytic cleavage of procaspase-8-10, which are required for initiation of apoptotic cell death (190). Activated caspase-8 stimulates apoptosis via two parallel cascades; namely, it can directly cleave and activate caspase-3, or alternatively, it can cleave Bid, a pro-apoptotic Bcl-2 family protein, which leads to the triggering of the mitochondria-mediated intrinsic apoptotic signaling. Oligomerization of caspase-8 at the cell membrane, using molecular chimeras of caspase-8 with either CD8 or Tac, induces caspase-8 autoactivation and apoptosis (191). In addition, overexpression of FADD or caspase-8 coupled to green fluorescent protein can cause apoptosis, independently of receptors, by forming novel cytoplasmic filaments, named as death effector filaments, which efficiently recruit and activate procaspase-8 (192).

Unlike growth factor receptors, showing intrinsic tyrosine kinase activity, death receptors lack enzymatic activity and depend on protein-protein interactions through their DDs for signal transmission. Thus, Fas/CD95-mediated
apoptotic signaling depends on the homotypic interactions between the DDs of Fas/CD95 and FADD, followed by the DED-mediated interactions between FADD and procaspase-8/-10 that lead to the formation of DISC and autoactivation of procaspase-8/-10. This process should require a high concentration of the above molecules in a specific and limited area of the cell membrane.

With the advent of the new millennium, we found in 2001 that treatment of human acute T-cell leukemia Jurkat cells and acute myeloid leukemia HL-60 cells with the antitumor ether lipid edelfosine induced apoptosis through the recruitment and clustering of Fas/CD95 death receptor in lipid rafts (193). This work unveiled a novel mechanism of action for an antitumor drug, which involved, for the first time, the participation of lipid rafts in cancer chemotherapy as well as in apoptosis regulation (98, 193–195). Translocation of Fas/CD95 into lipid rafts could be achieved following binding to its ligand cognate in mouse thymocytes (196) or through a ligand-independent way following appropriate pharmacological treatment of various cancer cells (154, 193, 197, 198). Subsequent studies demonstrated that edelfosine induced coclustering of Fas/CD95 and lipid rafts in different types of cancer cells, leading to the formation of lipid raft platforms that harbored a number of downstream signaling molecules triggering apoptosis (154, 199, 200) (Figs. 3, 4). Coclustering of lipid rafts and Fas/CD95 death receptor, leading to the recruitment of Fas/CD95 and downstream signaling molecules, forming the apoptotic DISC in aggregated lipid rafts, provide an explanation on how this death receptor-mediated apoptosis is triggered through the aggregation of rafts (Figs. 3, 4). Lipid rafts act as scaffolds where Fas/CD95 and downstream molecules are recruited and concentrated, leading to the accumulation of DISC complexes in a limited space of the cell membrane, thus facilitating protein-protein interactions and providing a membrane location where a large amount of complexes and, therefore, many caspase-8/-10 molecules showing a basal enzymatic activity are brought together so they can be autoproteolytically activated (Figs. 3, 4). Biochemical, genetic, and electron and fluorescence microscopy evidence indicates that Fas/CD95, FADD, and procaspase-8/-10 (that is all the DISC components) are recruited into lipid rafts during the induction of apoptosis by the ether lipid edelfosine (154, 195, 199, 200). Disruption of rafts by membrane cholesterol depletion abolishes DISC formation and Fas/CD95-mediated cell death (85, 98, 199, 200). Fas/CD95 localization to lipid rafts and efficient cell death signaling require S-palmitoylation of human Fas/CD95 at cysteine residue 199 (Cys-194 in mice), at the membrane proximal intracellular region, of the death receptor (201, 202). Despite the molecular and regulatory mechanisms involved in the recruitment of Fas/CD95 and downstream molecules into rafts being poorly understood, a number of proteins have been reported to modulate Fas/CD95 apoptotic signaling and recruitment in rafts, including the PI3K pathway and semaphorin [reviewed in (82, 85, 184)]. Inhibition of PI3K signaling by edelfosine or using specific inhibitors, LY294002 and wortmannin, induces redistribution of Fas/CD95 into lipid rafts in T-cell leukemia Jurkat and CEM cells or myeloid leukemia HL-60 cells (203). Treatment of MCL cells with the ether lipid edelfosine displaces PI3K/AKT signaling from lipid rafts, thus preventing its proper activation, and promotes the recruitment of Fas/CD95 in rafts (68). Incubation of human T-cell leukemia Jurkat cells with semaphorin 3A promotes translocation of Fas/CD95 into lipid rafts, showing that semaphorin 3A/neuropilin-1/plexin signaling rearranges lipid rafts, promoting Fas/CD95 clustering in rafts (204). Disruption of lipid rafts and interference with the actin cytoskeleton prevented Fas/CD95 clustering and apoptosis in Aplidin-treated T-cell leukemia Jurkat cells (205). Actin-linking proteins, ezrin, moesin, RhoA, and RhoGDI, were conveyed into Fas-enriched rafts in drug-treated leukemic cells (205), thus suggesting a major role of the actin cytoskeleton in the formation of Fas/CD95 caps in rafts. In fact, Fas/CD95 interacts with the actin cytoskeleton through ezrin in human T lymphocytes as a regulatory mechanism of the death receptor apoptotic pathway (206, 207).

DEATH-PROMOTING RAFT PLATFORMS AS THERAPEUTIC TARGETS IN CANCER THERAPY

The above-mentioned edelfosine-induced apoptosis through the recruitment and clustering of Fas/CD95 death receptor in lipid rafts (193) was the first evidence for the involvement of membrane rafts in cancer chemotherapy, and edelfosine became the first antitumor drug to act through lipid rafts, promoting cell death through raft-Fas/CD95 coclustering and inducing apoptosis in a wide range of tumor cells (97, 98, 154, 193, 200). This raft-mediated process induced by edelfosine opened up a novel raft-mediated mechanism through which an anticancer drug could lead to tumor cell demise and was found to occur in a wide variety of hematological cancer cells (96, 154, 193, 200, 208), thus leading to a new approach to target cancer cells (82, 209, 210). Because the addition of physiological ligands also induced death receptor translocation to lipid rafts (196), we concluded that edelfosine treatment exacerbated a physiological process that leads to apoptotic cell death. Furthermore, edelfosine was able to promote recruitment of Fas/CD95 in rafts and subsequent apoptosis in a number of cells that were resistant to the physiological ligand FasL/CD95L (82, 184), thus triggering a new way to activate Fas/CD95 independently of the natural ligand (85, 154, 197, 198). It might be envisaged that pharmacological activation of the Fas/CD95 system might bypass processes that could block the physiological death receptor stimulation, thus promoting a response in resistant cells. This suggests that edelfosine put in motion a series of processes that otherwise would remain dormant in the cancer cell, thus offering a new way to promote cell death in tumor cells. Edelfosine accumulated in rafts (96, 199, 208, 211), showed a high affinity for cholesterol (212, 213), enhanced membrane thickness (212, 213), and induced an increase in membrane fluidity (214, 215). Edelfosine-induced Fas/CD95 raft coclustering has become the epitome and paradigm of this new way of ligand-independent death.
Fig. 4. Time-related sequence of events in the formation of cell death-promoting rafts. Clustering of lipid rafts and S-palmitoylation are required for the redistribution of Fas/CD95 to lipid rafts. Clusters of rafts, generated by not yet clear protein-lipid and protein-protein interactions, offer a platform adequate for the recruitment of an increasing number of additional proteins in a rather protected and segregated membrane domain. Once Fas/CD95 has been recruited into rafts, multimeric complexes (DISC) are formed through homotypic DD and DED interaction motifs. This elevated concentration of procaspase-8 molecules in rafts leads to self-activation by auto-catalytic cleavage of procaspase-8, thus activating a caspase cascade and the initiation of the execution phase of apoptosis. See the text for further details.
receptor activation, through recruitment of death receptors in lipid rafts and subsequent triggering of apoptotic signaling. In the almost two decades since the first evidence for edelfosine-induced Fas/CD95 recruitment and activation in 2001, an increasing number of molecules and anti-tumor agents have been reported to promote apoptosis, at least in part, through the recruitment of death receptors in lipid rafts, as listed in Table 1. However, edelfosine can be considered as the lead compound and paradigm in the search for chemical agents promoting Fas/CD95 recruitment in rafts, and research on this ether lipid is paving the way to unravel the mechanisms underlying this novel raft-mediated regulation of cancer cell death.

The pioneering work with edelfosine also led to the first identification of the recruitment of downstream apoptotic signaling molecules, such as c-Jun N-terminal kinase (JNK) and Bid, in lipid rafts, thus connecting death receptor extrinsic and mitochondrial intrinsic apoptotic pathways (154).

