

Review

Toshiyuki Murai*

Cholesterol lowering: role in cancer prevention and treatment

Abstract: The accumulation of cholesterol is a general feature of cancer tissue, and recent evidence suggests that cholesterol plays critical roles in the progression of cancers, including breast, prostate, and colorectal cancers. The dysregulation of metabolic pathways, including those involved in cholesterol biosynthesis, is implicated in tumor development and cancer progression. Lipid rafts are highly dynamic cholesterol-enriched domains of the cell membrane, involved in various cellular functions, including the regulation of transmembrane signaling at the cell surface. It was recently demonstrated that lipid rafts also play critical roles in cancer cell adhesion and migration. This review focuses on our current understanding of how cholesterol regulation, lipid rafts, and dysregulated cholesterol biosynthesis contribute to cancer development and progression, and the therapeutic potential of cholesterol lowering for cancer prevention and treatment.

Keywords: lipid raft; microdomain; membrane remodeling; metabolic reprogramming; tumor invasion; statin.

DOI 10.1515/hsz-2014-0194

Received May 1, 2014; accepted June 15, 2014; previously published online September 2, 2014

Introduction

Cancer is a major public health concern in developed countries worldwide (Siegel et al., 2013). Altered cell adhesion and enhanced migratory ability are the most prominent features of cancer cells; these properties affect the cell's ability to invade tissue and metastasize (Hanahan and Weinberg, 2011). Cholesterol is required for the assembly and maintenance of cell membranes and modulates membrane fluidity and function, including transmembrane

signaling and cell adhesion to the extracellular matrix. In mammals, cholesterol is a precursor for bile acid and steroid hormone synthesis and is either absorbed from dietary sources or synthesized *de novo* in the liver, from which it is transported by apolipoproteins to the systemic circulation. Under physiological conditions, the circulating levels of cholesterol are regulated by its rate of biosynthesis. More than a century ago, cholesterol was observed to accumulate in malignant tissues (White, 1909; Jowett, 1931; Yasuda and Bloor, 1932), and this is now regarded as a general feature of cancer cells (Freeman and Solomon, 2004). Accumulating evidence suggests that cholesterol plays a critical role in cancer progression. This review focuses on the current understanding of the mechanisms by which cholesterol contributes to cancer development and the therapeutic potential of cholesterol lowering in cancer prevention and treatment.

Cholesterol metabolism in cancer

Cholesterol biosynthesis in cancer cells

The maintenance of cholesterol homeostasis is a fundamental requirement for the normal growth of eukaryotic cells, and the metabolic pathway of cholesterol synthesis is highly conserved, from yeast to humans (Figure 1). Cholesterol synthesis begins with the conversion of citrate, derived from the tricarboxylic acid (TCA) cycle in the mitochondria, to acetyl-coenzyme A (acetyl-CoA), followed by a cascade of enzymatic reactions in the endoplasmic reticulum known as the mevalonate pathway, in which acetyl-CoA is converted to lanosterol. This series of reactions is primarily regulated by a rate-limiting step catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34), which converts HMG-CoA to mevalonate. Finally, lanosterol is converted to cholesterol via the Bloch pathway or the Kandutsch-Russell pathway (Figure 1).

Various metabolic pathways have been implicated in the multistep development of tumors, and a metabolic

*Corresponding author: Toshiyuki Murai, Department of Microbiology and Immunology, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan, e-mail: murai@orgctl.med.osaka-u.ac.jp

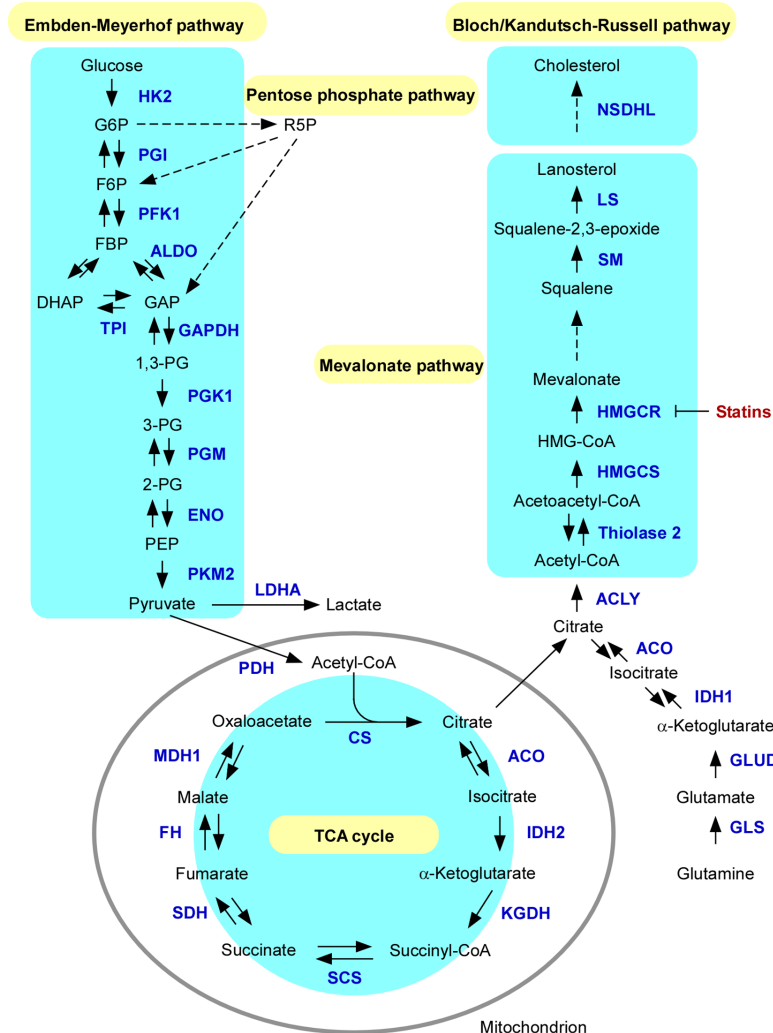


Figure 1 Metabolic pathways for cholesterol biosynthesis in cancer cells.

