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Thematic Review Series: Biology of Lipid Rafts

Lipid rafts as a therapeutic target

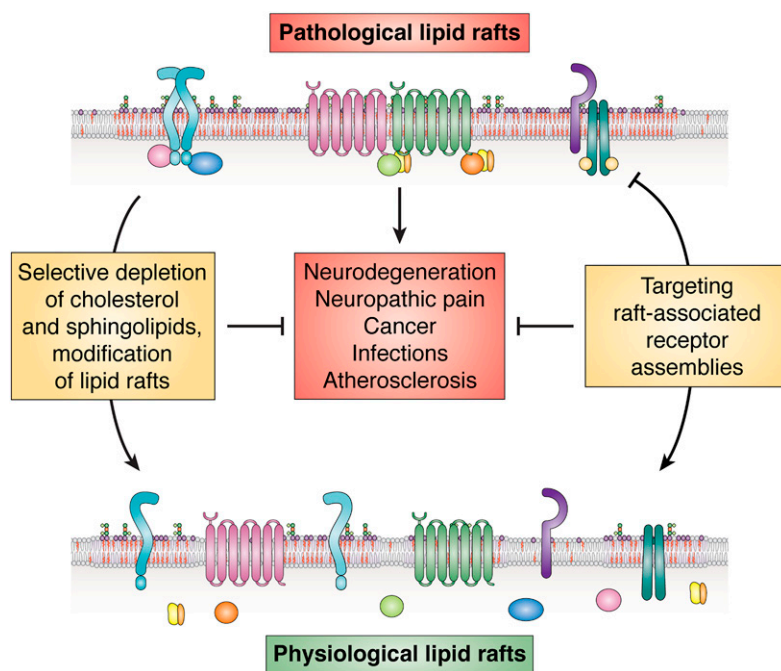
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Abstract Lipid rafts regulate the initiation of cellular metabolic and signaling pathways by organizing the pathway components in ordered microdomains on the cell surface. Cellular responses regulated by lipid rafts range from physiological to pathological, and the success of a therapeutic approach targeting “pathological” lipid rafts depends on the ability of a remedial agent to recognize them and disrupt pathological lipid rafts without affecting normal raft-dependent cellular functions. In this article, concluding the Thematic Review Series on Biology of Lipid Rafts, we review current experimental therapies targeting pathological lipid rafts, including examples of inflammarafts and clusters of apoptotic signaling molecule-enriched rafts. The corrective approaches include regulation of cholesterol and sphingolipid metabolism and membrane trafficking by using HDL and its mimetics, LXR agonists, ABCA1 overexpression, and cyclodextrins, as well as a more targeted intervention with apoA-I binding protein. Among others, we highlight the design of antagonists that target inflammatory receptors only in their activated form of homo- or heterodimers, when receptor dimerization occurs in pathological lipid rafts. Other therapies aim to promote raft-dependent physiological functions, such as augmenting caveolae-dependent tissue repair. The overview of this highly dynamic field will provide readers with a view on the emerging concept of targeting lipid rafts as a therapeutic strategy.—Sviridov, D., N. Mukhamedova, and Y. I. Miller. **Lipid rafts as a therapeutic target.** *J. Lipid Res.* 2020. 61: 687–695.

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Lipid rafts play a unique role in cell physiology providing a solid platform within a membrane where macromolecular complexes can assemble without battling forces of chaos in the disorderly liquid phase of the surroundings. The abundance and functional properties of lipid rafts can change rapidly in response to changing metabolic conditions, most likely representing a fundamentally important layer of fast physiological regulation, connecting and coordinating a broad range of metabolic and signaling pathways. At the same time, as described in review articles published in this series, dysregulation of lipid rafts plays a

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Abbreviations: AIBP, apoA-I binding protein; β CD, β -cyclodextrin; CASMERS, cluster of apoptotic signaling molecule-enriched rafts; Cav-1, caveolin-1; CIPN, chemotherapy-induced peripheral neuropathy; HIV, human immunodeficiency virus; M β CD, methyl- β -cyclodextrin; MOR, μ -opioid receptor; NPC, Niemann-Pick type C.