### Table 1. Recruitment of death receptors and downstream signaling molecules into lipid rafts by anticancer drugs and chemical agents in cancer cells

| Anticancer Drug | Cancer Cells | Death Receptors (and Downstream Signaling Molecules) | Recruited in Rafts | References |
|----------------|-------------|------------------------------------------------------|--------------------|------------|
| Cationic amphipathic lytic peptide, Avicin D Jurkat | Fas/CD95 | (279) |
| Aplidin Jurkat | Fas/CD95, DR5, TNF-R1 (FADD, procaspase-8, procaspase-10, JNK, Bid) | (205) |
| Anandamide Mz-ChA-1 | Fas/CD95 (FADD, procaspase-8, procaspase-7, Bid) | (299) |
| AKT signaling inhibition (AKT inh-VIII) Jurkat | DR4, DR5 | (300) |
| Anticancer Drug | Cancer Cells | Death Receptors (and Downstream Signaling Molecules) | Recruited in Rafts | References |
| Cisplatin, platinum(IV) complex LA-12 HCT116 | DR4, DR5 | (302) |
| Doxorubicin coupled to cell penetrating peptides (Dox-CPPs) MDA-MB-231 | DR4, DR5 | (306) |
| Ceramide* Jurkat | Fas/CD95 | (226–228) |
| Cisplatin | HT29 | Fas/CD95 (FADD, procaspase-8) | (234) |
| Cryptocaryone PC-3 | DR4, DR5 | (302) |
| Depsipeptide FR901228 DU-145 | DR4, DR5 | (304) |
| Docosahexanoic acid MDA-MB-231 | Fas/CD95 | (305) |
| Edelfosine EHEB | Fas/CD95 | (208) |
| Edelfosine coupled to cell penetrating peptides (Dox-CPPs) Jurkat | Fas/CD95 (FADD, procaspase-8, procaspase-10, JNK, Bid) | (154, 193) |
| Epirubicin MGC803 | DR4, DR5 | (307) |
| HS-275 LM7 | Fas/CD95 | (308) |
| HU-1 MGC803 | DR4, DR5 | (307) |
| PA567 MGC803 | DR4, DR5 | (307) |
| PEG signaling inhibition (LY294002, wortmannin) Jurkat, CEM | Fas/CD95, DR4, DR5 (FADD, procaspase-8, Bid) | (200) |
| Polymeric fluoropyrimidine F10 HL-60 | Fas/CD95 | (203, 297) |
| Quercetin HT-29 | DR4, DR5 | (310) |
| Resveratrol HT-29 | DR4, DR5 | (311) |
| Rituximab SW480 | Fas/CD95 (FADD, procaspase-8) | (312) |
| Stichoposide D K562, HL-60 | Fas/CD95 (ceramide synthase 6, p38 kinase, caspase-8) | (314) |
| TSWU-BR25 (a synthetic bichalcone analog) HT-29 | Fas/CD95 | (315) |
| Ursodeoxycholic acid (UDCA) SNU601, SNU638 | DR5, Fas/CD95 | (318, 319) |

*Acting not as an inducer but as an amplifier of a previous triggering of Fas/CD95 response by its cognate ligand or agonistic antibodies.

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**Notes:**
- CEM, human acute T-cell leukemia cell line; DU-145, human prostate tumor cells; EHEB, human chronic lymphocytic leukemia cell line; HCT116, human colon carcinoma cell line; HL-60, human acute myeloid leukemia; HT-29, human colon carcinoma cell line; Jurkat, human acute T-cell leukemia cell line; JVM-2, human MCL cell line; K562, human chronic myelogenous leukemia cell line; LM7, human osteosarcoma cell line; LNCaP, human prostate cancer cell line; MDA-MB-231, human breast cancer cells; MGC803, human gastric carcinoma cell line; MM144, human multiple myeloma cell line; MS-275, human melanoma cell line; MCF-7, human breast cancer cell line; MDA-MB-231, human breast cancer cells; MDDC-2K1, human monocyte cell line; Mz-ChA-1 cells, human cholangiocarcinoma cell line; PC-3, human prostate cancer cell line; Ramos, human Burkitt’s lymphoma cell line; SNU601, human gastric cancer cell line; SNU638, human gastric carcinoma cell line; SW480, human colon carcinoma cell line; TSWU-BR25, (E)-1-((4-(4-acetylphenyl)piperazin-1-yl)methyl)-4-hydroxy-5-methoxyphenyl)-3-(pyridin-3-yl)prop-2-en-1-one.
- *Acting not as an inducer but as an amplifier of a previous triggering of Fas/CD95 response by its cognate ligand or agonistic antibodies.
Interestingly, membrane-bound Fas ligand (FasL/CD95L) as well as Fas/CD95 death receptor and downstream signaling molecules, including Fas-associating DD-containing protein (FADD), procaspase-8, procaspase-10, JNK, and Bid, were also translocated into lipid rafts upon incubation of T-cell leukemic Jurkat cells with the cyclic depsipeptide antitumor drug Aplidin (205), an extremely potent and rapid apoptotic inducer on leukemic cells (216). Endogenous FasL/CD95L has also been localized in rafts in the human natural killer NK92 cell line (which expresses relatively high endogenous amounts of the protein) and in human T cells after activation (217). FasL/CD95L was also constitutively localized in lipid rafts of FasL-/CD95L- transfectants and primary T cells, and its localization to lipid rafts appeared to be predominantly mediated by the characteristic secondary T cells, and its localization to lipid rafts appeared to be predominantly mediated by the characteristic cytoplasmic proline-rich domain of FasL/CD95L because mutations of this domain resulted in reduced recruitment to lipid rafts and attenuated FasL/CD95L killing activity (218). Additional evidence has shown that human FasL/CD95L was palmitoylated at the cysteine residue 82, which is located at the N-terminal portion of the transmembrane domain, and this posttranslational modification was required for appropriate partitioning of FasL/CD95L into rafts for efficient FasL/CD95-mediated cell death and FasL/CD95L processing by a disintegrin and metalloproteinase 10 (ADAM10) (219).

Additional death receptors, such as TNFR1 (CD120a) (205, 220, 221) and TRAIL receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (200, 205, 222), have been shown to be recruited to lipid rafts to induce apoptosis. DR4 is also palmitoylated and confers an efficient TRAIL-induced cell death signaling (223). Fluorescence resonance energy transfer (FRET) assays have shown the molecular interaction between ganglioside GM3, abundant in lipid rafts and lymphoid cells (224, 225), and DR4 that was associated to TRAIL susceptibility of B-cell cancer cells, whereas this association was negligible in nontransformed cells (222).

It is noteworthy to mention that the initial redistribution of Fas/CD95 in rafts not only facilitates caspase-8 activation and apoptosis, but in turn it could be further favored by a caspase-8-mediated positive feed-forward loop involving ceramide formation through caspase-8-activated acidic sphingomyelinase that promotes the capping of Fas/CD95 in large patches of ceramide-rich rafts in the plasma membrane (182, 226–228). This latter process is supported by a decrease in Fas/CD95 capping and lipid raft association in cells defective in sphingomyelin synthase (229).

On these grounds, it could be envisaged that lipid rafts act as the linchpin from which a potent death signal is launched, becoming a promising anticancer target (82, 85). The onset of apoptosis can be triggered by an alteration of the overall balance between apoptotic and survival signals, due to a modification in the cell concentration of one of the two components of the apoptosis/survival ratio. We have found that this apoptosis/survival signaling balance could also be modified by a redistribution and local accumulation of apoptotic molecules in lipid rafts, setting apart apoptotic (Fas/CD95, FADD, caspase-8, caspase-10, JNK) from survival (ERK, AKT) signaling molecules, as has been shown by treatment of human leukemic cells with the ether lipid edelfosine (68, 89, 96, 154, 200, 208). This concentration of apoptotic molecules, segregated from survival signaling molecules, leads to a dramatic local change in the apoptosis/survival signal ratio in a specific subcellular structure that eventually triggers a cell death response.

**DIFFERENTIAL PRONENESS TO GENERATE DEATH-PROMOTING RAFT PLATFORMS BETWEEN HEMATOLOGICAL AND SOLID TUMOR CELLS AND RAFT-MEDIATED ORGANELLE CONNECTIONS**