G6P, glucose 6-phosphate; R5P, ribose 5-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; 1,3-PG, 1,3-bisphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; HK, hexokinase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; ALDO, aldolase; TPI, triosephosphate isomerase; GAPDH, GAP dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENO, enolase; PKM2, pyruvate kinase, muscle, M2 isoform; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; CS, citrate synthase; ACO, aconitase; IDH, isocitrate dehydrogenase; KGDH, α-ketoglutarate dehydrogenase; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; FH, fumarate; MDH, malate dehydrogenase; GLS, glutaminase; GLUD, glutamate dehydrogenase; ACLY, ATP citrate lyase; HMGCS, HMG-CoA synthase; HMGCR, HMG-CoA reductase; SM, squalene monooxygenase; LS, lanosterol synthase; NSDHL, NAD(P)H-dependent steroid dehydrogenase-like protein; TCA, tricarboxylic acid.

shift from catabolic to anabolic metabolism is a classic hallmark of cancer cells (Hanahan and Weinberg 2011). This metabolic reprogramming of cancer cells – resulting in increased glucose uptake and conversion to lactate by glycolysis – is known as the Warburg effect (Warburg et al., 1927; Warburg, 1956), and leads to the acidification of the tumor environment (Parks et al., 2011). Glucose is normally metabolized through the glycolytic pathway into two molecules of pyruvate, which are then converted to acetyl-CoA by a complex of pyruvate dehydrogenase

(PDH) in the mitochondria. Acetyl-CoA then enters the TCA cycle, resulting in the generation of substrates for oxidative phosphorylation. Cancer cells, in contrast, consume an excessive quantity of glucose, metabolizing it via both catabolic and anabolic pathways, resulting in the synthesis of large amounts of precursors for macromolecule biosynthesis; thus, these cells have highly proliferative phenotypes. An isozyme of the first enzyme in the glycolytic pathway, hexokinase 2 (HK2), is highly expressed in certain cancer cells and is thought to support anabolic

metabolism, including prostate cancer cells (Mathupala et al., 2006). The product of hexokinase catalysis, glucose 6-phosphate (G6P), undergoes one of two fates: glycolysis via the Embden-Meyerhof (EM) pathway or metabolism via the pentose phosphate pathway (PPP). The PPP is indispensable for NADPH homeostasis, and plays critical roles in highly proliferating cells such as cancer cells. In these cells, G6P is shunted through the PPP, producing NADPH, which is required for cholesterol biosynthesis. PPP intermediates can also re-enter the glycolytic pathway to produce pyruvate, which may also be utilized for cholesterol biosynthesis (Vander Heiden et al., 2010). In addition, cancer cells exhibit increased glutamine uptake and glutaminolysis, which replenish TCA cycle metabolites (DeBerardinis et al., 2007). Tumor hypoxia prevents pyruvate entry into the TCA cycle, and mitochondrial oxidative metabolism may be impaired in cancer cells as a result of mutations in enzymes involved in the TCA cycle or electron transport chain. Under these conditions, reductive carboxylation supports cell growth through the activity of a cytosolic isozyme of isocitrate dehydrogenase, IDH1, which catalyzes the first step in the production of acetyl-CoA from glutamine-derived α -ketoglutarate (Mullen et al., 2012) (Figure 1).

In addition to the shift in glucose and glutamine metabolism, increased lipid synthesis is also recognized as a common feature of the metabolic reprogramming in cancer cells (Hirsch et al., 2010). Many cancer cell types exhibit a reactivation of *de novo* fatty acid synthesis (Menendez and Lupu, 2007) and the enhanced expression of enzymes of the mevalonate pathway (Freed-Pastor et al., 2012). Most of the *de novo* synthesized fatty acids and cholesterol are derived from glucose, but in certain cancer cells they are synthesized from glutamine. Cholesterol synthesis is tightly regulated in normal cells, but dysregulated cholesterol synthesis and sterol-dependent proliferation are frequently found in various cancer cell types, and may lead to cancer progression (Hirsch et al., 2010). In addition, proliferating cancer cells exhibit increased HMG-CoA reductase and low-density lipoprotein (LDL) receptor activities, resulting in increased cholesterol levels and higher cholesterol consumption compared to normal proliferating cells (Ho et al., 1978; Azrolan and Coleman 1989).

Cancer-related regulators of cholesterol metabolism

The expression of HMG-CoA reductase is transcriptionally regulated by the sterol response element-binding proteins

(SREBPs). The SREBPs, which belong to the leucine zipper family of transcription factors, consist of three isoforms: SREBP-1a, -1c, and -2, and their regulation has been explored and recently reviewed (Brown and Goldstein, 2009). The upregulation of SREBPs in cancer cells leads to the activation of genes controlling lipid synthesis, such as HMG-CoA reductase, which is known to be increased in tumors (Azrolan and Coleman 1989). The tumor suppressor p53 maintains mitochondrial activity by regulating the expression of cytochrome c oxidase 2, and a loss of p53 causes a metabolic switch to glycolysis, providing an explanation for the Warburg effect (Matoba et al., 2006). P53 also serves as a co-activator of SREBPs (Freed-Pastor et al., 2012), potentially contributing to the regulation of cholesterol synthesis.

In addition to the classic transcriptional regulation of cholesterol metabolism by SREBPs, a class of non-coding RNAs termed microRNAs (miRNAs) has been identified as potent post-transcriptional regulators of genes involved in cholesterol metabolism (Moore et al., 2010). Recently, a number of miRNAs were found to be aberrantly expressed in cancer cells, and to play a role in regulating cancer progression (Huang and He, 2011). An intronic miRNA located within the gene encoding sterol-regulatory element-binding factor-2 (SREBF-2), miR-33, regulates cholesterol metabolism in concert with SREBPs, contributing to tumor growth (Rayner et al., 2010; Ibrahim et al., 2011), suggesting a therapeutic potential for miRNA modulation.

HMG-CoA reductase acts very early in the cholesterol biosynthesis pathway, during the third step in the mevalonate pathway, with over 20 subsequent enzymes required to produce cholesterol. How these later enzymes are regulated is largely unexplored, but there is growing evidence that some of them serve as flux-controlling points (Sharpe and Brown, 2013). In renal cancer cells, for example, another enzyme of the mevalonate pathway, squalene monooxygenase (SM), which converts squalene to squalene-2,3-epoxide (Figure 1), has been proposed as a second rate-limiting enzyme in cholesterol synthesis, and may contribute to renal cancer development (Gonzalez et al., 1979). In addition, in a breast cancer cell model of metastasis, an enzyme in the Kandutsch-Russell pathway, NAD(P)H-dependent steroid dehydrogenase-like protein (NSDHL) was found to translocate to the plasma membrane as its expression increases (Xue et al., 2013). This translocation may contribute to the signal transduction involved in cancer metastasis by facilitating the formation of lipid rafts, which are cholesterol-enriched cell-membrane domains.

Cholesterol lowering and cancer

Epidemiological studies originally revealed a negative correlation between serum cholesterol levels and the risk of certain type of cancers (Kritchevsky and Kritchevsky, 1992), although this may be an effect rather than a cause of cancer, and it is difficult to draw conclusions from epidemiological studies because of their intrinsic limitations. More recently, however, low plasma cholesterol levels were inversely correlated with the risk of prostate cancer (Pelton et al., 2012), and statin-induced cholesterol lowering was positively associated with a decrease in cancer incidence (Farwell et al., 2008). In addition, a metabolite of cholesterol contributes to breast cancer growth (Kiberstis, 2013). These findings support the notion that cholesterol lowering may be a useful strategy for cancer prevention and treatment.

