Editorial

Disrupting membrane lipids composition promotes tumorigenesis: the other dark side of cholesterol and the potential implication of gangliosides

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Within the four major families of biological compounds, lipids are likely the most unknown. This nescience is partly due to their structure heterogeneity when compared to carbohydrates, proteins and nucleic acids that inside the same family share similar structural features, and to the negative press that is done to them. However and despite this contempt, lipids are fundamental building blocks for cell shape and constitute the most important energy tank. Lack of knowledge of their benefits makes us forget that they also support a myriad of biological functions they endorse in the form of fat-soluble vitamins, steroid hormones (corticoids and sex steroids), pheromones, odors, second messengers (inositides, ceramides), and post-translational modifications (acylation, prenylation, cholesteroylation).

More than any other lipids considered unloved, cholesterol appears as the common enemy of Western societies, as evidenced by the great advertising support that is supposed to learn people how to fight it. Indeed, excess cholesterol is long-standing known to be a major actor of atherosclerosis responsible for the two main causes of deaths worldwide, ischemic heart disease and ischemic stroke, and characterized by accumulation of cholesterol with macrophages in blood vessel walls. Less known is the involvement of lipid metabolism disruption in tumorigenesis albeit it was long been observed that nutrient excess, consumption of cholesterol increased gastrointestinal cancer (1). A recent paper from the group of Peter Tontonoz (University of California, Los Angeles, USA) (2) points out the reciprocal relationship between the availability of cholesterol on the proliferation of intestinal stem cells (ISCs) and the activity of the phospholipid-remodeling enzyme lysophosphatidylcholine acyltransferase 3 (Lpcat3), an enzyme the authors identified as pivotal for membrane phospholipids composition (3). Interestingly, previous independent studies focusing phospholipase A2 (PLA2), an enzyme that hydrolyzes the acyl group at position sn-2 of phospholipids, and belonging as well as Lpcat3 to Lands' cycle, reported that this enzyme aggravates emergence of intestinal tumors in Apc mice, a model in which the Apc^{Min} mutation disrupts the mouse homologue of the human familial polypose gene, thus developing intestinal tumors (4,5). Based on these observations, Wang and collaborators reasoned that since (I) a fraction of ISCs give progenitors that in turn move from the crypt to the intestinal villi and differentiate into all cell types needed for gut function, and that (II) ISCs are at the origin for intestinal tumors developing in mice harboring APC mutations, a remodeling of phospholipid should be partly responsible for tumorigenesis. The authors took advantage of the two following animal models: first, tamoxifen-inducible intestine-specific Lpcat3 deficient mice generated by crossing $Lpcat3^{fl/fl}$ with Villin-CreERT2, and a second one generated by crossing the former with Apc^{min} mice. Then, they showed that a deficiency in Lpcat3 enhances proliferation of the ISCs via an increase in cholesterol biosynthesis. Previously, studies proposed that remodeling of phospholipids promotes intestinal tumorigenesis but without elucidating the mechanism. In this way, in Apc^{min} mice, over-expression of the secretory/membrane-bound phospholipase Pla2g2a reduces tumor number and enhances