It is interesting to note that the antitumor alkylphospholipid analog edelfosine, which shows a high affinity for cholesterol (212, 213, 230) and accumulates in lipid rafts (96, 154, 199, 231, 232), displays a rather differential mechanism of action to induce apoptosis in hematological and solid tumor cells (98, 233). Edelfosine accumulated at the cell surface lipid rafts in different human leukemic cells, leading to raft reorganization, coclustering of Fas/CD95 and rafts, and apoptosis (96, 154, 199, 200, 208). However, despite the fact that Fas/CD95 clustering in rafts has been reported in both hematological and solid tumor cells following treatment with distinct apoptosis inducers (84, 85, 234), edelfosine was mainly localized in the endoplasmic reticulum and triggered apoptosis through an endoplasmic reticulum stress response-mediated process in several human solid tumor cells, including human epithelial cervix carcinoma, pancreatic ductal adenocarcinoma, and Ewing’s sarcoma cells (235–237). Interestingly, edelfosine has also been localized in mitochondria in a number of solid tumor cells (238–240). Edelfosine-induced raft- and endoplasmic reticulum-mediated pro-apoptotic responses converge on mitochondria to proceed to apoptosis as the final outcome in both hematological and solid tumor cells (98, 200). Overexpression of Bcl-2 or Bcl-xL, protecting mitochondria, prevented edelfosine-induced apoptosis (200, 238, 240, 241). Thus, the current data are compatible with the notion that edelfosine is first accumulated in lipid rafts and then internalized into the endoplasmic reticulum and finally into mitochondria, but the timing of these processes is largely dependent on the cell type. This view is consistent with the fact that HeLa cells incorporated edelfosine via raft- and dynamin-mediated endocytosis (242), and it was mainly located in the endoplasmic reticulum and mitochondria of these cancer cells (233, 235, 238, 240); whereas the drug remained in cell surface raft clusters in T-cell leukemia, multiple myeloma, MCL, and chronic lymphocytic leukemia cells by the time apoptosis was induced (96, 154, 199, 208). Interestingly, experiments conducted in yeast mutants led us to delineate the course and road map of the journey of edelfosine from its transient location at the cell surface lipid rafts to its accumulation in the endoplasmic reticulum before promoting yeast cell death (232). Thus, the above data suggest that the accumulation of edelfosine in membrane rafts in hematological cancer cells is enough to induce coclustering of lipid rafts with Fas/CD95 and downstream signaling molecules, leading to...
a rather rapid apoptotic response, and thereby any putative subsequent event at the endoplasmic reticulum level would not be required in these cells. However, the above raft and Fas/CD95 coclustering process is apparently not sufficient for the induction of apoptosis in most solid tumor cells, and therefore edelfosine is subsequently incorporated into the endoplasmic reticulum and eventually into mitochondria, affecting processes taking place in both subcellular structures that ultimately induce endoplasmic reticulum- and/or mitochondria-mediated cell death. This road map in the transit of the antitumor ether lipid edelfosine within a tumor cell, from the cell surface membrane raft to the endoplasmic reticulum and mitochondria, explains the different timing and potency observed in the induction of apoptosis in different cancer cell types following edelfosine treatment (faster and more potent induction of apoptosis in hematological cancer cells than in solid tumor cells) (197, 200, 233, 235–237, 241). Thus, hematological cancer cells appear to be more prone to undergo apoptosis through a Fas/CD95-raft coclustering process than solid tumor cells. However, the molecular underpinnings for these differences are currently unknown. In agreement with the above reasoning and data, it is interesting to note that edelfosine treatment induces a redistribution of lipid rafts from the plasma membrane to mitochondria in HeLa cells, suggesting a raft-mediated link between plasma membrane and mitochondria (238). Taken together, these studies, carried out to unveil the mechanism of action of the alkylphospholipid edelfosine in its pro-apoptotic action against cancer cells, suggest a raft-mediated process that modulates cell death through a plasma membrane-endoplasmic reticulum-mitochondria connection. In this regard, communications between distinct organelles occur through direct contacts employing membranes and membrane proteins of different organelles. Membrane contacts have been reported to occur between endoplasmic reticulum and different organelles, including endoplasmic reticulum and mitochondria that modulate lipid transfer and metabolism (243–246). The so-called mitochondria-associated endoplasmic reticulum membranes are tethering sites between the endoplasmic reticulum and mitochondrial membranes that are enriched in cholesterol (5- to 7-fold higher levels in cholesterol compared with those in the endoplasmic reticulum) and are specialized raft-like subdomains of the endoplasmic reticulum membrane that regulate endoplasmic reticulum-mitochondria communications (244, 247–251). Thus, membrane rafts seem to play a major role in membrane tethering processes between two organelles, and therefore in organelle communication. It is tempting to suggest that membrane rafts could be involved in the transport of molecules, acting as cargo wagons from the cell surface to different subcellular organelles. The protein composition of mitochondria-associated endoplasmic reticulum membranes suggests that these membrane subdomains could play a role in apoptosis, autophagy, and cancer cell fate (252, 253). On these grounds, and together with a higher level of cholesterol and rafts in cancer cells, membrane rafts could play a major role in the regulation of cell death in tumor cells through the Fas/CD95 recruitment and formation of DISC and through molecule redistribution between different subcellular organelles, particularly, endoplasmic reticulum and mitochondria.

THE CASMER CONCEPT: A RAFT-BASED SUPRAMOLECULAR ENTITY ACTING AS A SIGNALING HUB IN DEATH RECEPTOR-MEDIATED APOPTOSIS

The accumulating evidence showing the recruitment and concentration of death receptors, together with downstream apoptotic signaling molecules, in aggregated rafts led us to coin the term CASMER as an acronym of “cluster of apoptotic signaling molecule-enriched rafts” (205, 254–256). CASMER refers to the recruitment of death receptors together with downstream signaling molecules in aggregated lipid rafts (Fig. 5). Thus, CASMER represents a novel raft-based supramolecular entity, acting as a death-promoting platform or scaffold where death receptors and downstream signaling molecules are brought together, thus facilitating protein-protein interactions and the transmission of apoptotic signals (255, 256). CASMER behaves as a major signaling hub connecting cell death signaling routes and as a linchpin from which a potent apoptotic signal is launched. Because CASMER is made of raft clusters, its lipid composition is crucial to provide enough fluidity to allow proteins to interact with each other, but enough rigidity to sort out this apoptotic signaling molecule assortment. In this regard, cholesterol is a critical constituent of rafts and its displacement disrupts CASMER formation (154, 193, 199, 200). As stated above, cancer cells seem to contain higher levels of intracellular cholesterol and cholesterol-rich lipid rafts than their normal counterparts, and thereby cancer cells could be suggested to show a proneness to form CASMERs. This leads to the notion that CASMER formation could be a major regulatory apoptotic signaling hub and a potential therapeutic target in cancer.

CASMER formation would lead to the recruitment and concentration of pro-apoptotic molecules in a limited area of the cell membrane where protein-protein interactions and the subsequent transmission of apoptotic signals would be greatly facilitated. The ability to promote CASMER formation, as well as CASMER protein composition, will depend on cell phenotype and triggering stimulus (255, 256). Most of the available data on CASMER formation derive from hematological cancer cells, suggesting that the CASMER system seems to operate more efficiently in hematopoietic tumor cells than in solid tumor cells, as discussed above on the functional generation of death-promoting rafts. A fundamental and basic protein composition of CASMER would include the recruitment of death receptors in aggregated rafts, but CASMERs could increase in complexity by the recruitment in rafts of additional downstream signaling molecules, including FADD and procaspase-8/-10, forming the DISC, as well as additional signaling molecules, some of which might change their regulatory features when redistributed in another microenvironment.
Fig. 5. The concept of the formation of CASMER. A number of death receptors and downstream signaling molecules, including DISC, are recruited and brought together in close proximity in large cholesterol- and sphingolipid-enriched lipid raft platforms (highlighted in green), thus facilitating protein-protein interactions, caspase-8 activation, and cross-talk signaling. The presence of Bid in CASMER facilitates the interaction between death receptor extrinsic apoptotic signaling and mitochondria-related intrinsic apoptosis signaling, thus potentiating apoptosis. Apoptotic signaling would be highly amplified by CASMER formation. See the text for further details.

In this way, treatment of leukemic Jurkat cells with edelfosine induced recruitment of heat shock protein 90 (Hsp90), JNK, Fas/CD95 death receptor, and downstream signaling apoptotic molecules in lipid rafts, but not the JNK regulators apoptosis signal-regulating kinase 1 (ASK1) and Daxx, or the survival signaling molecules, ERK and AKT (89). JNK and Hsp90 were recruited in lipid rafts after treatment of leukemic Jurkat cells with edelfosine, and Hsp90-JNK clusters were identified at the plasma membrane by immunoelectron microscopy (89). In this new lipid raft location, JNK is segregated from major JNK regulators, such as ASK1 and Daxx, being associated with and regulated by Hsp90, which usually acts as a survival signaling chaperone and is considered a cancer chemotherapeutic target. However, we found a chaperoning role of Hsp90 on JNK-mediated edelfosine-induced apoptosis when both Hsp90 and JNK were recruited in lipid rafts (89). Thus, Hsp90 is also able to promote and facilitate apoptosis when located in lipid rafts and under certain circumstances that concentrate apoptotic signaling molecules in rafts, thus chaperoning proteins that are in its vicinity. The differential translocation of signaling molecules to lipid rafts, forming CASMERs, leads to dramatic changes in the behavior of death/survival regulators and highlights that lipid rafts behave as a major modulator of apoptosis as well as a promising target in the treatment of leukemia and cancer in general. Interestingly, Bid, a protein acting as a bridge between Fas/CD95 signaling and mitochondria (257, 258), has been shown to be recruited in rafts following treatment of leukemic cells with different antitumor drugs (154, 200, 205, 254, 259). This highlights a major role of lipid rafts as a putative linker between death receptor-mediated extrinsic and mitochondria-mediated intrinsic signaling pathways in apoptosis, which might amplify the apoptotic response through both caspase- and mitochondria-related processes.