Breast cancer

Breast cancer is the most commonly diagnosed malignancy in women (Siegel et al., 2013). Dietary cholesterol intake is positively associated with the risk of breast cancer (Hu et al., 2012), and oxysterol 27-hydroxycholesterol (27HC), a primary metabolite of cholesterol (Figure 2A), increases tumor growth and metastasis in mouse models of breast cancer (Nelson et al., 2013). Furthermore, cholesterol is a precursor of estrogen, and high estrogen levels are associated with an increased risk of breast cancer (Yager and Davidson, 2006) (Figure 2A). Thus, the lowering of circulating cholesterol levels may be a useful strategy for breast cancer prevention or treatment.

Prostate cancer

Prostate cancer is the most commonly diagnosed cancer among men, and a leading cause of death in Western countries (Siegel et al., 2013). The increased cholesterol content of benign prostatic hyperplasia was reported 70 years ago (Swyer, 1942), and evidence of a role for cholesterol in prostate cancer has increased over the past decade (Pelton et al., 2012). In prostate cancer cells, fatty acid and cholesterol synthesis pathways are highly upregulated (Rossi et al., 2003; Platz et al., 2008). In SCID mice injected with prostate cancer cells, an elevation in circulating cholesterol increases the cholesterol content of lipid rafts, alters raft-mediated downstream signaling, and promotes tumor growth (Zhuang et al., 2005). These findings suggest that novel molecules involved in cholesterol-dependent

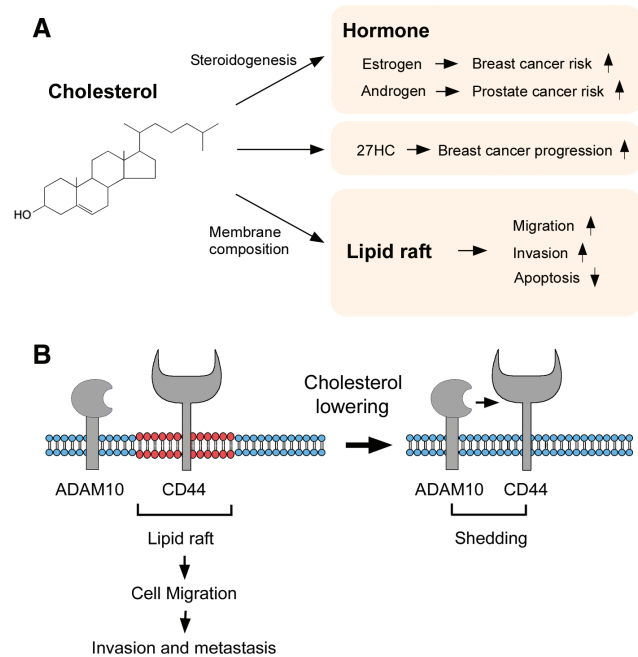


Figure 2 The roles of cholesterol in cancer.

(A) Potential roles of cholesterol in cancer. Estrogen and androgen affects breast and prostate cancer risk, and these hormones are produced by steroidogenesis, in which cholesterol is the precursor. A primary metabolite of cholesterol, 27-hydroxycholesterol (27HC), also increases tumor growth and metastasis in mouse models of breast cancer. Cholesterol is a major component of lipid rafts that regulate cancer cell migration, invasion, and apoptosis. (B) Proposed model for the mechanism underlying cancer cell migration, invasion, and metastasis regulated by cholesterol-rich membrane domains.

tumor progression may be identified in the lipid rafts. The mevalonate pathway, which promotes cholesterol accumulation, is highly upregulated in prostate cancers, and epidemiological studies have been performed to examine the association between high serum cholesterol and an increased risk of prostate cancer (Pelton et al., 2012). Treating prostate cancer cells with simvastatin, an inhibitor of cholesterol synthesis, lowers the cholesterol content of the plasma membrane and inhibits raft-mediated signaling related to cancer progression (Zhuang et al., 2005), while the long-term administration of statins reduces the risk of human prostate cancer (Solomon and Freeman, 2008).

Prostate cancer cells proliferate in response to androgen via the nuclear androgen receptor, and androgen is produced by steroidogenesis, in which cholesterol is the precursor (Figure 2A). Recent studies demonstrated that prostate cancer cells produce androgen by intratumoral steroidogenesis, leading to their enhanced proliferation (Locke et al., 2008). Thus, cholesterol may play multiple roles in promoting prostate cancer, supporting the

potential use of cholesterol-reducing treatments for this disease.

Colorectal cancer

Colorectal cancer is the second leading cause of cancer death in the world (Siegel et al., 2013). Colorectal cancer is a distinct type of genetic disease in which not one, but several mutations are required, and the majority of colorectal cancer occurs without identifiable genetic risk factors, suggesting that environmental factors are critical in the development of this disease (Vogelstein and Kinzler, 1993). One of the major environmental factors that affect the colorectal cancer risk is dietary fat, and high levels of serum cholesterol are associated with an increased risk of colorectal cancer (Holtzman et al., 1987). In contrast, the long-term use of statins is associated with a significantly reduced relative risk of colorectal cancer (Poynter et al., 2005). Bile acids are synthesized from cholesterol mostly in the liver, and an increased incidence of colorectal cancer is associated with high levels of bile acids. Thus, bile acids may function as cancer promoters in the colon (Bernstein et al., 2005).

Colitis-associated cancer is the type of colorectal cancer which is preceded by inflammatory bowel disease (IBD) including ulcerative colitis (UC), and the risk of colorectal cancer for any patient with UC is estimated to be 18% after 30 years of disease (Grivennikov, 2013). The disruption of intestinal epithelial lipid rafts is a feature of inflammatory conditions in IBD, suggesting the possibility that the role of cholesterol relates to the chronic inflammation step toward cancer initiation (Bowie et al., 2012).

Lipid rafts and cancer

Structure and function of lipid rafts

Lipid rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched membrane domains that compartmentalize certain cellular processes (Pike, 2006). The traditional model of cellular membrane structure put forward by Singer and Nicolson, known as the “fluid mosaic model,” proposed that globular proteins float in a lipid bilayer with an amphipathic structure (Singer and Nicolson, 1972). The model was later improved by Simons and van Meer, who suggested the existence of microdomains, or “lipid rafts” in the plasma membrane of epithelial cells (Simons and van Meer, 1988). In the

current understanding of the lipid raft model, cholesterol- and sphingolipid-enriched microdomains of the plasma membrane assume a biophysical state comparable to a liquid-ordered (L_o) phase floating within a liquid-disordered (L_d) membrane phase (Simons and Ikonen, 1997). A major subclass of lipid rafts is the caveolae, flask-shaped plasma membrane invaginations characterized by an abundance of caveolin (Parton et al., 2006). Lipid rafts consist of assemblies of cholesterol, sphingolipids (including sphingomyelin and gangliosides), and certain types of proteins (Simons and Ikonen, 1997). Sphingolipids contain saturated fatty acyl chains in their structure, which allow cholesterol to be tightly intercalated into sphingolipid assemblies to form L_o microdomains. The most important properties of lipid rafts are that they are small, dynamic, and heterogeneous, and can selectively recruit certain classes of proteins (Resh, 1999; Simons and Toomre, 2000). Proteins with raft affinity include glycosylphosphatidylinositol-anchored proteins, palmitoylated proteins, doubly acylated proteins, such as Src family kinases (SFKs), and transmembrane proteins such as CD44 (Brown and London, 2000; Fessler and Parks, 2011). Lipid rafts are implicated in various physiological cellular processes, such as protein membrane trafficking and signal transduction (Simons and Toomre, 2000; Hanzal-Bayer and Hancock, 2007).