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key role in the pathogenesis of hematopoietic, neurological, inflammatory, and infectious diseases, as well as that of cancer. The emerging physiological and pathological roles of lipid rafts point to an exciting possibility to target lipid rafts for therapeutic purposes. Targeting an early step in pathogenesis has a significant advantage of addressing “a root” of the problem and mitigating diverse consequences of lipid raft pathology. For example, targeting lipid rafts in neurodegenerative diseases may simultaneously reduce amyloidogenic protein misfolding and processing as well as neuroinflammation, two key elements of pathogenesis of neurodegeneration. Targeting lipid rafts in infectious diseases can simultaneously mitigate the infection and its metabolic comorbidities. Given a key role of inflammation in a multitude of pathological processes, targeting rafts to moderate the inflammation may have a broad utility.

However, targeting rafts is not without problems. *Primum non nocere*, “first, do no harm.” The question that inevitably comes to mind, is it really possible to target lipid rafts, an essential component in the plasma membrane organization and the platform for a multitude of physiologic processes, to achieve a therapeutic effect without significant adverse impact? Two observations indicate that this might be a realistic possibility. First, somewhat surprisingly, most raft-associated pathologies are caused by “excessive” lipid rafts: elevated raft abundance or increased raft stability, or both. Further, β -cyclodextrins (β CDs) are an effective tool to deplete cells of cholesterol and indiscriminately destroy rafts. Although at high concentrations they may be cytotoxic, when used at lower concentrations they still destroy rafts, but have remarkably few adverse effects *in vitro* and *in vivo*. This points to the existence of significant redundancy and/or backup mechanisms supporting the physiological role of rafts. Second is spatial and temporal heterogeneity of the lipid rafts in relation to their size, stability, structure, and, ultimately, function. Raft heterogeneity is determined by a repertoire of lipids and proteins in the rafts and opens, at least theoretically, a possibility to selectively target one subset of lipid rafts and not the other, one cell function and one cell type, but not all of them. The goal of this review article is to demonstrate that recent advances in understanding lipid raft regulation point to the possibility of targeting excessive or pathological lipid rafts as a viable therapeutic strategy.

REGULATION OF LIPID RAFTS

There are two major mechanisms that regulate dynamic remodeling of lipid rafts. One mechanism relies on the availability of lipids that are critical for raft structure, principally, cholesterol and sphingolipids. Depletion of plasma membrane cholesterol using methyl- β CD (M β CD) is a classical method to break down lipid rafts, significantly attenuating all signaling originating from rafts. Inhibition of cholesterol biosynthesis also lowers lipid raft cholesterol content and alters raft-originated signaling (1). Enrichment of membranes with ceramides, either directly or via depletion of sphingomyelin, displaces cholesterol from rafts altering their properties (2, 3). Monounsaturated fatty

acids inhibit raft formation (4), while polyunsaturated fatty acids stabilize it (5). Thus, simple interventions acting on membrane lipids robustly modify lipid rafts and their protein cargo with consequent changes in signal transduction (6). Another mechanism regulating raft organization depends on changes in the cytoskeleton. Recent findings indicate that the structural and functional properties of lipid rafts depend upon interactions with and dynamic rearrangement of the cytoskeleton (7). For example, β -actin remodeling modulates raft abundance and changes their properties (8). The two mechanisms are not mutually exclusive and can be used to selectively target pathological subsets of lipid rafts in one cell type or cell types harboring pathological rafts.

PATHOLOGICAL LIPID RAFTS

For the purpose of this article, the definition of pathological lipid rafts is rather teleological, referring to lipid rafts in inflammatory or activated or transformed cells under pathological conditions, and to a lesser degree to their specific structural characteristics. Emerging new techniques will allow for a more detailed characterization of the composition and biophysical features of altered lipid rafts under various pathological conditions. Pathological lipid rafts serve the purpose of organizing metabolic and signaling processes leading to diseases states. We posit that operating within the framework of pathological lipid rafts, with the examples of inflammarafts and clusters of apoptotic signaling molecule-enriched rafts (CASMERs) given below, can be useful in discussing therapeutic targeting of lipid rafts.