The concept of CASMER could serve as a stark example of how proteins located in lipid rafts might behave differently depending on their close microenvironment. Proteins are packed in a rather reduced space when located in lipid raft platforms, thus potentiating protein-protein and protein-lipid interactions. On these grounds, CASMERs serve as a hub in death receptor-mediated apoptosis signaling and as a connecting platform among different subcellular...
structures to facilitate cross-talk processes occurring during apoptosis.

Overall, lipid rafts display manifold functions to modulate cell fate, namely: 1) concentrate apoptotic molecules in a limited region of the cell facilitating protein-protein interactions; 2) sort out cell death and survival signaling pathways in different membrane domains, thus increasing their own efficiency; 3) provide a platform to recruit Hsps and other signaling molecules to protect and potentiate apoptotic signaling; 4) provide a scaffold to harbor proteins required to efficiently convey cell death signaling and to transport these signals toward the interior of the cell; and 5) transfer molecules and modulate contacts between the membranes of different organelles.

RAFTS AND METASTASIS

Hyaluronan or hyaluronic acid (HA) is a major glycosaminoglycan component of the ECM with diverse and often opposing functions (260). HA plays pivotal roles in inflammation and cancer through its association with the transmembrane receptor CD44 (261), an 80–95 kDa type-I transmembrane glycoprotein that is the major cell-surface receptor for HA and that is implicated in a wide variety of biological processes, including cell adhesion and migration (262, 263). CD44 plays a prominent role in tumor cell signaling (264) and is an important marker for various cancer stem cells, including pancreatic (265–267) and gastric (268, 269) cancer stem cells. Cell surface adhesion receptor CD44 is a multifunctional receptor that controls many biological functions involved in cancer cell adhesion and migration (270), is highly expressed in many cancers, and regulates metastasis, although the underlying mechanisms leading to cancer cell detachment from the tumor mass and invasion of the surrounding ECM are not totally elucidated. Its interaction with appropriate ECM ligands promotes the migration and invasion processes involved in metastases. In order to exert its role, CD44 undergoes sequential proteolytic cleavages in the ectodomain (release of soluble CD44 responsible for the dynamic regulation of the interaction between CD44 and the ECM during cell migration on an HA-containing substrate) and intramembranous domain (release of the CD44 intracellular domain that is translocated to the nucleus and activates transcription) (271). CD44 cleavage, shedding, and elevated levels of soluble CD44 in the serum of cancer patients are observed in a variety of human cancers (272) and can be considered a marker of tumor burden and metastasis in several cancers (261).

Despite numerous reports demonstrating that CD44 is present in lipid rafts, the role of lipid rafts in cancer cell adhesion and migration remains to be fully understood (273) (Fig. 3). Unlike CD44, which is present in lipid rafts, its processing enzyme, ADAM10, is largely in non-raft fractions (270). Displacement of CD44 from lipid raft to non-raft membrane domains, where ADAM10 is located, makes CD44 accessible to ADAM10 and thereby to CD44 shedding (274) (Fig. 3). The proteolytic cleavage of CD44 from the cell surface plays a critical role in the migration of tumor cells (275), and lipid rafts modulate CD44 function, playing a critical role in regulating the accessibility of CD44 to sheddingases and other proteins that regulate its function (273, 276) (Fig. 3). Localization of CD44 outside lipid raft in human glioblastoma cells has been shown to induce metalloproteinase-mediated CD44 shedding and tumor cell migration (277). Disruption of lipid rafts by extracting cellular membrane cholesterol with methyl-β-cyclodextrin in glioma and pancreatic cancer cells resulted in increased CD44 shedding mediated by ADAM10 and affected tumor cell migration (270). The cholesterol-lowering medication simvastatin has also been reported to enhance CD44 shedding and affect the stimulation of glioma cell migration by hyaluronan oligosaccharides (274, 277, 278). Additional evidence suggests that CD44 must relocalize outside lipid rafts to drive cell migration in breast cancer cell migration (279) (Fig. 3). Palmitoylation of two CD44 cysteine residues at positions 286 and 295 confers high affinity for cholesterol-enriched lipid rafts, thus leading to the localization of CD44 in lipid rafts, which inversely regulate breast cancer cell migration (276). CD44 palmitoylation leading to lipid raft localization negatively regulates interactions with its migratory binding partner, ezrin, and inversely modulates breast cancer cell migration (276). CD44 is predominantly located in rafts, whereas ezrin is in non-raft compartments (279). Translocation of CD44 into lipid rafts attenuated CD44-ezrin binding and hepatocellular carcinoma invasion and metastasis (280). Thus, CD44 localization in rafts limits associations with its cytoskeletal linker binding partner, ezrin (279) (Fig. 3), thus restricting CD44-ezrin interaction and promotion of breast cancer cell migration (279). On these grounds, lipid rafts sequester CD44 to limit cell migration. Thus, the localization of CD44 in lipid rafts is a major regulator of cell migration and cancer metastasis and this could have implications for rafts as pharmacological targets to downregulate cancer cell migration.

Lipid rafts seem to play a role in the localization, regulation and proper function of additional proteins that are involved in cancer metastasis, including integrins (281, 282) and ion channels (283, 284). In this regard, CD44 has also been reported to colocalize with MMP-9 in lipid rafts, which plays a major role in tumor invasion (285, 286). The SK3 channel, a calcium-activated potassium channel, controls constitutive Ca2+ entry and cancer cell migration through an interaction with the Ca2+ channel Orai1 within lipid rafts (283). This localization of an SK3-Orai1 complex seemed essential to control cancer cell migration, and following treatment with the alkyl-lipid Ohmline, the SK3-Orai1 complex moved away from lipid rafts, and SK3-dependent Ca2+ entry, migration, and bone metastases were subsequently impaired (86, 283, 287). Furthermore, voltage-gated potassium channels, which are suggested to be involved in cancer metastasis and proliferation (288), have also been detected in different types of lipid rafts (289).

In addition, an increasing number of proteins involved in the development of several malignant cancers, tumor cell invasion, and metastasis are being associated with lipid
rafts (290), such as the GPI-anchored cell membrane receptor urokinase-type plasminogen activator receptor (uPAR) (286, 291) and the type 1 transmembrane glycoprotein mucin 1 (MUC1) (292, 293), further supporting a major role for lipid rafts in tumor progression. MUC1 is often overexpressed in metastatic cancers and used as a diagnostic marker for metastatic progression (294).

CONCLUDING REMARKS

The advent of the concept of lipid rafts in 1997 (19) has changed our view of the role cellular membranes in cell signaling regulation. Over the last two to three decades, a great deal of evidence has led to the conclusion that these membrane domains act as platforms for the recruitment of signaling processes that regulate cell fate, thus suggesting the presence of different types of lipid rafts, including survival- and apoptotic-promoting rafts (Fig. 3). Increasing evidence shows that membrane rafts provide platforms where a number of receptors and downstream signaling molecules are brought together, thus facilitating and fostering their interaction in a transient manner and eventually leading to the onset of a potent signaling pathway. Cancer cells show high levels of intracellular cholesterol and lipid rafts as compared with their non-tumorigenic counterparts, thus suggesting that formation of cholesterol-rich rafts is potentiated in tumor cells. Proteins and signaling processes involved in cancer development and progression, such as the IGF system and the PI3K/AKT pathway, as well as in metastasis (MUC1, CD44), are dependent on or modulated by lipid rafts (Fig. 3). This could be an explanation for the higher cholesterol and raft levels found in cancer cells, thus favoring their cell survival and dissemination. However, the presence of lipid rafts can also become an Achilles' heel for cancer cells. Evidence collected in the last two decades highlights the critical role of the recruitment of death receptors and downstream signaling molecules in lipid rafts for mounting an apoptotic response (Figs. 3, 4) as well as the crucial role of the so-called CASMERS, recruiting and concentrating death receptors and downstream signaling molecules and acting as the linchpin from which a potent death signal is launched (Fig. 5). Cancer is perhaps one of the most complicated diseases to treat because of its genetic heterogeneity and complexity, and different tumors show distinct types of genetic alterations, oncogenic signaling, metabolic features, and epigenetic changes, which are responsible for tumorigenesis, and additional mutations or resistance processes can come up along the course of the disease, thus driving tumor progression. In this regard, an appealing approach to combat cancer would be to set in motion the apoptotic machinery of the cancer cell to induce its own demise (255, 256). Because CASMER formation directly recruits and triggers the death receptor apoptotic machinery, the potential for CASMER-based therapies seems very promising in the treatment of cancer, in a rather general way, as well as in other pathologies where stimulation of apoptosis is defective (255, 256). CASMER-mediated direct activation of apoptosis opens a new avenue in cancer therapy, which would be apparently independent of tumor suppressor genes (e.g., p53) and sensors, which are frequently mutated in cancer (82, 255). Nevertheless, a putative limitation could reside in the feasibility to generate CASMER and in its heterogeneity in both composition and ability to generate apoptotic signals in different malignant cell populations. Ideally, optimization of this raft-mediated approach of promoting cancer cell suicide by apoptosis could be useful for a large variety of different tumors. Membrane rafts housing survival signaling might be important for cancer development and drug-resistance, and membrane rafts housing apoptotic signaling might provide a new avenue to promote cell death in cancer cells. Membrane rafts are heterogeneous in terms of their protein and lipid contents, leading to the existence of functionally distinct raft populations (295, 296), acting as signaling hubs that can cross-talk or coalesce to modulate cell function. Furthermore, the protein profile of membrane rafts varies both spatially and temporally, and actin cytoskeleton, posttranslational protein modifications as well as protein-protein and protein-lipid interactions play pivotal roles in generating the precise raft architecture. Membrane rafts can also be localized to different regions of the cell and can behave as cargo wagons playing a critical role in the communication between different cellular compartments, transporting molecules and modulating the function of each subcellular organelle. The fact that the antitumor ether lipid edelfosine can reorganize membrane raft composition, displacing PI3K/AKT survival signaling from rafts in MCL, leading to AKT inhibition, while it induces recruitment of Fas/CD95 and downstream cell death signaling molecules in rafts, suggests that modulation of survival and apoptotic signaling routes via rafts might be a promising and appealing approach, as well as a pharmacologically viable strategy, for cancer therapy. However, the physicochemical principles responsible for compartmentalization in membrane rafts and the molecular mechanisms by which they are functionalized remain to be elucidated. On these grounds, several open questions in the membrane domain field need to be answered before we move forward implementing the above mentioned raft-mediated therapeutic approach. What are the mechanisms that drive raft formation and determine their properties? How many different raft subtypes can coexist in a cancer cell type? What is the role of complex lipidome in the generation of different membrane rafts? What is the role of cholesterol and membrane lipidomics in spatial raft coalescence and raft-mediated connections between different subcellular organelles? How is membrane compartmentalization integrated into cellular signaling? Are survival-promoting rafts different from the apoptotic-promoting rafts? Can these survival- and apoptotic-promoting rafts interact, influence, or transform each other? How are these raft-mediated signaling processes turned on and off? What are the mechanisms involved in the recruitment of Fas/CD95 and downstream signaling molecules in lipid rafts leading to a functional cell death-promoting raft platform? Although many questions remain to be solved, modulation of survival and apoptotic signaling through membrane rafts could be a
promising and appealing approach in the treatment of cancer, highlighting the potential of lipid rafts as a novel therapeutic target in cancer therapy. 