Cholesterol depletion is often used as a method for investigating the role of lipid rafts *in vitro*, although these studies are limited by nonspecific effects that can be caused by severe cholesterol depletion. The results of these studies suggest that cholesterol is a crucial component of cell membranes and contributes to the organization of lipid rafts. In particular, cholesterol contributes to the organization of lipid rafts that contain a large number of cancer-related signaling and adhesion molecules. Table 1 summarizes the cancer-related proteins that were found to be associated with lipid rafts for example in an unbiased proteomics analysis of these structures (Foster et al., 2003; Baruthio et al., 2008).

Lipid rafts and cancer cell migration and invasion

There is growing interest in targeting lipid rafts for cancer prevention and treatment, because of their role in regulating various steps of cancer progression, including cancer cell migration and invasion (Murai, 2012). Cell adhesion is a key factor associated with the metastatic spread of cancer cells, and regulating this process is critical for the therapeutic intervention of cancer. CD44 is a major cell

Table 1 Cancer-related proteins associated with lipid rafts.

Protein	Function	References
Raft markers		
Flotillin-1	Breast cancer grade	Lin et al., 2011
Flotillin-2	Breast cancer metastasis	Berger et al., 2013
Flotillin-2	Melanoma progression	Hazarika et al., 2004
Caveolin-1	Tumor promoter	Williams and Lisanti, 2005
Cell adhesion molecules		
CD44	Tumor cell migration	Murai et al., 2011
N-cadherin	Cell-cell junction	Causeret et al., 2005
β1 Integrin	Lipid-raft signaling	Wary et al., 1998
Oncogenes		
H-Ras	Growth signaling	Roy et al., 1999
Src	Transformation	Oneyama et al., 2008
Csk	Transformation	Oneyama et al., 2008
Cbp	Transformation	Oneyama et al., 2008
Fyn	Integrin signaling	Wary et al., 1998
Lyn	Lipid-raft signaling	Young et al., 2003
Lck	Leukemia invasion	Jiang et al., 2008
Receptors		
EGFR	Growth factor signaling	Waugh et al., 1999
uPAR	Apoptosis	Chetty et al., 2010
Apoptotic molecules		
Fas	Apoptosis	Gajate and Mollinedo, 2001
TRAIL	Apoptosis	Gajate et al., 2004
FADD	Apoptosis	Gajate and Mollinedo, 2007
Caspase-8	Apoptosis	Gajate and Mollinedo, 2007
Bid	Apoptosis	Gajate and Mollinedo, 2007
JNK	Apoptosis	Nieto-Miguel et al., 2008

EGFR, epidermal growth factor receptor; uPAR, urokinase-type plasminogen activator receptor; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand; FADD, Fas-associated death domain protein; Bid, BH3-interacting domain death agonist; JNK, *c-jun* N-terminal kinase.

adhesion receptor expressed in cancer cells and implicated in cancer cell migration, invasion, and metastasis (Lesley et al., 1993). However, despite numerous reports demonstrating that CD44 is present in lipid rafts (Murai, 2012), the role of lipid rafts in cancer cell adhesion and migration has not been elucidated.

It was recently demonstrated that lipid rafts play a crucial role in the localization and functionality of CD44 (Murai et al., 2011) (Figure 2B). Treating human glioma cells with the lipid-raft-disrupting agent methyl-β-cyclodextrin (MβCD), which extracts cellular membrane cholesterol, results in increased CD44 shedding mediated by a disintegrin and metalloproteinase 10 (ADAM10) (Murai et al., 2011). Moreover, the CD44 shedding induced by cholesterol lowering is not limited to glioma cells, but is also seen in other tumor cells such as pancreatic cancer cells (Murai et al., 2011). CD44 shedding can also be induced by filipin, a polyene macrolide antibiotic that binds cholesterol and disperses it in the membrane, thereby disrupting lipid rafts by a different mechanism from MβCD (Murai et al., 2011). The cholesterol-lowering clinical medicine

simvastatin also enhances CD44 shedding, and concomitantly blocks the stimulation of glioma cell migration by hyaluronan oligosaccharides or epidermal growth factor (EGF) (Sugahara et al., 2003; Murai et al., 2004, 2006, 2009). Taken together, these results suggest that cholesterol lowering results in disordered CD44 localization, raft-dependent CD44 shedding, and the suppression of tumor cell migration (Adler, 2011). Cholesterol lowering also enhances the CD44-mediated adhesion of lymphocytes, suggesting that lipid rafts regulate lymphocyte interactions under physiological flow conditions (Murai et al., 2013a,b). The lipid raft affiliation of CD44 is likely to occur through its palmitoylation, which may affect malignancy of breast cancer (Babina et al., 2014).

Studies on other membrane proteins in cancer cells revealed that depleting the cholesterol in cell membranes also triggers the shedding of L1-cell adhesion molecule (L1-CAM) (Mechtersheimer et al., 2001), amyloid precursor protein (APP) (Kojro et al., 2001), interleukin-6 (IL-6) receptor (Matthews et al., 2003), CD30 (von Tresckow et al., 2004), and lipoprotein receptor-related protein-1

(LRP-1) (Selvais et al., 2011) (Table 2). These proteins are shed by the action of ADAM family proteinases, triggered by cholesterol lowering with M β CD or other lipid-raft disrupting agents, such as filipin or statins. In addition, type XVII and type XXIII collagens are shed from keratinocytes upon treatment with M β CD or filipin (Zimina et al., 2005; Veit et al., 2007). These findings indicate that lipid rafts may play a critical role in regulating the accessibility of sheddases to their substrate proteins under constitutive and induced conditions.