Inflammarafts

The term inflammaraft was introduced to emphasize the role of enlarged lipid rafts harboring activated receptors and adaptor molecules and serving as a scaffold to organize the cellular inflammatory response (9). TLR4 is a prototypic inflammatory receptor, which is dimerized in response to ligand activation, the process that requires a lipid raft microenvironment. An increased abundance of lipid rafts, for example due to deficiency of ABCA1 and ABCG1 transporters (10), and the increased number of TLR4 dimers do not only reflect a ligand-induced TLR4 receptor activation event, but also indicate the permissive membrane microenvironment that supports assembly of other inflammatory receptor complexes. In this context, stimuli-mediated dimerization of TLR4 (11–14) and IFN γ receptor (15, 16), association of TREM2 with the adaptor molecule DAP12 (17), and assembly of the NADPH oxidase complex (18), among other inflammatory processes, lead to lipid raft clustering into larger and more stable inflammaraft units, pathological rafts. Depletion of cholesterol and/or sphingolipids from the plasma membrane disrupts inflammarafts. Thus, targeting cholesterol efflux agonists to inflammatory cells, for example via apoA-I binding protein (AIBP) (the treatment highlighted in a separate section below), could serve as a therapeutic strategy to reduce inflammation by targeting lipid rafts in a specific subset of cells.

CASMERs

The CASMER designates a supramolecular signaling hub playing a central role in death receptor-mediated apoptosis and localizing in lipid rafts (19, 20). The aggregated rafts forming CASMERs allow for an increased complexity of recruited proteins, which include the death receptors, Fas/CD95 and TNFR1 (CD120a) (19, 21), and the TRAIL receptors, TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (19, 22), as well as downstream signaling molecules, including FADD, procaspase-8, and procaspase-10, forming the death-inducing signaling complex (20, 23, 24). It is remarkable that signaling molecules might change their regulatory features when redistributed between a raft and a non-raft microenvironment (25). Compared with normal cells, cancer cells contain higher levels of cholesterol, facilitating clustering of cholesterol-rich lipid rafts to form CASMERs. Thus, formation of CASMERs as a major regulatory apoptotic signaling pivot makes them another example of a distinctive subset of pathological rafts, a potential therapeutic target in cancer. However, the therapeutic strategy here would be to promote recruitment of death receptors to CASMERs rather than to disrupt CASMERs, as is highlighted with an example of edelfosine in the section below.

EXPERIMENTAL LIPID RAFT THERAPEUTICS

AIBP

AIBP (gene name *APOA1BP*, also known as *NAXE*) was discovered in a yeast two-hybrid screen of proteins that bind apoA-I (26) and shown to promote cholesterol efflux from endothelial cells, macrophages, and microglia to apoA-I and/or HDL (14, 27–29). AIBP also binds to TLR4 (14). Surface expression of TLR4, which is localized to inflamarrafts, is rapidly increased in activated cells, for example, in macrophages stimulated with LPS (14), until TLR4 dimers are internalized via endocytosis (30). The increased TLR4 expression increases binding of recombinant AIBP to activated inflammatory cells, and this leads to enhanced cholesterol efflux and reduced abundance of inflamarrafts (14). The TLR4 binding affords selectivity to an AIBP mode of action: recombinant AIBP has little effect on nonactivated cells, while reversing pathological changes in lipid rafts back to the levels observed in nonactivated cells. A single intrathecal dose of AIBP reverses tactile allodynia (pain response to a light touch) in mouse models of chemotherapy-induced peripheral neuropathy (CIPN) and arthritis, with the therapeutic effect lasting as long as over 2 months in the CIPN model. This remarkable therapeutic effect is accompanied by no adverse effects of AIBP on motor or sensory function in mice (14). Inhaled AIBP reduces LPS-induced acute lung injury in mice (29) and AAV-mediated sustained expression of secreted AIBP reduces hyperlipidemia and atherosclerosis in *Ldlr*^{-/-} mice fed a Western type diet (31, 32) and human immunodeficiency virus (HIV) replication in humanized mice (33). Although experimental data provide evidence for TLR4-mediated targeting of AIBP to inflammatory cells (14), other components

of inflamarrafts may mediate this targeting as well, depending on the cell type and specific pathologic conditions. By the virtue of affecting lipid raft composition and abundance, in addition to TLR4 dimerization (14), AIBP likely inhibits other receptors, enzymes, and channels localized to inflamarrafts, but this hypothesis needs experimental validation.

