E. Sottocornola,
B. Berra

Modulation of membrane lipid raft by omega-3 fatty acids and possible functional implication in receptor tyrosine-kinase activation

Progress in Nutrition
Vol. 10, N. 4, 210-213, 2008

Titolo
Modulazione dei raft lipidici di membrana da parte degli acidi grassi omega-3 e possibili implicazioni funzionali sulla attivazione di recettori tirosin-chinasici

Key words
Lipid raft, omega-3 PUFA, receptor tyrosine-kinase, ErbB receptor, mammary adenocarcinoma

Parole chiave
Raft lipidici, omega-3 PUFA, recettori tirosin-chinasici, recettori ErbB, adenocarcinoma mammario

Institute of General Physiology and Biological Chemistry "G. Esposito"
University of Milan

Indirizzo per la corrispondenza:
Prof. Bruno Berra
Institute of General Physiology and Biological and Chemistry "G. Esposito"
University of Milan
Via Trentacoste, 2 - 20134 Milano
Tel. 02.503.15781
Fax 02.503.15775
E-mail: bruno.berra@unimi.it

Summary
Current understanding of biologic membrane structure and function is largely based on the concept of lipid rafts. Lipid rafts are composed primarily of tightly packed, liquid-ordered sphingolipids/cholesterol/saturated phospholipids that float in a sea of more unsaturated and loosely packed, liquid-disordered lipids. Lipid rafts have important clinical implications because many important membrane-signalling proteins are located within the raft regions of the membrane, and alterations in the raft structure can alter the activity of these signalling proteins. Because rafts are lipid-based, their composition, structure, and function are susceptible to manipulation by dietary components such as omega-3 polyunsaturated fatty acids and by cholesterol depletion. Important components of lipid rafts are receptor tyrosine-kinase, such as the ErbB receptor superfamily. Here we review how alteration of raft lipids affects the raft/nonraft localization and hence the function of several proteins involved in cell signalling, focusing our discussion on two members of the ErbB receptors: EGFR and ErbB2, that are specifically involved in the development of many solid tumors, such as mammary adenocarcinomas.

Riassunto
Ad oggi, la comprensione della struttura e della funzionalità della membrana plasmatica non può prescindere dal concetto dei 'raft' lipidici. I raft lipidici sono costituiti principalmente da sfingolipidi/colesterolo/fosfolipidi saturi strettamente impaccati in una fase liquido-ordinata che flottano all'interno della restante massa dei lipidi di membrana, maggiormente insaturi e costituenti la fase lipidica liquido-disordinata. I raft lipidici presentano importanti implicazioni cliniche poiché numerose importanti proteine deputate alla trasduzione del segnale sono in essi localizzate, e alterazioni della struttura dei raft possono alterare la trasduzione del segnale da parte di queste proteine. Dal momento che la massa critica dei raft è fondamentalmente costitutita da lipidi, la loro composizione, struttura e funzione è suscettibile a manipolazioni da parte di lipidi di derivazione dietetica quali gli acidi grassi poliinsaturi omega-3 e dalla deplezione di colesterolo. I recettori della super-famiglia ErbB sono tra le tirosin-chinasi recettoriali che

segregano nei raft. Sarà in questa sede trattato come l'alterazione dei lipidi dei raft possa alterare la localizzazione raft/non-raft e, di conseguenza, la funzione di numerose proteine coinvolte nella trasduzione del segnale, con particolare attenzione proprio a due recettori della famiglia ErbB: EGFR ed ErbB2, che sono specificamente coinvolti nello sviluppo di numerosi tumori solidi, tra i quali l'adenocarcinoma mammario.

What lipid rafts are?

A provisional contemporary definition of rafts that embodies their complexity is: "Membrane rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions". The origin of the raft hypothesis (1) can be traced to the perplexing discovery that glycosphingolipids cluster in the Golgi apparatus before being sorted to the apical surface of polarized epithelial cells (2). Subsequent studies established that glycosphingolipid clusters tend to be insoluble in Triton X-100 at 4°C, forming detergent-resistant membranes (DRM), have a light buoyant density on sucrose gradients and are rich in both cholesterol and glycosylphosphatidyl inositol (GPI)-anchored proteins (3). The raft hypothesis was bolstered by the observation that synthetic membranes composed of glycosphingolipids and cholesterol recapitulate the detergent-resistant characteristics of the glycosphingolipid clusters (4, 5), suggesting that lipid lateral heterogeneity occurs spontaneously as a function of the lipid composition of the membrane. Simons and Ikonen originally proposed that lipid "…lateral organization probably results from preferential packing of sphingolipids and cholesterol into moving platforms, or rafts, onto which specific proteins attach within the bilayer" (1).