REFERENCES

1. Swinnen, J. V., K. Brusselmans, and G. Verhoeven. 2006. Increased lipogenesis in cancer cells: new players, novel targets. *Curr. Opin. Clin. Nutr. Metab. Care.* 9: 358–365.

2. Cheng, C., F. Geng, X. Cheng, and D. Guo. 2018. Lipid metabolism reprogramming and its potential targets in cancer. *Cancer Commun. (Lond.)* 38: 27.

3. Chapman, D. 1975. Phase transitions and fluidity characteristics of lipids and cell membranes. *Q. Rev. Biophys.* 8: 185–235.

4. Jaipuria, G., T. Ukar-Godec, and M. Zweckstetter. 2018. Challenges and approaches to understand cholesterol-binding impact on membrane protein function: an NMR view. *Cell. Mol. Life Sci.* 75: 2137–2151.

5. Chimento, A., I. Casaburi, P. Avena, F. Trotta, A. De Luca, V. Rago, V. Pezzi, and R. Siringioni. 2019. Cholesterol and its metabolites in tumor growth: therapeutic potential of statins in cancer treatment. *Front. Endocrinol. (Lausanne).* 9: 807.

6. Koudova, A., F. P. Guengerich, and P. Soucek. 2017. The role of oysterx in human cancer. *Trends Endocrinol. Metab.* 28: 485–496.

7. Wu, Q., T. Ishikawa, R. Siringioni, H. Tang, J. G. McDonald, I. S. Yuhanna, B. Thompson, L. Girard, C. Mineo, R. A. Breken, et al. 2013. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell. Reports.* 5: 637–645.

8. Raccosta, L., E. Ikonen, D. Maggioni, C. Lanterna, E. J. Villablanca, A. Paniccia, A. Musumeci, E. Chiricozzi, M. L. Trincavelli, S. Daniele, et al. 2013. The cholesterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils. *J. Exp. Med.* 210: 1711–1728.

9. Soricini, M., G. Corna, M. Moresco, N. Colletta, U. Restuccia, D. Maggioni, L. Raccosta, C. V. Lin, F. Invernizzi, R. Crocchiolo, et al. 2016. 24-Hydroxycholesterol participates in pancreatic neuroendocrine tumor development. *Proc. Natl. Acad. Sci. USA.* 113: E6219–E6227.

10. Mollinedo, F. 2019. Neutrophil degranulation, plasticity, and cancer metastasis. *Trends Immunol.* 40: 228–242.

11. Baek, A. E., Y. A. Yu, S. He, S. E. Wardell, C. Y. Chang, S. Kwon, R. V. Pillai, H. B. McDowell, J. W. Thompson, L. G. Dubois, et al. 2013. The cholesterol metabolite 27-hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. *Nat. Commun.* 4: 864.

12. Vedin, L. L., S. A. Lewandowski, P. Parini, J. A. Gustafsson, and K. R. Steffensen. 2009. The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. *Carcinogenesis.* 30: 575–579.

13. Vedin, L. L., J. A. Gustafsson, and K. R. Steffensen. 2013. The oxysterol receptors LXRalpha and LXRbeta suppress proliferation in the colon. *Mol. Carcinog.* 52: 835–844.

14. Theodoratou, E., S. M. Farrington, A. Teneva, G. McNeill, R. Cemarski, R. A. Barnetson, M. F. Porteous, M. G. Dunlop, and H. Campbell. 2008. Modification of the inverse association between dietary vitamin D intake and colorectal cancer risk by a FokI variant supports a chemoprotective action of Vitamin D intake mediated through VDR binding. *Int. J. Cancer.* 123: 2170–2179.

15. Li, H., M. J. Stampfer, J. B. Hollis, L. A. Mucci, J. M. Gaziano, D. Hunter, E. L. Giovannucci, and J. Ma. 2007. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and the risk of breast cancer. *J. Natl. Cancer Inst.* 99: 1318–1323.

16. Vedin, L. L., J. A. Gustafsson, and K. R. Steffensen. 2013. The oxysterol receptors LXRalpha and LXRbeta suppress proliferation in the colon. *Mol. Carcinog.* 52: 835–844.

17. Theodoratou, E., S. M. Farrington, A. Teneva, G. McNeill, R. Cemarski, R. A. Barnetson, M. F. Porteous, M. G. Dunlop, and H. Campbell. 2008. Modification of the inverse association between dietary vitamin D intake and colorectal cancer risk by a FokI variant supports a chemoprotective action of Vitamin D intake mediated through VDR binding. *Int. J. Cancer.* 123: 2170–2179.

18. Li, H., M. J. Stampfer, J. B. Hollis, L. A. Mucci, J. M. Gaziano, D. Hunter, E. L. Giovannucci, and J. Ma. 2007. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and the risk of breast cancer. *J. Natl. Cancer Inst.* 99: 1318–1323.

19. Yamasaki, I., M. Tombolou, G. Kadikouly, A. Dommez, S. Cagirgan, and Z. Bolaman. 2008. Cholesterol levels in patients with multiple myeloma. *Ann. Hematol.* 87: 223–228.

20. Rose, G., and M. J. St.Phalle. 1980. Plasma lipids and mortality: a source of error. *Lancet.* 1: 525–526.

21. Danilo, C., and P. G. Frank. 2012. Cholesterol and breast cancer development. *Curr. Opin. Pharmacol.* 12: 677–682.

22. Chen, H., S. Qin, M. Wang, T. Zhang, and S. Zhang. 2015. Association between cholesterol intake and pancreatic cancer risk: evidence from a meta-analysis. *Sci. Rep.* 5: 8243.

23. Freeman, M. R., and K. R. Solomon. 2011. Cholesterol and benign prostate disease. *Differentiation.* 82: 244–252.
Pollak, M. 2012. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat. Rev. Cancer. 12: 159–169.

Huó, H., X. Guo, S. Hong, M. Jiang, X. Liu, and K. Liao. 2003. Lipid rafts/caveolae are essential for insulin-like growth factor-I receptor signaling during STS-I preepoxidation differentiation induction. J. Biol. Chem. 278: 11561–11569.

Matthews, L. C., M. J. Taggart, and M. Westwood. 2005. Effect of cholesterol depletion on mitogenesis and survival: the role of caveolar and noncaveolar domains in insulin-like growth factor-mediated cellular function. Endocrinology. 146: 5463–5473.

Panetta, D., C. Biedli, S. Repetto, R. Cordera, and D. Maggi. 2004. IGF-I regulates caveolin 1 and IRS1 interaction in caveolae. Biochim. Biophys. Res. Commun. 316: 240–243.