LXR agonists and ABC transporters

LXR is a transcriptional regulator of ABCA1 and ABCG1 (among other genes), and, in the presence of an agonist, it significantly stimulates expression and abundance of these cholesterol transporters. ABCA1 is a key regulator of both cholesterol availability and actin polymerization and regulates the abundance of lipid rafts through both mechanisms. The “lipid” mechanism relies on the central role of ABCA1 and ABCG1 in cholesterol efflux. Thus, reduced abundance of ABCA1 increases the amount of cellular cholesterol potentiating formation of lipid rafts and vice versa (34). The same mechanism is probably also responsible for the increased abundance of lipid rafts in ABCG1- or ABCA1/ABCG1-deficient macrophages (10). The “cytoskeleton” mechanism relies on the ability of ABCA1 to activate the small GTPase Cdc42, which stimulates polymerization of actin (35–37), with a subsequent negative effect on raft abundance (8). The connection between ABCA1 and rafts is reciprocal: ABCA1 determines the abundance of rafts (38); but at the same time, activity and stability of ABCA1 is determined by the abundance of rafts (39). LXR agonists have been shown to reduce the abundance of lipid rafts in vitro and in vivo (40–42). Given that LXR regulates the expression of many genes and is involved in regulation of multiple pathways, selectivity of the effect of LXR agonists on lipid rafts and the contribution of raft-dependent effects to overall outcome are difficult to ascertain. The ability of LXR agonists to reduce inflammatory signaling is well documented, but it involves both raft-dependent and raft-independent mechanisms (43).

Activating LXR, however, is not the only way to increase ABCA1 abundance. Adenoviral overexpression of ABCA1 in endothelial cells reduces lipid raft-dependent inflammatory signaling (44). Knockout of miR-33, a potent negative regulator of both ABCA1 and ABCG1 expression, increases expression of these transporters in cardiac fibroblasts reducing lipid raft abundance, proliferation of these cells, and cardiac fibrosis (45). Another way to increase the abundance of ABCA1 is to enhance its stabilization with HDL or HDL mimetics (46).

HDL and HDL mimetics

HDL and lipid-free apoA-I are the main acceptors of cholesterol in the reverse cholesterol transport pathway. Whether they remove cholesterol directly from lipid rafts or after transfer of cholesterol to other membrane locations is a contentious issue, but there is little doubt that the end result is a reduction of lipid raft abundance (47). Furthermore, apoA-I stabilizes ABCA1 (48), an additional mechanism of reducing lipid raft abundance, which may or may not be related to cholesterol efflux. Elevating HDL levels,

providing that this does not impair HDL functionality, has a multitude of beneficial effects and some of them may be related to reducing the abundance and/or cholesterol content of lipid rafts. Numerous reports have demonstrated that exposure of macrophages, monocytes, neutrophils, endothelial cells, and adipocytes to HDL or apoA-I leads to a reduction of lipid raft abundance and broad inhibition of various raft-dependent inflammatory responses in vitro and in vivo (47, 49–52). Infusion of HDL mimetics (reconstituted HDL or apoA-I mimetic peptides) has similar anti-inflammatory effects (51, 53), reduces platelet activation (54), and is generally anti-atherogenic (55, 56). High levels of HDL inversely associate with risk of cancer (57), and HDL mimetics that stimulate cholesterol efflux are used as anti-cancer therapy (58); however, the direct involvement of lipid rafts in the anti-cancer activity of HDL is yet to be verified. High levels of HDL are associated with reduced risk of infectious disease (59), consistent with the role of lipid rafts in pathogenesis of many infections.

Statins

Statins are competitive inhibitors of HMG-CoA reductase, a rate-limiting enzyme of the cholesterol biosynthesis pathway. Inhibition of cholesterol biosynthesis often results in cholesterol deficiency and reduction of the abundance and/or changing properties of lipid rafts. Simvastatin lowers raft cholesterol content, inhibits Akt/PKB pathway signaling, and induces apoptosis in prostate cancer cells (1). Treatment with simvastatin induces shedding of CD44, a raft-associated adhesion molecule involved in tumor metastasis (60). Entrance of HIV into macrophages through lipid rafts is inhibited when raft abundance is reduced by lovastatin (61). It has to be recognized, however, that inhibition of HMG-CoA reductase by statins also reduces the concentration of intermediates of the cholesterol biosynthesis pathway, such as isoprenoids, which are metabolically active in pathways unrelated to lipid rafts. Reduced levels of cholesterol and intermediates of the mevalonate pathway have raft-independent effects, such as attenuation of cell growth or inhibition of DNA repair (62). Thus, statins, as well as HDL and its mimetics and LXR agonists, have broad effects on systemic cholesterol metabolism and limited selectivity in targeting pathological lipid rafts.