It is now well established that all cellular membranes of mammalian as well as of *Drosophila*, *Dictyostelium*, and yeast, are not homogeneous in their lipid and protein distribution, but instead are composed of diverse, ever-changing patches, called domains. Domains exist in a bewilderind array of sizes, stabilities, lipid and protein compositions, and functionalities. Some domains are macroscopic and stable for extended time periods (such as the apical–basolateral specializations and junctional complexes in epithelial cells and clathrin-coated pits) and so are isolated and well defined, but the major proportions of most membranes are composed of tiny (on the nanometer scale) and unstable (existing for less than microseconds) microdomains (like rafts, caveolae, and glycosynapses), and, as a result, their investigation and comprehension is very difficult.

Lipid rafts are in a liquid-ordered state and are envisioned to float in a sea of liquid-disordered phospholipids, poor of cholesterol and sphingolipids. They are enriched in tightly packed, saturated fatty acyl chains, whereas the non-raft regions are enriched in loosely packed, polyunsaturated chains.

It is established that rafts incorporate many diverse signalling receptors, downstream signal molecules, and signaling associated adaptor proteins. Included in an ever expanding list of raft-associated signalling proteins are high-affinity IgE receptors (FcR), insulin receptors, T-cell antigen receptors, G-protein-coupled receptors, epidermal growth factor (EGF) receptors, and several kinases and phosphatases. Lipid rafts are therefore postulated to serve as platforms for protein activity by accumulating specific proteins usually in-

volved in cell signalling. Recent investigations have shown that movement of proteins into or out of lipid rafts can be modified by changes in the lipid microenvironment.

Lipid rafts and omega-3 long chain fatty acids

The lipid composition of cancer and non-cancer cell membranes are notably different (6). It has been suggested that increased proportions of saturated fatty acid in cancer cells alter lipid raft structure and protein composition in a manner that enhances cancer cell survival by promoting growth, escaping immune surveillance, or preventing apoptosis (7). Indeed, increased activity levels of the fatty acid synthase (FAS) has been found in numerous malignancies (7). Moreover, cancer cells have higher levels of cholesterol, that is perhaps the most important component of rafts.

As rafts are lipid-based, their composition, structure and function are susceptible to manipulation by dietary components such as omega-3 polyunsaturated fatty acids (PUFAs), cholesterol depletion, manipulation of glycosphingolipid expression.

Dietary lipids and lipid emulsions are known to have many effects upon cellular functions. PUFAs have been reported to alter synthesis of lipid mediators such as prostaglandins and to modulate expression of many gene products involved in inflammatory and immunologic processes. These and other long-chain fatty acids are known to localize to cell membranes and to alter their properties. The exact mechanisms whereby long-chain fatty acids modulate cell functions are not entirely clear. However, an extensive body of scientific evidence suggests that PUFAs affect cellular functions by modulating the structure and function of specific lipid domains, such as lipid rafts, within the plasma membrane (8).

A number of recent in vitro studies using model membranes and cell culture systems have demonstrated that PUFAs can greatly reduce raft formation and can displace signalling proteins (9, 10). Changes in the nature and abundance of functional proteins in lipid rafts, can alter cell physiology and hence the development and progression of various diseases. Experimental evidences indicate that lipid raft signalling proteins can be modulated in inflammation and cancer models by the omega-3 PUFAs, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are found in high concentrations in fish oils. DHA is of special interest because it is the longest (22 carbons) and the most unsaturated (6 double bonds) fatty acid commonly found in membranes. DHA is a perturbing fatty acid, significantly altering the basic properties of cell membranes, including acyl chain order and fluidity, phase behaviour, elastic compressibility, ion permeability, fusion, flip-flop, and the activity of many resident proteins (11).

The shorter chain omega-3 PUFA, alfa-linolenic acid, can be desaturated and elongated to DHA and EPA in vivo. Therefore alfa-linolenic acid may also exhibit beneficial effects on health similar to EPA and DHA. However, there is concern that patients with a variety of diseases may have decreased capacity to elongate alfa-linolenic acid to EPA and DHA. Therefore, alfa-linolenic acid should be used with caution as sole source of omega-3 PUFAs.

Recent studies have demonstrated that EPA and DHA can displace several raft-associated signalling proteins from membrane rafts (12, 13).