Lipid rafts and apoptosis

In addition to increased proliferation and invasion, reduced apoptosis is another hallmark of cancer cells, and contributes to the drug resistance and poor prognosis of cancer treatments (Hanahan and Weinberg, 2011). Lipid rafts are reported to serve as platforms for the accumulation of signaling molecules involved in cancer cell apoptosis. An anti-tumor agent, edelfosine (1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine), was found to induce the translocation of the death receptor Fas/CD95 into lipid rafts, followed by Fas/CD95 co-clustering and activation, leading to tumor cell death (Gajate and Mollinedo, 2001). The edelfosine-induced co-clustering of Fas/CD95 in lipid rafts was later shown to be independent of its cognate ligand (Gajate et al., 2004). In the intrinsic apoptosis pathway, the functions of the Fas receptor and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors 1 and 2 were also found to depend on their translocation into lipid rafts, as the depletion of membrane cholesterol attenuated the apoptosis mediated by these factors (Gajate et al., 2004). Subsequent studies revealed that other apoptosis-related signaling molecules, such as Fas-associated death domain protein (FADD), caspase-8, BH3-interacting domain death agonist (Bid),

and *c-jun* N-terminal kinase (JNK) are also translocated to lipid rafts upon treatment with anti-tumor agents (Gajate and Mollinedo, 2007; Nieto-Miguel et al., 2008; Mollinedo et al., 2010a,b) (Table 1). In addition, certain cancer cell types with higher membrane cholesterol levels are more sensitive to the cholesterol-depletion-induced apoptosis, suggesting that apoptosis is another target in the therapeutic application of cholesterol lowering for cancer (Li et al., 2006).

Cancer prevention and therapy

Statins

Statins are HMG-CoA reductase inhibitors with cholesterol-lowering properties. Statins inhibit the conversion of HMG-CoA to mevalonic acid by serving as structural analogues of HMG-CoA. Several different compounds in this drug class have been developed: atorvastatin (Lipitor), cerivastatin (Baycol; withdrawn from the market in 2001), fluvastatin (Lescol), lovastatin (Mevacor), mevastatin (Compactin), pravastatin (Pravachol), rosuvastatin (Crestor), and simvastatin (Zocor). Statins lower cholesterol content and are thus widely prescribed for the treatment of cardiovascular diseases. Furthermore, prevention studies in which high-risk individuals were treated with statins confirmed the efficacy of these drugs in preventing cardiovascular diseases (Collins et al., 2002). In addition, a retrospective study demonstrated that statins may be effective for preventing neurodegenerative diseases, including Alzheimer's disease (Jick et al., 2000). Although the various population-based reports of the effects of statins on cancer are controversial, recent epidemiologic studies suggest that statins inhibit the progression of certain cancers (Solomon and Freeman, 2008).

Table 2 Transmembrane proteins that undergo shedding upon cholesterol depletion.

Protein	Cell type	Reagent	Sheddases	References
CD44	Glioblastoma, pancreatic carcinoma	M β CD, simvastatin, filipin	ADAM10	Murai et al., 2011
L1-CAM	Breast carcinoma	M β CD	ADAM10	Mechtersheimer et al., 2001
APP	Astrocytoma	M β CD, lovastatin	ADAM10	Kojro et al., 2001
IL-6 receptor	AMoL, hepatoma	M β CD	ADAM 10, 17	Matthews et al., 2003
CD30	Lymphoma	M β CD, lovastatin, filipin	ADAM17	von Tresckow et al., 2004
LRP-1	Fibrosarcoma	M β CD, lovastatin	ADAM 12, MT1-MMP	Selvais et al., 2011
Collagen XVII	Keratinocyte	M β CD, filipin	ADAM17	Zimina et al., 2005
Collagen XXIII	Keratinocyte	M β CD, filipin	Furin	Veit et al., 2007

L1-CAM, L1-cell adhesion molecule; APP, amyloid precursor protein; IL-6, interleukin-6; AMoL, acute monocytic leukemia; LRP-1, lipoprotein receptor-related protein-1; MT1-MMP, membrane-type 1 matrix metalloproteinase.

Recent evidence suggests that statins block the adhesion and migration processes of cancer cells by disrupting membrane lipid rafts (Murai, 2012), supporting the potential therapeutic use of cholesterol lowering for suppressing various pathogenic features of cancer cells. In addition, dysregulation of the mevalonate pathway promotes transformation, suggesting that HMG-CoA reductase is a candidate metabolic oncogene, and providing further rationale for the exploration of statins as anticancer agents (Clendening et al., 2010).

Non-statin cholesterol-lowering agents

Although statins are effective cholesterol-lowering agents, they elicit many side effects. In addition to the statin target enzyme HMG-CoA reductase, many other enzymes are involved in the multistep pathway leading to cholesterol synthesis. Inhibitors that target enzymes required for the conversion of lanosterol to cholesterol may also be effective for lowering cholesterol (Trapani et al., 2011). In addition, another class of compounds that prevent cholesterol absorption from dietary intake may also be useful cholesterol-lowering agents with anticancer activity. For example, ezetimibe (Zetia), a cholesterol uptake-blocking drug, decreases circulating cholesterol levels and reduces the growth of prostate tumors by inhibiting tumor angiogenesis (Solomon et al., 2009).

Conclusion

Here I have summarized the roles of cholesterol and its lowering on cancer cell adhesion and migration, focusing on the current state of knowledge, with particular emphasis on lipid rafts. Although a number of recent studies have linked these membrane structures to the pathogenesis of cancer cells, many questions about the nature of lipid rafts remain unanswered. Future studies will be required to elucidate the mechanism(s) underlying the lipid-raft-mediated regulation of cancer cell adhesion and migration. These investigations and other studies of cholesterol regulation and metabolism will provide new insights into the mechanisms of cancer pathogenesis and a wealth of new targets for cancer treatment and prevention.

Acknowledgments: This work was supported in part by Grants-in-Aid for Scientific Research, Category (C) and Grants-in-Aid for Scientific Research on Priority Areas, System Cell Engineering by Multi-scale Manipulation,

from the Ministry of Education, Culture, Sports, Science and Technology of Japan, All Japan Coffee Association, and Kieikai Research Foundation.