Xu, L., X. Qu, X. Hu, Z. Zhu, C. Li, E. Li, Y. Ma, N. Song, and Y. Liu. 2013. Lipid raft-regulated IGF-1R activation antagonizes TRAIL-induced apoptosis in gastric cancer cells. FEBS Lett. 587: 3815–3823.

Yi, J., M. A. Sokolovsky, and L. E. Samuelson. 2019. TCR microclusters form spatially segregated domains and sequentially assemble in calcium-dependent kinetic steps. Nat. Commun. 10: 277.

Mollinedo, F., J. de la Iglesia-Vicente, C. Gajate, A. Estella-Hernando de Monroy, J. A. Villa-Pelagín, M. A. Camanero, and M. J. Blanco-Prieto. 2010. Lipid raft-targeted therapy in multiple myeloma. Oncogene. 29: 3748–3757.

Gajate, C., and F. Mollinedo. 2011. Lipid rafts and Fas/CD95 signaling in cancer chemotherapy. Recent Pat. Anticancer Drug Discov. 6: 274–283.

Gajate, C., and F. Mollinedo. 2014. Lipid rafts, endoplasmic reticulum and mitochondria in the antitumor action of the alkylphospholipid analog edelfosine. Anticancer Agents Med. Chem. 14: 509–527.

Surmacz, E. 2003. Growth factor receptors as therapeutic targets: strategies to inhibit the insulin-like growth factor I receptor. Oncogene. 22: 6589–6597.

Christopoulos, P. F., F. Masouei, and M. Koutsilieris. 2015. The role of the insulin-like growth factor-I system in breast cancer. Mol. Cancer. 14: 43.

Biegls, L. H. E. Johnsen, K. Maes, E. M. Van Valkenhorst, M. T. Overgaard, M. Nyegaard, C. A. Conover, K. Vanderkerken, and E. De Bruyne. 2016. The insulin-like growth factor system in multiple myeloma: diagnostic and therapeutic potential. Front. Oncol. 6: 4575–4579.

Allard, J. B., and C. Duan. 2018. IGF-binding proteins: why do they exist and why are there so many? Front. Endocrinol. (Lausanne). 9: 117.

Adams, T. E., V. C. EPA, T. P. Garrett, and G. W. Ward. 2000. Structure and function of the type I insulin-like growth factor receptor. Cell. Mol. Life Sci. 57: 1056–1067.

Pollak, M. N. S. Schernhammer, and S. E. Hankinson. 2004. Insulin-like growth factors and neoplasia. Nat. Rev. Cancer. 4: 505–518.

Pollak, M. 2012. The insulin receptor/insulin-like growth factor receptor family as a therapeutic target in oncology. Clin. Cancer Res. 18: 40–50.

Sun, W. Y., H. Y. Yun, Y. J. Song, H. Kim, O. J. Lee, S. J. Nam, and J. S. Koo. 2015. Insulin-like growth factor I receptor expression in breast cancer tissue and mammographic density. Mol. Clin. Oncol. 3: 572–580.

Trojan, J. J., F. Cloix, M. Y. Ardoürel, M. Chatel, and D. D. Anthony. 2007. Insulin-like growth factor type I biology and targeting in malignant gliomas. Neuroscience. 145: 795–811.

Nakajima, N., K. Kozu, S. Kobayashi, R. Nishiyama, R. Okubo, Y. Akai, M. Moriyama, and N. Kinukawa. 2016. The expression of IGF-1R in Helicobacter pylori-infected intestinal metaplasia and gastric cancer. J. Clin. Biochem. Nutr. 627–644.

Rahajandari, N., W. C. Lin, B. L. Wedhe, A. A. Triplett, and K. U. Wenger. 2017. Autocrine IGF1 signaling mediates pancreatic tumor cell dormancy in the absence of oncogenic drivers. Cell Reports. 18: 2243–2255.

Sprynski, A. C., D. Hose, L. Caillot, T. Reme, J. D. Shaughnessy, Jr., B. Barlogie, A. Seckinger, J. Moreaux, M. Hundemer, M. Mollinedo, F., J. de la Iglesia-Vicente, C. Gajate, A. Estella-Hernando de Monroy, J. A. Villa-Pelagín, M. A. Camanero, and M. J. Blanco-Prieto. 2010. Lipid raft-targeted therapy in multiple myeloma. Oncogene. 29: 3748–3757.

Bähr, C., and B. Groner. 2005. The IGF-1 receptor and its contributions to metastatic tumor growth-novel approaches to the inhibition of IGF-1R function. Growth Factors. 23: 1–14.

Chng, W. J., A. Gualberto, and R. Fonseca. 2006. IGF-1R is overexpressed in poor-prognostic subtypes of multiple myeloma. Leukemia. 20: 174–176.

Ye, C. D., K. H. Park, C. K. Park, S. H. Lee, S. J. Kim, H. K. Yoon, Y. S. Lee, E. J. Lee, K. Y. Lee, and T. J. Kim. 2015. Expression of insulin-like growth factor 1 receptor (IGF-1R) predicts poor responses to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in non-small cell lung cancer patients harboring activating EGFR mutations. Lung Cancer. 87: 311–317.

Heskamp, S., O. C. Boerman, J. D. Molkenboer-Kuenen, C. A. Wauters, L. J. Strobbe, C. M. Mandigers, P. Bult, W. J. Oven, W. T. van der Graaf, and H. W. van Laarhoven. 2015. Upregulation of IGF-1R expression during neoadjuvant therapy predicts poor outcome in breast cancer patients. PLoS One. 10: e0117745.

Iams, W. T., and C. M. Loyd. 2015. Molecular pathways: clinical applications and future direction of insulin-like growth factor-1 receptor pathway blockade. Clin. Cancer Res. 21: 4270–4277.
140. Yamaoka, T., S. Kusumoto, K. Ando, M. Ohba, and T. Ohmori. 2018. Receptor tyrosine kinase-targeted cancer therapy. *Int. J. Mol. Sci.* **19**: E3491.

141. Yuan, J., Z. Yin, K. Tao, G. Wang, and J. Gao. 2018. Function of insulin-like growth factor 1 receptor in cancer resistance to chemotherapy. *Cell Oncol.* **41**: 41–47.

142. Rodríguez-Viciana, P., P. H. Warne, R. Dhand, B. Vankaevebroeck, I. Gout, M. J. Fry, M. D. Waterfield, and J. Downward. 1994. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* **370**: 527–532.

143. Murillo, M. M., S. Rana, B. Spencer-Dene, E. Nye, G. Stamp, and J. Downward. 2018. Disruption of the interaction of Ras with PI3 kinase induces regression of EGFR-mutant driven lung cancer. *Cell Rep.* **25**: 3545–3553.e2.

144. Mollinedo, F., and C. Gajate. 2019. Novel therapeutic approaches for pancreatic cancer by combined targeting of RAF→ERK→MEK signaling and autophagy survival response. *Ann. Transl. Med.* **7**: S153.

145. Gao, X., P. R. Lowry, X. Zhou, C. Depuy, Z. Wei, G. W. Wong, and J. Zhang. 2011. PI3K/Akt signaling requires spatial compartmentalization in plasma membrane microdomains. *Proc. Natl. Acad. Sci. USA* **108**: 14509–14514.

146. Chapuis, N., J. Tamburini, P. Cornillet-Lefèvre, L. Gillot, V. Bardey, L. Willems, S. Park, A. S. Green, N. Ifrah, F. Dreyfus, et al. 2017. Allosteric IGF-1/IGF-1R signaling is responsible for constitutive PI3K/Akt activation in acute myeloid leukemia: therapeutic value of neutralizing anti-IGF-1R antibody. *Haematologica* **95**: 415–423.

147. Rieder, S., C. W. Michalski, H. Friess, and J. Kleeff. 2011. Insulin-like growth factor signaling as a therapeutic target in pancreatic cancer. *Anticancer Agents Med. Chem.* **11**: 427–433.

148. Gussott, S., C. E. Jenkins, S. H. Lam, V. Giambra, M. Pollak, and A. P. Weng. 2016. IGF1R derived PI3K/AKT signaling maintains growth in a subset of human T-cell acute lymphoblastic leukemias. *PLoS One.* **11**: e0161158.

149. Gao, X., and J. Zhang. 2008. Spatiotemporal analysis of differential Akt regulation in plasma membrane microdomains. *Mol. Biol. Cell* **19**: 3546–3557.

150. Gao, X., and J. Zhang. 2009. Akt signaling dynamics in plasma membrane microdomains visualized by FRET-based reporters. *Commun. Integr. Biol.* **2**: 32–34.

151. Lasserre, R., X. J. Guo, F. Conchonaud, Y. Hamon, O. Hawchar, A. M. Bernard, S. M. Soudja, P. F. Lenne, H. Rigneault, D. Olive, et al. 2010. Autoimmune IGF-1/IGF-1R signaling can induce regression of EGFR-mutant lung cancer. *Cell Rep.* **25**: 3545–3553.e2.