β Cyclodextrins

Treatment with β CDs is a common method to deplete cholesterol from the plasma membrane, leading to destruction of lipid rafts as well as redistribution of intracellular cholesterol (63). However, the mechanism of β CD action is more complex, and depending on dose and exposure, β CDs have intracellular effects. Following endocytosis, β CDs promote cholesterol transfer from late endosomes to lysosomes and its processing in the lysosomes (64), thus alleviating cholesterol storage disorders, such as Niemann-Pick type C (NPC) disease (65), activates the LXR (66) and AMPK/autophagy (67) pathways. LXR activation is due to β CD-induced upregulation of 27-hydroxycholesterol, an LXR agonist, resulting in macrophage transcriptional reprogramming and enhanced cholesterol efflux (66). These

are interesting findings, although the exact mechanism of β CD-induced production of 27-hydroxycholesterol is not entirely clear. In animal models, therapeutic effects of 2-hydroxypropyl- β CD have been demonstrated in treatment of NPC disease (68–70) and atherosclerosis (66). Initial results of clinical trials exploring intrathecal 2-hydroxypropyl- β CD in treatment of NPC patients have been promising (71), and the results of a phase 2b/3 clinical trial are expected in late 2020.

A targeted approach has been proposed by Lee et al. (72) who describe a nanoassembly consisting of M β CD conjugated with hyaluronic acid-ceramide, targeting the particle to the CD44 receptor present in many tumors. These nanoparticles disrupt lipid rafts and exert pro-apoptotic and anti-proliferation activity in vitro and are more selective and active than “untargeted” M β CD in tumor-bearing mice.

Sphingolipid inhibitors and modulation of phospholipid composition

In addition to cholesterol, sphingolipids are the essential component of lipid rafts and modulation of their metabolism is a promising direction in lipid raft regulation. In systemic lupus erythematosus patients, CD4⁺ T cells are characterized by defects in the lipid raft localization and function of key TCR signaling molecules. This is likely due to increased levels of cholesterol glycosphingolipids (GM1, Gb3, and lactosylceramide) in the plasma membrane, associated with increased expression of LXR β and its target genes *NPC1* and *NPC2*, but not *ABCA1* or *ABCG1*. Remarkably, in vitro, a clinically approved inhibitor of glycosphingolipid synthesis, *N*-butyldeoxynojirimycin, corrects CD4⁺ T cell signaling and functional defects (73). Inhibition of glycosylceramide synthesis in adipocytes prevents iNKT cell activation and effector function in adipose tissue (74). In mouse models, a related inhibitor of sphingolipid biosynthesis, *N*-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin, reduces diet-induced liver steatosis, inflammation, and fibrosis, characteristic of human nonalcoholic steatohepatitis (75), and improves biliary lipid secretion (76).

Other phospholipid constituents of lipid rafts are also important for maintaining raft structure and therefore could be targeted for therapeutic purposes. Lipid rafts are rich in phospholipids with long-chain saturated fatty acids, and enrichment of cells with poly- or monounsaturated fatty acids, which can be achieved by dietary means, leads to incorporation of these fatty acids into cellular phospholipids and to changes in the properties of the plasma membrane and, specifically, rafts (77–79). This approach has been used for therapeutic purposes [for review see (78, 80)] mainly in cancer, but the contribution of changes in lipid rafts in the context of complex pleiotropic effects of various phospholipids on cell metabolism is difficult to elucidate.

CASMER agonists

Edelfosine, a synthetic analog of lysophosphatidylcholine, is a potent inducer of apoptosis through the recruitment and clustering of Fas/CD95 and other death receptors in

CASMERs (23, 81). Edelfosine accumulates in lipid rafts (24) due to high affinity to cholesterol and disturbs the cholesterol-sphingomyelin interaction in the membrane (82, 83). Edelfosine treatment can both augment the action of the physiologic death receptor ligand FasL/CD95L, promoting a response in otherwise resistant cancer cells, and induce ligand-independent death receptor activation. Many other chemotherapy drugs having compound anticancer effects possess the ability to recruit death receptors and downstream signaling molecules into CASMERs, as summarized in a recent review article (25).