References

- Adler, E.M. (2011). Lost without a raft. *Sci. Signal.* 4, ec24.
- Azrolan, N.I. and Coleman, P.S. (1989). A discoordinate increase in the cellular amount of 3-hydroxy-3-methylglutaryl-CoA reductase results in the loss of rate-limiting control over cholesterol synthesis in a tumour cell-free system. *Biochem. J.* 258, 421–425.
- Babina, I.S., McSherry, E.A., Donatello, S., Hill, A.D., and Hopkins, A.M. (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.
- Baruthio, F., Quadroni, M., Rüegg, C., and Mariotti, A. (2008). Proteomic analysis of membrane rafts of melanoma cells identifies protein patterns characteristic of the tumor progression stage. *Proteomics* 8, 4733–4747.
- Berger, T., Ueda, T., Arpaia, E., Chio, I.I.C., Shirdel, E.A., Jurisica, I., Hamada, K., You-Ten, A., Haight, J., Wakeham, A., et al. (2013). Flotillin-2 deficiency leads to reduced lung metastases in a mouse breast cancer model. *Oncogene* 32, 4989–4994.
- Bernstein, H., Bernstein, C., Payne, C.M., Dvorakova, K., and Garewal, H. (2005). Bile acids as carcinogens in human gastrointestinal cancers. *Mutat. Res.* 589, 47–65.
- Bowie, R.V., Donatello, S., Lyes, C., Owens, M.B., Babina, I.S., Hudson, L., Walsh, S.V., O'Donoghue, D.P., Amu, S., Barry, S.P., et al. (2012). Lipid rafts are disrupted in mildly inflamed intestinal microenvironments without overt disruption of the epithelial barrier. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302, G781–793.
- Brown, M.S. and Goldstein, J.L. (2009). Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J. Lipid Res.* 50, S15–S27.
- Brown, D.A. and London, E. (2000). Structure and function of sphingolipid- and cholesterol-rich membrane rafts. *J. Biol. Chem.* 275, 17221–17224.
- Causeret, M., Taulet, N., Comunale, F., Favard, C., and Gauthier-Rouvière, C. (2005). N-cadherin association with lipid rafts regulates its dynamic assembly at cell-cell junctions in C2C12 myoblasts. *Mol. Biol. Cell* 16, 2168–2180.
- Chetty, C., Lakka, S.S., Bhoopathi, P., Gond, C.S., Veeravalli, K.K., Fassett, D., Klopfenstein, J.D., Dinh, D.H., Gujrati, M., and Rao, J.S. (2010). Urokinase plasminogen activator receptor and/or matrix metalloproteinase-9 inhibition induces apoptosis signaling through lipid rafts in glioblastoma xenograft cells. *Mol. Cancer Ther.* 9, 2605–2617.
- Clendening, J.W., Pandya, A., Boutros, P.C., El Ghamrasni, S., Khosravi, F., Trentin, G.A., Martirosyan, A., Hakem, A., Hakem, R., Jurisica, I., et al. (2010). Dysregulation of the mevalonate pathway promotes transformation. *Proc. Natl. Acad. Sci. USA* 107, 15051–15056.
- Collins, R., Armitage, J., Parish, S., Sleight, P., and Peto, R.; Heart Protection Study Collaborative Group (2002). MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in

- 20536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 360, 7–22.
- DeBerardinis R.J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., and Thompson, C.B. (2007). Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc. Natl. Acad. Sci. USA* 104, 19345–19350.
- Farwell, W.R., Scranton, R.E., Lawler, E.V., Lew, R.A., Brophy, M.T., Fiore, L.D., and Gaziano, J.M. (2008). The association between statins and cancer incidence in a veterans population. *J. Natl. Cancer Inst.* 100, 134–139.
- Fessler, M.B. and Parks, J.S. (2011). Intracellular lipid flux and membrane microdomains as organizing principles in inflammatory cell signaling. *J. Immunol.* 187, 1529–1535.
- Foster, L.J., de Hoog, C.L., and Mann, M. (2003). Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc. Natl. Acad. Sci. USA* 100, 5813–5818.
- Freed-Pastor, W.A., Mizuno, H., Zhao, X., Langerød, A., Moon, S.H., Rodriguez-Barrueco, R., Barsotti, A., Chicas, A., Li, W., Polotskaia, A., et al. (2012). Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell* 148, 244–258.
- Freeman, M.R. and Solomon, K.R. (2004). Cholesterol and prostate cancer. *J. Cell. Biochem.* 91, 54–69.
- Gajate, C. and Mollinedo, F. (2001). The antitumor ether lipid ET-18- OCH_3 induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells. *Blood* 98, 3860–3863.
- Gajate, C. and Mollinedo, F. (2007). Edelfosine and perifosine induce selective apoptosis in multiple myeloma by recruitment of death receptors and downstream signaling molecules into lipid rafts. *Blood* 109, 711–719.
- Gajate, C., del Canto-Jañez, E., Acuña, A.U., Amat-Guerri, F., Geijo, E., Santos-Beneit, A.M., Veldman, R.J., and Mollinedo, F. (2004). Intracellular triggering of Fas aggregation and recruitment of apoptotic molecules into Fas-enriched rafts in selective tumor cell apoptosis. *J. Exp. Med.* 200, 353–365.
- Gonzalez, R., Carlson, J.P., and Dempsey, M.E. (1979). Two major regulatory steps in cholesterol synthesis by human renal cancer cells. *Arch. Biochem. Biophys.* 196, 574–580.
- Grivennikov, S.I. (2013). Inflammation and colorectal cancer: colitis-associated neoplasia. *Semin. Immunopathol.* 35, 229–244.
- Hanahan, D. and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674.
- Hanzal-Bayer, M.F. and Hancock, J.F. (2007). Lipid rafts and membrane traffic. *FEBS Lett.* 581, 2098–2104.
- Hazarika, P., McCarty, M.F., Prieto, V.G., George, S., Babu, D., Koul, D., Bar-Eli, M., and Duvic, M. (2004). Up-regulation of Flotillin-2 is associated with melanoma progression and modulates expression of the thrombin receptor protease activated receptor 1. *Cancer Res.* 64, 7361–7369.
- Hirsch, H.A., Iliopoulos, D., Joshi, A., Zhang, Y., Jaeger, S.A., Bulyk, M., Tschlis, P.N., Liu, S., and Struhl, K. (2010). A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. *Cancer Cell* 17, 348–361.
- Ho, Y.K., Smith, R.G., Brown, M.S., and Goldstein, J.L. (1978). Low-density lipoprotein (LDL) receptor activity in human acute myelogenous leukemia cells. *Blood* 52, 1099–1114.
- Holtzman, E.J., Yaari, S., and Goldbourt, U. (1987). Serum cholesterol and the risk of colorectal cancer. *N. Engl. J. Med.* 317, 114.
- Hu, J., La Vecchia, C., de Groh, M., Negri, E., Morrison, H., and Mery, L.; Canadian Cancer Registries Epidemiology Research Group (2012). Dietary cholesterol intake and cancer. *Ann. Oncol.* 23, 491–500.
- Huang, S. and He, X. (2011). The role of microRNAs in liver cancer progression. *Br. J. Cancer* 104, 235–240.
- Ibrahim, A.F., Weirauch, U., Thomas, M., Grünweller, A., Hartmann, R.K., and Aigner, A. (2011). MicroRNA replacement therapy for miR-145 and miR-33a is efficacious in a model of colon carcinoma. *Cancer Res.* 71, 5214–5224.
- Jiang, G., Freywald, T., Webster, J., Kozan, D., Geyer, R., DeCoteau, J., Narendran, A., and Freywald A. (2008). In human leukemia cells ephrin-B-induced invasive activity is supported by Lck and is associated with reassembling of lipid raft signaling complexes. *Mol. Cancer Res.* 6, 291–305.
- Jick, H., Zornberg, G.L., Jick, S.S., Seshadri, S., and Drachman, D.A. (2000). Statins and the risk of dementia. *Lancet* 356, 1627–1631.
- Jowett, M. (1931). The phosphatide and cholesterol contents of normal and malignant human tissues. *Biochem. J.* 25, 1991–1998.
- Kiberstis, P.A. (2013). Cholesterol and cancer. *Sci. Signal.* 6, ec292.
- Kojro, E., Gimpl, G., Lammich, S., März, W., and Fahrenholz, F. (2001). *Proc. Natl. Acad. Sci. USA* 98, 5815–5820.
- Kritchevsky, S.B. and Kritchevsky, D. (1992). Serum cholesterol and cancer risk: an epidemiologic perspective. *Annu. Rev. Nutr.* 12, 391–416.
- Lesley, J., Hyman, R., and Kincade, P.W. (1993). CD44 and its interaction with extracellular matrix. *Adv. Immunol.* 54, 271–335.
- Li, Y.C., Park, M.J., Ye, S.K., Kim, C.W., and Kim, Y.N. (2006). Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. *Am. J. Pathol.* 168, 1107–1118.
- Lin, C., Wu, Z., Lin, X., Yu, C., Shi, T., Zeng, Y., Wang, X., Li, J., and Song, L. (2011). Knockdown of FLOT1 impairs cell proliferation and tumorigenicity in breast cancer through upregulation of FOXO3a. *Clin. Cancer Res.* 17, 3089–3099.
- Locke, J.A., Guns, E.S., Lubik, A.A., Adomat, H.H., Hendy, S.C., Wood, C.A., Ettinger, S.L., Gleave, M.E., and Nelson, C.C. (2008). Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res.* 68, 6407–6415.
- Mathupala, S.P., Ko, Y.H., and Pedersen, P.L. (2006). Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* 25, 4777–4786.
- Matthews, V., Schuster, B., Schütze, S., Bussmeyer, I., Ludwig, A., Hundhausen, C., Sadowski, T., Saftig, P., Hartmann, D., Kallen, K.J., et al. (2003). *J. Biol. Chem.* 278, 38829–38839.
- Matoba, S., Kang, J.G., Patino, W.D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P.J., Bunz, F., and Hwang, P.M. (2006). p53 regulates mitochondrial respiration. *Science* 312, 1650–1653.
- Mechtersheimer, S., Gutwein, P., Agmon-Levin, N., Stoeck, A., Oleszewski, M., Riedle, S., Postina, R., Fahrenholz, F., Fogel, M., Lemmon, V., et al. (2001). Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J. Cell Biol.* 155, 661–673.