Lipid raft and ErbB tyrosine-kinase receptors

The ErbB superfamily of receptors, particularly EGFR and ErbB2, are actively involved in mammary gland development and differentiation during pregnancy and lactation and in the uncontrolled growth of mammary adenocarcinomas characterized by poor prognosis and drug resistance. Many are the literature data describing ErbB receptor compartmentalization in and out raft and the mechanisms of their homo-/hetero-

dimerization and activation (14), but very little is known about the regulatory effect of omega-3 PUFA supplementation. Shley et al. (15) demonstrated that treatment of the MDA-MB-231 human breast cancer cell line with a mixture of EPA and DHA induced the decrease of sphingomyelin, cholesterol and diacylglicerol as well as EGFR in lipid raft, although phosphorylation of both EGFR and p38 MAPK were sensibly increased. Activation of EGFR and p38 MAPK was associated with apoptosis of MDA-MB-231 cells, suggesting that the alteration of lipid composition of membrane raft is able to modify EGFR signalling transduction pathways and cell fate.

We analyzed the role of gangliosides, an important lipid component of rafts, in the association of the ErbB2, EGFR and shc-p66 (protein correlated with the ErbB2 signal transduction pathway) with lipid rafts in mammary epithelial HC11 cells (16). Scanning confocal microscopy experiments together with the analysis of membrane fractions obtained by linear sucrose gradient ultracentrifugation revealed the preferential association of ErbB2/EGFR and ganglioside GM3 in lipid raft. In addition, after activation of EGFR with EGF, shc-p66 was preferentially enriched in lipid rafts. Blocking of endogenous ganglioside synthesis by (+/-)-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride (PDMP) induced a drastic cell-surface redistribution of ErbB2, EGFR and Shc-p66, within the Triton-soluble fractions. This redistribution was partially reverted when exogenous GM3 was added to ganglioside-depleted HC11 cells. The results point out the key role of ganglioside GM3 in retaining ErbB2/EGFR and signal-transduction-correlated proteins in lipid rafts, suggesting the possible existence of different pool of receptors at the plasma membrane levels.

Bibliografia

1. Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997; 387: 569-72.
2. Simons K, van Meer G. Lipid sorting in epithelial cells. Biochemistry 1988; 27: 6197–202.
3. Brown D. GPI-anchored proteins and detergent-resistant membrane domains. Braz J Med Biol Res. 1994; 27: 309–15.
4. Dietrich C, Bagatolli LA, Volovyk ZN, et al. Lipid rafts reconstituted in model membranes. Biophys J 2001; 80: 1417–28.
5. Dietrich C, Volovyk ZN, Levi M, Thompson NL, Jacobson K. Partitioning of Thy-1, GM1, and cross-linked phospholipids into lipid rafts reconstituted in supported model membrane monolayers. Proc Natl Acad Sci USA 2001; 98: 10642–7.
6. Meng X, Riordan NH, Riordan HD. Cell membrane fatty acid composition differs between normal and malignant cell lines. P R Health Sci J 2004; 23: 103-6.
7. Rakheja D, Kapur P, Hoang MP, Roy LC, Bennett MJ. Increased ratio of saturated to unsaturated C18 fatty acids in colonic adenocarcinoma: implications for cryotherapy and lipid raft function. Med Hypothesis 2005; 65: 1120-3.
8. Chen W, Jump DB, Esselman WJ, Busik JV. Inihibition of cytokine signalling in human retinal endothelial cells through modification of caveolae/lipid rafts by docosaexaenoic acid. Invest. Ophthalmol. Vis Sci 2007; 48: 18-26.
9. Stulnig TM, Huber J, Leitinger N. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. J Biol Chem 2001; 276: 37335-40.
10. Stulnig TM, Berger M, Sigmund T, Raederstorff D, Stockinger H, Walhausl W. Polyunseturated fatty acids inhibit T cell signal transduction by modification of detergent-insoluble membrane domains. J Cell Biol 1998; 143: 637-44.
11. Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassal SR. Docosaexaenoic acid affects cell signalling by altering lipid rafts. Reprod Nutr Dev 2005; 45: 559-79.
12. Ma DW, Seo J, Davidson LA, et al. N-3 PUFA alter caveolae lipid composition and resident protein localization in mouse colon. FASEB J 2004; 18: 1040-2.
13. Li Q, Wang M, Tan L, et al. Docosahexaenoic acid changes lipid composition and interleukin-2 receptor signalling in membrane rafts. J Lipid Res 2005; 46: 1904-13.
14. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature 2001; 2: 127-37.
15. Schley PD, Brindley DN, Field CJ. (n-3) PUFA alter lipid raft composition and decrease epidermal growth factor receptor levels in lipid rafts of human breast cancer cells. J Nutr 2007; 137: 548-53.
16. Sottocornola E, Misasi R, Mattei V, et al. Role of gangliosides in the association of ErbB2 with lipid rafts in mammary epithelial HC11 cells. FEBS J 2006; 273; 1821-30.