152. Rüdiger, C., B. Sion, G. Marceau, C. Damon, K. Mouzat, F. Caira, et al. 2010. Involvement of PI3K-Akt-Bad pathway in apoptosis induced by 2,6-di-O-methyl-beta-cyclodextrin, not 2,6-di-O-methyl-alpha-cyclodextrin, through cholesterol depletion from lipid rafts on plasma membranes in cells. *J. Invest. Dermatol.* **130**: 6068–6077.

153. Desruelles, G. Fromont, N. Prevarskaya, C. Slomianny, and D. Gkika. 2017. Membrane lipid rafts and cancer 631.
181. Gucciardi, M. E., and G. J. Gores. 2009. Life and death by death receptors. *FASEB J.* 23: 1625–1637.
182. Wajant, H. 2014. Principles and mechanisms of CD95 activation. *Biol. Chem.* 395: 1401–1416.
183. Fouquie, A., L. Debure, and P. Legembre. 2014. The CD95/CD95L signaling pathway: a role in carcinogenesis. *Biochim. Biophys. Acta.* 1851: 130–141.
184. Mollinedo, F., and C. Gajate. 2017. Fas/CD95, lipid rafts, and cancer. In *TRAIL, Fas Ligand, TNF andTLR in Cancer*. O. Micheau, editor. Springer International Publishing AG, Cham, Switzerland. 187–227.
185. Tibbets, M. D., L. Zheng, and M. J. Lenardo. 2003. The death effector domain protein family: regulators of cellular homeostasis. *Nat. Immunol.* 4: 404–409.
186. Kischkel, F. C., S. Hellbardt, I. Behrmann, M. Germer, M. Pavlitia, P. H. Krammer, and M. E. Peter. 1995. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J.* 14: 5579–5588.
187. Muzzo, M. B. R. Stockwell, H. R. Stennicke, G. S. Salvesen, and V. M. Dixit. 1998. An induced proximity model for caspase-8 activation. *J. Biol. Chem.* 273: 2926–2930.
188. Schleicht, K., U. Warnken, N. Fricker, S. Ozturk, P. Richter, K. Kammener, M. Schnorler, P. H. Krammer, and I. N. Lavrik. 2012. Stoichiometry of the CD95 death inducing signaling complex: experimental analysis and modeling evidence for a death effector domain chain model. *Mol. Cell.* 47: 306–319.
189. Dickens, L. S., R. S. Boyd, R. Jukes-Jones, M. A. Hughes, G. L. Robinson, L. Fairall, J. W. Schwabe, K. Cain, and M. Macfarlane. 2012. A death effector domain chain DISC model reveals a crucial role for caspase-8 chain assembly in mediating apoptotic cell death. *Mol. Cell.* 47: 291–305.
190. Ohtsuka, A., C. Germain, A. G. Tremblay, V. Blais, J. B. Denault, G. S. Salvesen, and D. R. Green. 2010. Inducible dimerization and inducible cleavage reveal a requirement for both processes in caspase-8 activation. *J. Biol. Chem.* 285: 16632–16642.
191. Martin, D. A., R. M. Siegel, L. Zheng, and M. J. Lenardo. 1998. Membrane oligomerization and cleavage activates the caspase-8 Fas-associated via death-domain (FADD) death-executing function. *Cell Death Dis.* 16: 11010–11016.
192. Guardiola-Serrano, F., A. Procopio, R. Lazzarini, M. R. Rippo, R. Testa, M. Marra, L. Tamagnone, and A. Catalano. 2008. Semaphorin3A signaling controls Fas (CD95)-mediated apoptosis by promoting Fas translocation into lipid rafts. *Blood.* 111: 2290–2299.
193. Gajate, C., and F. Mollinedo. 2005. Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. *J. Biol. Chem.* 280: 11641–11647.
194. Palatto, S., A. M. Gimamiarioli, M. Logozzi, F. Lozupone, P. Matarrese, F. Luciani, M. Falchi, W. Malorni, and S. Fais. 2000. CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. *EMBO J.* 19: 5123–5134.
195. Fais, S., A. De Milito, and F. Lozupone. 2005. The role of FAS to FasL interaction in FAS-mediated apoptosis. *Apoptosis.* 10: 941–947.
196. Mollinedo, F., J. de la Iglesia-Vicente, C. Gajate, A. Estella-Hermoso de Mendoza, J. A. Villapulgarin, M. de Frias, G. Roux, J. Gil, D. Colomer, M. A. Campanero, et al. 2010. In vitro and In vivo selective antitumor activity of Edelfosine against mantle cell lymphoma and chronic lymphocytic leukemia involving lipid rafts. *Clin. Cancer Res.* 16: 2046–2054.
197. Mollinedo, F., C. Gajate, S. Martin-Santamaría, and F. Gago. 2004. ET-18-OCH3 (edelfosine): a selective antitumour lipid targeting apoptosis through intracellular activation of Fas/CD95 death receptor. *Curr. Med. Chem.* 11: 3163–3184.
198. Mollinedo, F. 2008. Death receptors in multiple myeloma and therapeutical opportunities. In *Myeloma Therapy. Pursuing the Plasma Cell*. S. Loniard, editor. Humana Press, Totowa, NJ. 393–419.
199. Hargoutt, L., A. A. R. M. van der Luit, S. R. Vink, J. B. Klarenbeek, D. Perrisoud, D. E. Solary, M. Verheij, and W. J. van Blitterswijk. 2007. A new class of anticancer alklyphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells. *Mol. Cancer Ther.* 6: 2357–2375.
200. Ausili, A., A. Torrecillas, F. J. Aranda, F. Mollinedo, C. Gajate, S. Corbalan-Garcia, A. de Godos, and J. C. Gomez-Fernandez. 2008. Edelfosine is incorporated into rafts and alters their organization. *J. Phys. Chem. B.* 112: 11643–11654.
201. Ausili, A., P. Martinez-Valera, A. Torrecillas, V. Gomez-Murcia, A. M. de Godos, S. Corbalan-Garcia, J. A. Teruel, and J. C. Gomez Fernandez. 2018. Anticancer agent edelfosine exhibits a high affinity for cholesterol and disorganizes liquid-ordered membrane rafts. *Lipids. Membranes. Langmuir.* 3: 833–846.
202. Hac-Wydro, K., P. Dynamoic-Latka, P. Wydro, and K. Bak. 2011. Edelfosine disturbs the spinning myosin-arginine cholesterol membrane system in a cholesterol-dependent way - the Langmuir monolayer study. *Colloids Surf. B Biointerfaces.* 88: 635–640.
203. Castro, B. M., A. Fedorov, V. Hormillos, J. Delgado, A. U. Acuna, F. Mollinedo, and M. Prieto. 2013. Edelfosine and mitofusine effects on lipid raft properties: membrane biophysics in cell death by antitumor lipids. *J. Phys. Chem. B.* 117: 7929–7940.
204. Gajate, C., F. An, and F. Mollinedo. 2003. Rapid and selective apoptosis in human leukemic cells induced by Aplidine through a Fas/CD95- and mitochondrial-mediated mechanism. *Clin. Cancer Res.* 9: 1535–1545.
205. Cahuzac, N., W. Baum, V. Kirkin, F. Conchonaud, L. Wawrezinieck, D. Marguet, O. Janssen, M. Zornig, and A. O. Hueber. 2006. Fas ligand is localized to membrane rafts, where it displays increased cell death-inducing activity. *Blood.* 107: 2384–2391.
206. Nachbur, U., D. Kassahn, S. Youcef, D. F. Legler, and T. Brunner. 2006. Postranscriptional regulation of Fas (CD95) ligand killing activity by lipid rafts. *Blood.* 107: 2790–2796.
207. Guardiola-Serrano, F., A. Rossin, N. Cahuzac, K. Luckerath, I. Guardiola-Serrano, F., A. Rossin, N. Cahuzac, K. Luckerath, I. Mesler, S. Mailfert, D. Marguet, M. Zornig, and A. O. Hueber. 2010. Palmitoylation of human FasL modulates its cell death-inducing function. *Cell Death Dis.* 1: e88.
208. Lotocki, G., O. F. Alonso, W. D. Dietrich, and R. W. Keane. 2004. Tumor necrosis factor receptor 1 and its signaling intermediates are recruited to lipid rafts in the traumatized brain. *J. Neurosci.* 24: 11010–11016.
Lipid rafts and cancer
262. Oriani-Rousseau, V., and J. Sleeman. 2014. CD44 is a multidomain signaling platform that integrates extracellular matrix cues with growth factor and cytokine signals. Adv. Cancer Res. 123: 231–254.

263. Murai, T. 2015. Lipid raft-mediated regulation of hyaluronan-CD44 interactions in inflammation and cancer. Front. Immunol. 6: 401.

264. Skandalis, S. S., T. T. Karalis, A. Chatzopoulos, and N. K. Karamanos. 2019. Hyaluronan-CD44 axis orchestrates cancer stem cell functions. Cell. Signal. 63: 109377.

265. Li, C., D. G. Heidt, P. Dalerba, C. F. Burant, L. Zhang, V. Adsay, M. Wicha, M. F. Clarke, and D. M. Simeone. 2007. Identification of pancreatic cancer stem cells. Cancer Res. 67: 1039–1047.