Targeting lipid raft-organized receptor complexes

Numerous therapeutic receptor antagonists, in the form of either a small molecule, peptide, or antibody, target a single-molecule receptor. However, upon activation, many of these receptors localize to lipid rafts and dimerize or form heteromeric receptor complexes. Capitalizing on the knowledge that one of the important therapeutic targets, CXCR4, localizes to and dimerizes in lipid rafts of tumor cells, a recent work describes the design of a liposome presenting the CXCR4 binding peptide DVI (L-DV1) as a 3D molecular array of varying density (84). The authors have identified the DVI density of 24,000 molecules per square micrometer, corresponding to a 45 Å distance between DVI peptides on the liposome surface, as the most effective formulation in treatment of triple negative breast cancer. These specific L-DVIs significantly reduce cancer migration and inhibit metastasis from a primary tumor in mice for 27 days (84). This design of L-DVIs does not encapsulate a chemotherapeutic, preventing off-target toxicity of peptide-functionalized liposomes, which mirror the presentation of CXCR4 dimers in the membrane of cancer cells. In addition, L-DVIs likely target only tumor, but not bystander, cells in which altered lipid rafts organize CXCR4 in the manner that is amenable to L-DV1 binding.

Another chemokine receptor, CCR5, often clusters with the μ -opioid receptor (MOR) in lipid rafts of neurons and glial cells (85), resulting in cross-desensitization. Via CCR5 and other receptor signaling, proinflammatory cytokines and chemokines counteract the analgesia produced by opioids (86). These findings led to the design of a bivalent ligand, MCC22, for the treatment of CIPN-associated pain. MCC22 consists of MOR agonist and CCR5 antagonist pharmacophores connected through a 22-atom spacer and, thereby, targets the MOR-CCR5 heteromer (87). Intrathecal delivery of MCC22 decreases CIPN-associated spinal neuroinflammation, hyperalgesia, and, unlike morphine, MCC22 does not exhibit tolerance to its analgesic effect or rewarding properties (88).

Targeting raft scaffolding proteins

Raft scaffolding proteins, caveolins 1 and 2 and flotillins 1 and 2, are essential for maintaining raft structure and are regulated by several miRNAs [for review see (89)]. Physiological regulation and experimental modulation of these miRNAs control a wide range of cellular functions, carcinogenesis and metastasis, spermatogenesis, inflammation, insulin sensitivity, fibrosis, and resistance to pathogens.

Although therapeutic use of miRNAs is complicated by the fact that they often have several targets, this approach may be considered in the context of “raft therapy”.

Caveolin gene therapy

So far, we discussed strategies to reduce the abundance of pathological lipid rafts associated with inflammatory disease, infection, or cancer. However, recovery of organ function, for example, recovery of brain structural and functional plasticity after traumatic brain injury or stroke, often benefits from an opposite, maintaining the integrity of lipid rafts, or at least of a specific raft subset, such as caveolae (90). Lipid rafts support response to intracellular signals, modulation of cytoskeletal dynamics, and tethering of the cytoskeleton to the plasma membrane, which generate a cellular polarity that promotes neuronal growth and plasticity. In this context, upregulating the expression of the scaffolding and cholesterol-binding lipid raft-localized protein caveolin-1 (Cav-1) provides multiple beneficial effects on neuronal function and axonal growth. Neuron-targeted overexpression of Cav-1 in adult and aged mice increases lipid rafts and expression of raft-localized growth-promoting receptors, augments structural and functional hippocampal neuroplasticity, and improves hippocampal-dependent contextual fear learning and memory (91). In this work, Cav-1 overexpression has been achieved by stereotaxic injections of AAV9 in which Cav-1 expression is driven by the neuron-specific synapsin promoter (91). Cav-1 expression is reduced in the brain of type 2 diabetes patients and *db/db* (*Lep^{r^{db}}*) diabetic mice and corresponds with recognition memory deficits. Restoration of Cav-1 levels in the brains of male *db/db* mice using AAV-Cav-1 rescues learning and memory deficits and reduces APP, BACE-1, and p-tau levels in the brain (92). Recent clinical success of AAV-mediated gene therapy and the technological innovation have made therapeutic AAV drug development a reality, particularly for nervous system disorders where routine drug delivery routes have severe limitations (93).

At first, the therapeutic effect of Cav-1 overexpression seems to be at odds with the therapeutic effects of agents designed to reduce lipid rafts. However, as we discussed above, not all rafts are equal and the perception of lipid rafts as being “good” or “bad” and “deserving” to be up-regulated or disrupted depends on the physiological or pathological processes they support in a given cell type at a given time and metabolic circumstances, ranging from tissue repair to initiating inflammatory signaling, cancerous growth, or facilitating infection. In addition, different types of lipid rafts (flat rafts versus caveolae) likely support different cellular functions in different cells. In part, these differences are defined by the different proteins localized to flat lipid rafts and to caveolae. Flat rafts commonly host G_i , G_o , G_q , SRC and SYK kinase GRB2, ERK2, and GPI-anchored protein, whereas caveolae often contain G_q , SRC kinases, eNOS, PI3K, PKC, and uPAR (94). As evidenced from data collected in this article, caveolae seem to be mostly involved in homeostatic functions, whereas the flat rafts organize inflammatory and apoptotic signaling, although this division is not absolute.