tumor resistance (6). In contrast, loss of the phospholipase increased tumor formation (7). Nevertheless, none of these studies pointed out the potential involvement of cholesterol in intestinal tumor emergence. Wang and collaborators undertook a gene ontology analysis in Lpcat3-deficient crypts that unveiled an increased-expression of genes responsible for cholesterol biosynthesis, and consequently a higher amount of free cholesterol. Mice deficient for Lpcat3 exhibited nuclear accumulation of the transcription factor SREBP-2, a master driver of cholesterol biosynthesis, making sense to the monitoring of enhanced cholesterol. These observations were strengthened by use of cholesterol inhibitors such as simvastatin and Ro48, an inhibitor of lanosterin synthase. The latter when administered to Lpcat3 deficient mice significantly reduced the height of the crypts, proliferation of cells in jejunum crypts and expression of the Olfm4 specific ISC marker. At last, by generating Srebf2 Tg $Apc^{min/+}$ mice, it was observed that these mice developed more tumors than $Apc^{min/+}$ control. Therefore, the paper concludes that in response to phospholipid remodeling, cholesterol acts as a mitogen: cholesterol is an active actor of ISC proliferation and in all likelihood an initiator of intestinal tumorigenesis. Yet, a major point, not least, remains to be elucidated: how the remodeling of phospholipids affects the synthesis of cholesterol. A possible explanation would be that SREBP-2 subcellular localization is perturbed in conditions of decreased activity of Lpcat3. The authors showed that Lpcat3 deficiency dramatically increases localization of SREBP-2 in the nucleus. However, a disturbance in the membrane of the endoplasmic *reticulum* (ER), in which many enzymes responsible for cholesterol synthesis including HMG-CoA reductase and lanosterin synthase are located, and of Golgi apparatus has not been envisioned by the authors. All three members of the SREBP family, SREBP-1a, SREBP-1c, and SREBP-2 are generated as inactive precursors embedded in the membrane of the ER. Maturation of these precursors requires their translocation from the ER to the Golgi membrane where are located the proteases S1P and S2P (site-1 and site-2 proteases) that cleave SREBP-2 precursor into matured fragments. Two factors control this process: the protein SCAD capable to sense cholesterol and derivatives concentration, and the inhibitory protein Insig to which SCAP binds. SCAP and Insig therefore prevent SREBPs to reach the Golgi. In conditions of low cholesterol, SREBP-2 is translocated into the nucleus where it associates to sterol regulatory element (SRE) found in promoters of genes encoding LDLR (cholesterol uptake) and enzymes involved in cholesterol biosynthesis including the key-rate limiting enzyme HMG-CoA reductase. A remodeling of ER to Golgi phospholipids may result in a default of processing and subcellular location of SREBP-2, and to a disturbed transport in the nucleus.

Phospholipids and cholesterol are not the only ones to play a leading role in membrane dynamics and architecture. Glycosphingolipids (GSLs) are essential compounds for the organization, the maintenance and the functions of lipid rafts (lipid microdomains) in plasma membrane. Cholesterol is rather equally distributed between the two leaflets of the plasma membrane: it interacts preferentially with phosphatidylethanolamine and phosphatidylserine in the inner leaflet and with phosphatidylcholine and sphingomyelin in the outer one. Importantly, cholesterol also needs interactions with GSLs, especially gangliosides, the sialic acid containing GSLs, exclusively found in the outer leaflet. Within the membrane these components tightly interact through their hydrophobic lateral chains to cluster in lipid rafts. In a somewhat disappointing way, while investigating lipid composition disruption in ISCs, Wang and collaborators did not look at the levels of GSLs/gangliosides. Yet and in the context of the paper, highly proliferative cells like cancer cells are not only characterized by the need of an accelerated biosynthesis of lipid compounds so as to respond to increased membrane surface, but also by the necessity to remodel lipid rafts. Lipid rafts are crucial for signal transduction and cell signaling since they gather together receptors tyrosine kinases (RTKs) such as growth factor receptors and intracellular signaling components of mitogen pathways (8). Thus, within lipid rafts gangliosides interact with signaling molecules including RTKs and integrins, transmembrane receptors particularly active in signal transduction. Even if to date no data regarding Lpcat3 and gangliosides are available, a connection between this group of GSLs and lysophosphatidylcholine was drawn. It was reported that lysophosphatidylcholine-stimulated EGF signaling is inhibited by GM3 strengthening the role of gangliosides in cell transduction (9). Therefore, interactions between gangliosides and RTKs play an important role in the fine tuning of signal transduction by regulating RTK signaling (10). A large number of reports have underlined the role of complex gangliosides, especially GD3 and GD2, in several cancers from neuro-ectoderm origin, such as melanoma or neuroblastoma. These papers demonstrated the implication of complex gangliosides in cell proliferation, migration, tumor growth and angiogenesis. The effects mainly result from the interaction of complex gangliosides with signaling molecules as it was clearly demonstrated in melanoma where GD3 expression results in a high level of phosphorylation and activation of the major adaptor proteins paxillin, p130Cas