- Menendez, J.A. and Lupu, R. (2007). Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat. Rev. Cancer* 7, 763–777.
- Mollinedo, F., de la Iglesia-Vicente, J., Gajate, C., Estella-Hermoso de Mendoza, A., Villa-Pulgarin, J.A., Campanero, M.A., and Blanco-Prieto, M.J. (2010a). Lipid raft-targeted therapy in multiple myeloma. *Oncogene* 29, 3748–3757.
- Mollinedo, F., de la Iglesia-Vicente, J., Gajate, C., Estella-Hermoso de Mendoza, A., Villa-Pulgarin, J.A., de Frias, M., Roué, G., Gil, J., Colomer, D., Campanero, M.A., et al. (2010b). 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.
- Moore, K.J., Rayner, K.J., Suárez, Y., and Fandernández-Hernando, C. (2010). microRNAs and cholesterol metabolism. *Trends Endocrinol. Metab.* 21, 699–706.
- Mullen, A.R., Wheaton, W.W., Jin, E.S., Chen, P.H., Sullivan, L.B., Cheng, T., Yang, Y., Linehan, W.M., Chandel, N.S., and DeBerardinis, R.J. (2012). Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature* 481, 385–388.
- Murai, T. (2012). The role of lipid rafts in cancer cell adhesion and migration. *Int. J. Cell Biol.* 2012, 763283.
- Murai, T., Miyazaki, Y., Nishinakamura, H., Sugahara, K.N., Miyauchi, T., Sako, Y., Yanagida, T., and Miyasaka, M. (2004). Engagement of CD44 promotes Rac activation and CD44 cleavage during tumor cell migration. *J. Biol. Chem.* 279, 4541–4550.
- Murai, T., Miyauchi, T., Yanagida, T., and Sako, Y. (2006). Epidermal growth factor-regulated activation of Rac GTPase enhances CD44 cleavage by metalloproteinase disintegrin ADAM10. *Biochem. J.* 395, 65–71.
- Murai, T., Hokonohara, H., Takagi, A., and Kawai, T. (2009). Atomic force microscopy imaging of supramolecular organization of hyaluronan and its receptor CD44. *IEEE Trans. NanoBiosci.* 8, 294–299.
- Murai, T., Maruyama, Y., Mio, K., Nishiyama, H., Suga, M., and Sato, C. (2011). Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration. *J. Biol. Chem.* 286, 1999–2007.
- Murai, T., Sato, C., Sato, M., Nishiyama, H., Suga, M., Mio, K., and Kawashima, H. (2013a). Membrane cholesterol modulates the hyaluronan-binding ability of CD44 in T lymphocytes and controls rolling under shear flow. *J. Cell Sci.* 126, 3284–3294.
- Murai, T., Sato, M., Nishiyama, H., Suga, M., and Sato, C. (2013b). Ultrastructural analysis of nanogold-labeled cell surface microvilli in liquid by atmospheric scanning electron microscopy and their relevance in cell adhesion. *Int. J. Mol. Sci.* 14, 20809–20819.
- Nelson, E.R., Wardell, S.E., Jasper, J.S., Park, S., Suchindran, S., Howe, M.K., Carver, N.J., Pillai, R.V., Sullivan, P.M., Sondhi, V., et al. (2013). 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* 342, 1094–1097.
- Nieto-Miguel, T., Gajate, C., Gonzalez-Camacho, F., and Mollinedo, F. (2008). Proapoptotic role of Hsp90 by its interaction with c-Jun N-terminal kinase in lipid rafts in edelfosine-mediated antileukemic therapy. *Oncogene* 27, 1779–1787.
- Oneyama, C., Hikita, T., Enya, K., Dobenecker, M.W., Saito, K., Nada, S., Tarakhovskiy, A., and Okada, M. (2008). The lipid raft-anchored adaptor protein Cbp controls the oncogenic potential of c-Src. *Mol. Cell* 30, 426–436.
- Parks, S.K., Chiche, J., and Pouyssegur, J. (2011). pH control mechanisms of tumor survival and growth. *J. Cell. Physiol.* 226, 299–308.
- Parton, R.G., Hanzal-Bayer, M., and Hancock, J.F. (2006). Biogenesis of caveolae: a structural model for caveolin-induced domain formation. *J. Cell Sci.* 119, 787–796.
- Pelton, K., Freeman, M.R., and Solomon, K.R. (2012). Cholesterol and prostate cancer. *Curr. Opin. Pharmacol.* 12, 751–759.
- Pike, L.J. (2006). Rafts defined: a report on the Keystone symposium on lipid rafts and cellular function. *J. Lipid Res.* 47, 1597–1598.
- Platz, E.A., Clinton, S.K., and Giovannucci, E. (2008). Association between plasma cholesterol and prostate cancer in the PSA era. *Int. J. Cancer* 123, 1693–1698.
- Poynter, J.N., Gruber, S.B., Higgins, P.D., Almog, R., Bonner, J.D., Rennert, H.S., Low, M., Greenson, J.K., and Rennert, G. (2005). Statins and the risk of colorectal cancer. *New Engl. J. Med.* 352, 2184–2192.
- Rayner, K.J., Suárez, Y., Dávalos, A., Parathathm, S., Fitzgerald, M.L., Tamehirom, N., Fisherm, E.A., Moore, K.J., and Fernández-Hernando, C. (2010). MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* 328, 1570–1573.
- Resh, M.D. (1999). Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. *Biochim. Biophys. Acta* 1451, 1–16.
- Rossi, S., Graner, E., Febbo, P., Weinstein, L., Bhattacharya, N., Onody, T., Bubley, G., Balk, S., and Loda, M. (2003). Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. *Mol. Cancer Res.* 1, 707–715.
- Roy, S., Luetterforst, R., Harding, A., Apolloni, A., Etheridge, M., Stang, E., Rolls, B., Hancock, J.F., and Parton, R.G. (1999). Dominant-negative caveolin inhibits H-Ras function by disrupting cholesterol-rich plasma membrane domains. *Nat. Cell Biol.* 1, 98–105.
- Selvais, C., D'Auria, L., Tyteca, D., Perrot, G., Lemoine, P., Troeberg, L., Dedieu, S., Noël, A., Nagase, H., Henriot, P., et al. (2011). Cell cholesterol modulates metalloproteinase-dependent shedding of low-density lipoprotein receptor-related protein-1 (LRP-1) and clearance function. *FASEB J.* 25, 2770–2781.
- Sharpe, L.J. and Brown, A.J. (2013). Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). *J. Biol. Chem.* 288, 18707–18715.
- Siegel, R., Naishadham, D., and Jemal, A. (2013). Cancer statistics, 2013. *CA Cancer J. Clin.* 63, 11–30.
- Simons, K. and Ikonen, E. (1997). Functional rafts in cell membranes. *Nature* 387, 569–572.
- Simons, K. and Toomre, D. (2000). Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* 1, 31–39.
- Simons, K. and van Meer, G. (1988). Lipid sorting in epithelial cells. *Biochemistry* 27, 6197–6202.
- Singer, S.J. and Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* 175, 720–731.
- Solomon, K.R. and Freeman, M.R. (2008). Do the cholesterol-lowering properties of statins affect cancer risk? *Trends Endocrinol. Metab.* 19, 113–121.
- Solomon, K.R., Pelton, K., Boucher, K., Joo, J., Tully, C., Zurakowski, D., Schaffner, C.P., Kim, J., and Freeman, M.R. (2009). Ezetimibe is an inhibitor of tumor angiogenesis. *Am. J. Pathol.* 174, 1017–1026.
- Sugahara, K.N., Murai, T., Nishinakamura, H., Kawashima, H., Saya, H., and Miyasaka, M. (2003). Hyaluronan oligosaccharides