TABLE 1. Experimental therapies targeting lipid rafts


Agent	Mechanism	Disease	References
AIBP	Targeting cholesterol efflux to inflammatory cells and reduction of inflamma rafts	Atherosclerosis, acute lung injury, neuropathic pain, cancer, HIV	(14, 29, 31–33, 97)
LXR agonists	Induction of ABCA1 and ABCG1 transcription	Angiogenesis, neurodegeneration, infection, inflammation, thrombogenesis	(3, 40–43)
ABC transporter overexpression	Induction of cholesterol efflux and cholesterol depletion; cytoskeleton rearrangement	Inflammation, cardiac fibrosis	(45, 46)
HDL and mimetics	Induction of cholesterol efflux and cholesterol depletion; stabilization of ABCA1	Inflammation, atherosclerosis, cancer, infection	(47, 49–58)
Cyclodextrins	Cholesterol sequestration	Niemann-Pick type C disease, cancer	(71, 72)
Statins	Cholesterol depletion	Atherosclerosis, infections, inflammation, cancer	(1, 60–62)
Sphingolipid inhibitors	Sphingolipid depletion and immune cell inactivation	Lupus erythematosus, steatohepatitis	(73, 75)
Dietary phospholipids	Enrichment with unsaturated fatty acids	Cancer, inflammation	(78, 80)
Edelfosine	Clustering death receptors in CASMERs	Cancer	(23, 24, 81)
miRNAs	Caveolin and flotillin scaffold depletion	Cancer	(89)
L-DV1	Liposomes with 3D molecular array of ligands targeting raft-associated CXCR4 dimers in tumor cells	Cancer	(84)
Caveolin gene therapy	Tissue repair	Traumatic brain injury, memory loss	(91, 92)

Rafts in targeted drug delivery

Finally, rafts may be utilized not only as a target for therapy, but as a target for drug delivery. Given the unique lipid and protein composition of lipid rafts and the presence of endocytic machinery, rafts can be exploited for targeted delivery of drugs even when they are not aimed at modulating rafts themselves [just like microbes do (95)]. This approach may allow targeting of drugs not only to a specific cell type or cells in a specific state, but potentially to deliver drugs to a specific intracellular compartment. Lipid-coated liquid perfluorocarbon nanoparticles complexed with $\alpha\beta3$ -integrin ligands are specifically targeted to lipid rafts in $\alpha\beta3$ -integrin-expressing melanoma cells followed by delivery of lipophilic substances to the target cell via intracellular trafficking through lipid raft-dependent processes without internalization of the nanoparticle itself (96).

CONCLUDING REMARKS

The central role of lipid rafts in the pathogenesis of a broad range of pathological conditions makes them an attractive therapeutic target. Lipid rafts are targeted by a number of experimental therapies in a broad range of diseases with various degrees of success (Table 1). Success of a therapeutic approach targeting lipid rafts, however, critically depends on the ability to distinguish between the physiological and pathological functions of rafts, preserving the former and altering the latter. Several therapeutic approaches seemingly achieved this selectivity, exploiting the spatial and temporal heterogeneity of lipid rafts in one cell type and/or compositional differences of lipid rafts in different cell types or cell states. Raft protein and lipid constituents have been successfully targeted to reduce or elevate raft abundance or to modify their structural and functional properties leading to modification of pathological pathways originating from lipid rafts and providing a significant

therapeutic benefit. Targeting distinctive sets of proteins and protein complexes in lipid rafts of cancer cells and activated myeloid cells are examples of how targeting “raft disrupting” therapy to pathological, but not physiological, lipid rafts can be done with sufficient selectivity. In a number of instances, however, the mechanistic basis of selectivity is yet to be established despite promising therapeutic outcomes, highlighting limitations in our understanding of lipid raft heterogeneity and regulation. Overall, the “Lipid Raft Therapy” has important hurdles to overcome before it becomes a mainstream therapeutic approach; but even now, it shows remarkable promise. 

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