and FAK (11). In breast cancer cells, the expression of the GD3S and complex gangliosides results in the acquisition of higher proliferative capacity of tumor cells in the absence of growth factors. GD3S expressing cells bypass the need of growth factors by a specific and constitutive activation of c-Met receptor and activation of PI3K/Akt and Erk/MAPK pathways. GD3S expression also enhanced tumor growth in SCID mice, and a higher expression of *ST8SIA1* in "basal-like" sub-type of human breast tumors was observed (12). The decrease of GD2 expression by silencing of the GM2/GD2 synthase or competition assays using anti-GD2 mAbs reversed the proliferative phenotype as well as c-Met phosphorylation (13) demonstrating the involvement of GD2 in breast cancer cell proliferation *via* the constitutive activation of c-Met.

According to their expression pattern and localization, GSLs including stage-specific embryonic antigens (SSEA)-3 and SSEA-4, and complex gangliosides such as GD3 and GD2, have been used as stem cells markers, including cancer stem cells (CSCs) (14). Recently, the disialoganglioside GD2 was identified as a new specific cell surface marker of $CD44^{hi}CD24^{lo}$ breast CSCs from human breast cancer cell lines and patient samples that are capable of forming mammospheres and initiating tumors (15). Gene expression analysis revealed that several glycosyltransferases encoding genes involved in GD2 biosynthesis (*ST3GAL5*, *B4GALNT1*, and *ST8SIA1*) are highly expressed in CSCs (15,16). The reduction of GD2 expression by *ST8SIA1* knockdown reduced mammosphere formation and cell motility, and completely abrogated tumor formation *in vivo*, changing the phenotype from CSC to non-CSC (15,16). Moreover, the induction of epithelial-mesenchymal transition (EMT) in transformed human mammary epithelial cells dramatically increased GD3 synthase (GD3S) as well as GD2 expression, whereas the inhibition of GD3S compromised EMT initiation and maintenance and prevented metastasis (17). High-grade bladder cancer (BLCA) cells also show high expression of GD2 together with a mesenchymal and proliferative phenotype, and CSC properties (18).

GD3S alone can sustain CSC properties and promote malignant cancer characteristics. GD3 was shown to be associated with epithelial growth factor receptor (EGFR) and activated EGFR signaling in breast CSCs. In parallel, GD3S knockdown enhanced cytotoxicity of Gefitinib in resistant cells, showing that GD3S can contribute to Gefitinib-resistance in EGFR-positive breast cancer cells (19). GD3S and GD3 disialoganglioside are also highly expressed in glioblastoma multiforme (GBM) CSCs (GSC), a grade IV astrocytoma, playing a key role in glioblastoma tumorigenicity. GD3S is increased in neurospheres and human GBM tissues, but not in normal brain tissues, and suppression of GD3S results in decreased GSC-associated properties. In addition, anti-GD3 mAbs induce inhibition of GBM tumor growth *in vivo* (20).

Altogether, these findings underline the role of gangliosides in CSC maintenance and properties, as well as in tumorigenicity and tumor formation. According to the important role of phospholipids and cholesterol in lipid raft formation, it would be really interesting to address the question of lipid raft function in ISCs activation in Lpcat3 deficient mice and the relationship between cholesterol biosynthesis and lipid raft organization in this process. In this way, a tentative link has been drawn between cholesterol and gangliosides. It was shown that lack of GM2/GD2 synthase dramatically reduced accumulation of cholesterol (21). Like part of SREBP-2 processing, GD2 and GD3 synthases are localized in the Golgi. Perturbing phospholipids composition may affect gangliosides synthesis as it should interfere with SREBP-2 maturation. Nevertheless, a second study indicated that biosynthesis and concentration of cholesterol were unaffected in animals lacking GM2/GD2 synthase concluding that plasma membrane cholesterol status is unaffected by gangliosides (22). It is to note that both studies were performed in neurological diseases models, far from the topic of the paper from Wang and collaborators, since while gangliosides are distributed in all cell types, they are more abundant in the cells of the central nervous system. Therefore, more have to be done to determine whether gangliosides and cholesterol metabolisms are linked and if it has an impact on intestinal tumorigenesis.

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