- induce CD44 cleavage and promote cell migration in CD44-expressing tumor cells. *J. Biol. Chem.* 278, 32259–32265.
- Swyer, G.I.M. (1942). The cholesterol content of normal and enlarged prostates. *Cancer Res.* 21, 372–375.
- Trapani, L., Segatto, M., Ascenzi, P., and Pallottini, V. (2011). Potential role of nonstatin cholesterol lowering agents. *IUBMB Life* 63, 964–971.
- Vander Heiden, M.G., Locasale, J.W., Swanson, K.D., Sharfi, H., Heffron, G.J., Amador-Noguez, D., Christofk, H.R., Wagner, G., Rabinowitz, J.D., Asara, J.M., et al. (2010). Evidence for an alternative glycolytic pathway in rapidly proliferating cells. *Science* 329, 1492–1499.
- Veit, G., Zimina, E.P., Franzke, C.W., Kutsch, S., Siebolds, U., Gordon, M.K., Bruckner-Tuderman, L., and Koch, M. (2007). Shedding of collagen XXIII is mediated by furin and depends on the plasma membrane microenvironment. *J. Biol. Chem.* 282, 27424–27435.
- Vogelstein, B. and Kinzler, K.W. (1993). The multistep nature of cancer. *Trends Genet.* 9, 138–141.
- von Tresckow, B., Kallen, K.J., von Strandmann, E.P., Borchmann, P., Lange, H., Engert, A., and Hansen, H.P. (2004). Depletion of cellular cholesterol and lipid rafts increases shedding of CD30. *J. Immunol.* 172, 4324–4331.
- Warburg, O. (1956). On the origin of cancer cells. *Science* 123, 309–314.
- Warburg, O., Wind, F., and Negelein, E. (1927). The metabolism of tumors in the body. *J. Gen. Physiol.* 8, 519–530.
- Wary, K.K., Mariotti, A., Zurzolo, C., and Giancotti, F.G. (1998). A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell* 94, 625–634.
- Waugh, M.G., Lawson, D., and Hsuan, J.J. (1999). Epidermal growth factor receptor activation is localized within low-buoyant density, non-caveolar membrane domains. *Biochem. J.* 337, 591–597.
- White, C.P. (1909). On the occurrence of crystals in tumours. *J. Pathol. Bacteriol.* 13, 3–10.
- Williams, T.M. and Lisanti, M.P. (2005). Caveolin-1 in oncogenic transformation, cancer, and metastasis. *Am. J. Physiol. Cell. Physiol.* 288, C494–506.
- Yager, J.D. and Davidson, N.E. (2006). Estrogen carcinogenesis in breast cancer. *New Engl. J. Med.* 354, 270–282.
- Yasuda, M. and Bloor, W.R. (1932). Lipid content of tumors. *J. Clin. Invest.* 11, 677–682.
- Young, R.M., Holowka, D., and Baird, B. (2003). A lipid raft environment enhances Lyn kinase activity by protecting the active site tyrosine from dephosphorylation. *J. Biol. Chem.* 278, 20746–20752.
- Xue, T., Zhang, Y., Zhang, L., Yao, L., Hu, X., and Xu, L.X. (2013). Proteomic analysis of two metabolic proteins with potential to translocate to plasma membrane associated with tumor metastasis development and drug targets. *J. Proteome Res.* 12, 1754–1763.
- Zhuang, L., Kim, J., Adam, R.M., Solomon, K.R., and Freeman, M.R. (2005). Cholesterol targeting alters lipid raft composition and cell survival in prostate cancer cells and xenografts. *J. Clin. Invest.* 115, 959–968.
- Zimina, E.P., Bruckner-Tuderman, L., and Franzke, C.W. (2005). Shedding of collagen XVII ectodomain depends on plasma membrane microenvironment. *J. Biol. Chem.* 280, 34019–34024.