266. Li, C. J., C. Lee, and D. M. Simeone. 2009. Identification of human pancreatic cancer stem cells. Methods Mol. Biol. 568: 161–175.

267. Fitzgerald, T. L., and J. A. McCubre. 2014. Pancreatic cancer stem cell association with cell surface markers, prognosis, resistance, metastasis and treatment. Adv. Biol. Regul. 56: 45–50.

268. Takaishi, S., T. Okumura, S. Tu, S. S. Wang, W. Shibata, R. Vigneshwaran, S. A. Gordon, Y. Shimada, and T. C. Wang. 2009. Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells. 27: 1006–1020.

269. Watanabe, T., T. Okumura, K. Hirano, T. Yamaguchi, S. Sekine, T. Nagata, and K. Tsukada. 2017. Circulating tumor cells expressing cancer stem cell marker CD44 as a diagnostic biomarker in cancer patients with breast cancer. Oncotarget. 8: 281–298.

270. Murai, T., Y. Maruyama, K. Mio, H. Nishiyama, M. Suga, and C. Sato. 2011. Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration. J. Biol. Chem. 286: 1999–2007.

271. Nagano, O., and H. Saya. 2004. Mechanism and biological significance of CD44 cleavage. Cancer Sci. 95: 950–953.

272. Okitani, I., J. S. H. Kimchi, A. K. Godwin, D. R. Emlet, M. Holgado-Madruga, I. S. Lanham, C. J. Joynes, K. T. Vo, A. Guha, et al. 2002. Proteolytic cleavage of the CD44 adhesion molecule in multiple human tumors. Am. J. Pathol. 160: 441–447.

273. Murai, T. 2015. Cholesterol lowering: role in cancer prevention and treatment. Biol. Chem. 396: 1–11.

274. Murai, T. 2012. The role of lipid rafts in cancer cell adhesion and migration. Int. J. Cell Biol. 2012: 763283.

275. Sugahara, K. N., T. Murai, H. Nishinakamura, H. Kawashima, H. Saya, and M. Miyasaka. 2003. Hyaluronan oligosaccharides induce CD44 cleavage and promote cell migration in CD44-expressing tumor cells. J. Biol. Chem. 278: 32259–32265.

276. Babina, I. S., E. A. McSherry, S. Donatello, A. D. Hill, and A. M. Hopkins. 2014. A novel mechanism of regulating breast cancer cell migration via palmitoylation-dependent alterations in the lipid raft affiliation of CD44. Breast Cancer Res. 16: R19.

277. Murai, T., Y. Miyazaki, H. Nishinakamura, K. N. Sugahara, T. Miyauchi, Y. Sako, T. Yanagida, and M. Miyasaka. 2004. Engagement of CD44 promotes Rac activation and CD44 cleavage during tumor cell migration. J. Biol. Chem. 279: 4541–4550.

278. Murai, T., T. Miyauchi, T. Yanagida, and Y. Sako. 2006. Epidermal growth factor-regulated activation of Rac GTPase enhances CD44 cleavage by metalloproteinase integrasin ADAM10. Biochem. J. 395: 65–71.

279. Donatello, S., I. S. Babina, L. D. Hazelwood, A. D. Hill, I. N. Rabi, and A. M. Hopkins. 2012. Lipid raft association restricts CD44-ezrin interaction and promotion of breast cancer cell migration. Am. J. Pathol. 181: 2172–2187.

280. Yang, Z., W. Qin, Y. Chen, B. Yuan, X. Song, B. Wang, F. Shen, J. Fu, and H. Wang. 2018. Cholesterol inhibits hepatocellular carcinoma invasion and metastasis by promoting CD44 localization in lipid rafts. Cell. 172: 66–77.

281. Huang, Q., H. M. Shen, G. Shui, M. R. Wenk, and C. N. Ong. 2006. Emodin inhibits tumor cell adhesion through disruption of the membrane lipid raft-associated integrin signaling pathway. Cancer Res. 66: 5807–5815.

282. Wang, R., J. Bi, K. K. Ampah, X. Ba, W. Liu, and X. Zeng. 2013. Lipid rafts control human melanoma cell migration by regulating focal adhesion disassembly. Biochem. Biophys. Acta. 1833: 3195–3205.

283. Chantôme, A., M. Potier-Cartereau, L. Clarysse, G. Fromont, S. Marianne-Lambot, M. Gueguinou, J. C. Pages, C. Collin, T. Oullier, A. Girault, et al. 2013. Pivotal role of the lipid raft SK3/Orai1 complex in human cancer cell migration and bone metastases. Cancer Res. 73: 4852–4861.

284. Stock, C., and A. Schubach. 2015. Ion channels and transporters in metastasis. Biochem. Biophys. Acta. 1848: 2638–2646.
305. Ewaschuk, J. B., M. Newell, and C. J. Field. 2012. Docosahexanoic acid improves chemotherapy efficacy by inducing CD95 translocation to lipid rafts in ER(-) breast cancer cells. *Lipids*. 47: 1019–1030.

306. Aroui, S., S. Brahim, J. Hamelin, M. De Waard, J. Breard, and A. Kenani. 2009. Conjugation of doxorubicin to cell penetrating peptides sensitizes human breast MDA-MB 231 cancer cells to endogeous TRAIL-induced apoptosis. *Apoptosis*. 14: 1352–1365.

307. Xu, L., X. Qu, Y. Zhang, X. Hu, X. Yang, K. Hou, Y. Teng, J. Zhang, K. Sada, and Y. Liu. 2009. Oxaliplatin enhances TRAIL-induced apoptosis in gastric cancer cells by CBL-regulated death receptor redistribution in lipid rafts. *FEBS Lett.* 583: 943–948.

308. Gmeiner, W. H., J. Jennings-Gee, C. H. Stuart, and T. S. Pardee. 2015. Thymineless death in F10-treated AML cells occurs via lipid raft depletion and Fas/FasL co-localization in the plasma membrane with activation of the extrinsic apoptotic pathway. *Leuk. Res.* 39: 229–235.

309. Pahoulia, F. H., K. G. Drosopoulos, L. Doubravska, L. Andera, and A. Pintzas. 2007. Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. *Mol. Cancer Ther.* 6: 2591–2599.

310. Delmas, D., C. Rebe, S. Lacour, R. Filomenko, A. Athias, P. Gambert, M. Cherkaoui-Malki, B. Jannin, L. Dubrez-Daloz, N. Latruffe, et al. 2003. Resveratrol-induced apoptosis is associated with Fas redistribution in the rafts and the formation of a death-inducing signaling complex in colon cancer cells. *J. Biol. Chem.* 278: 41482–41490.

311. Delmas, D., C. Rebe, S. Lacour, R. Filomenko, A. Athias, P. Gambert, M. Cherkaoui-Malki, B. Jannin, L. Dubrez-Daloz, N. Latruffe, et al. 2003. Resveratrol-induced apoptosis is associated with Fas redistribution in the rafts and the formation of a death-inducing signaling complex in colon cancer cells. *J. Biol. Chem.* 278: 41482–41490.

312. Stel, A. J., B. Ten Cate, S. Jacobs, J. W. Kok, D. C. Spierings, M. Dondorf, W. Helfrich, H. C. Klein-Nelemans, L. F. de Leij, S. Witte, et al. 2007. Fas receptor clustering and involvement of the death receptor pathway in rituximab-mediated apoptosis with concomitant sensitization of lymphoma B cells to fas-induced apoptosis. *J. Immunol.* 178: 2287–2295.

313. Yun, S. H., E. S. Park, S. W. Shin, M. H. Ju, J. Y. Han, J. S. Jeong, S. H. Kim, V. A. Stonik, J. Y. Kwak, and J. I. Park. 2015. By activating Fas/ceramide synthase 6/p38 kinase in lipid rafts, stichoposide D inhibits growth of leukemia xenografts. *Oncotarget.* 6: 27596–27612.

314. Lin, M. L., S. S. Chen, and T. S. Wu. 2015. Synthetic bichalcone TSWU-BR23 induces apoptosis of human colon cancer HT-29 cells by p53-mediated mitochondrial oligomerization of BAX/BAK and lipid raft localization of CD95/FADD. *Anticancer Res.* 35: 5407–5416.

315. Elyassaki, W., and S. Wu. 2006. Lipid rafts mediate ultraviolet light-induced Fas aggregation in M624 melanoma cells. *Photochem. Photobiol.* 82: 787–792.

316. Lim, S. C., H. Q. Duong, J. E. Choi, T. B. Lee, J. H. Kang, S. H. Oh, and S. I. Han. 2011. Lipid raft-dependent death receptor 5 (DR5) expression and activation are critical for ursodeoxycholic acid-induced apoptosis in gastric cancer cells. *Carcinogenesis.* 32: 723–731.

317. Wang, S. C., and S. I. Han. 2015. Ursodeoxycholic acid effectively kills drug-resistant gastric cancer cells through induction of autophagic death. *Oncol. Rep.* 34: 1261–1268.