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COMMENTARY

Raft platforms highly enriched in cholesterol: major scaffolds for IL-6 signalling assembly with implications in inflammation and cancer

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Interleukin-6 (IL-6) is a pleiotropic cytokine with complex and major roles in inflammation, which could be linked to the different ways IL-6 signals at the plasma membrane. In this issue of FEBS Journal, Woo and co-workers present evidence for the involvement of Eps15 homology domain-containing protein 1 (EHD1)-mediated lipid raft platforms, highly enriched in cholesterol, in the IL-6 signalling pathway. Because of the strong connection between IL-6, inflammation and cancer, one implication of this report is that agents or approaches targeting cholesterol-rich raft platforms may assist the development of novel strategies to treat inflammatory and malignant diseases.

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Interleukin-6 signalling

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biologic activities and a high clinical relevance. IL-6 signalling is mediated by the binding of IL-6 to the membrane-bound IL-6 receptor α (IL-6Rα; gp80; CD126). The complex IL-6/IL-6Rα associates with the membrane-bound signal-transducing receptor glycoprotein 130 (gp130; CD130), which then dimerizes and induces Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) signalling [1]. Only a few cell types express IL-6Rα, but practically every cell type in the body expresses gp130, except mature neutrophils, despite their IL-6Rα expression [2]. IL-6Rα exists in two forms, namely a membrane-bound and a soluble form, with the latter being generated by proteolytic cleavage or alternative splicing [1,3]. IL-6Rα can be cleaved by the membrane-bound metalloprotease a disintegrin and metalloprotease 17 (ADAM17) to generate soluble IL-6Rα (sIL-6Rα) [4]. Interaction between IL-6 and membrane-bound IL-6Rα leads to the so-called classical or *cis*-signalling, whereas IL-6 binding to the sIL-6Rα leads to the IL-6/sIL-6Rα complex that can interact with gp130 and

Abbreviations
ADAM17, a disintegrin and metalloprotease 17; CTB, cholera toxin subunit B; EHD1, Eps15 homology domain-containing protein 1; gp130, glycoprotein 130; IL-6, interleukin-6; IL-6Rα, IL-6 receptor α; JAK, Janus kinase; sIL-6Rα, soluble IL-6Rα; SIM, structured illumination microscopy; STAT3, signal transducer and activator of transcription 3.

thus stimulates cells that only express gp130 through a process called *trans*-signalling. Classical IL-6 signalling is involved in regenerative and anti-inflammatory activities of the cytokine, whereas *trans*-signalling is linked to pro-inflammatory reactions. Because IL-6Rα is only expressed in certain cells (classical *cis*-signalling), such as hepatocytes and some leukocyte populations, while gp130 is ubiquitously expressed, *trans*-signalling greatly expands the spectrum of IL-6 actions and target cells [3]. The ratio of membrane-bound IL-6Rα to gp130 on the cell surface determines how the cell senses classical and *trans*-signalling [3].

IL-6 as a disease marker and the inflammation-cancer link

IL-6 levels in the bloodstream are as low as 1–5 pg·mL^{-1}, but this level can rise hundred- or thousandfold under some inflammatory states, indicating that IL-6 serum levels is a major alarm signal in several pathological states, including inflammation and infection [1]. Epidemiological studies have shown that chronic inflammation predisposes individuals to various types of cancer [5], and neutrophils, as major inflammatory cells, have been postulated to play a major role in cancer progression and metastasis [6]. This association between inflammation and cancer (Fig. 1) is reflected by the high IL-6 levels in the tumour microenvironment, and by the fact that IL-6 signalling is aberrantly hyperactivated in chronic inflammation and several types of cancer [7].

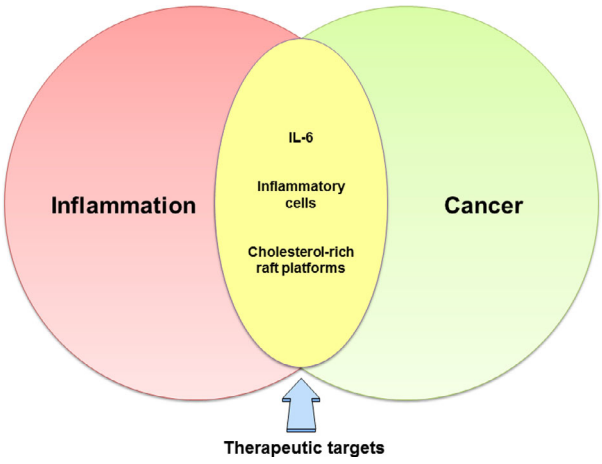

Fig. 1. Some aspects shared by the inflammation and cancer interplay, and putative targets in therapy. IL-6, inflammatory cells and cholesterol-rich raft platforms (highlighted with yellow background at the intersection between inflammation and cancer) are involved in both inflammation and cancer, becoming putative therapeutic targets in the treatment of inflammatory and malignant diseases. See text for further details.

Lipid rafts and IL-6 signalling

Lipid rafts are small (10–200 nm) heterogeneous sterol- and sphingolipid-enriched domains that can coalesce to form large platforms suitable to recruit proteins for efficient signal transduction, thus compartmentalizing signalling processes [8], and acting as hubs for different signalling pathways and processes [9]. Cholesterol-rich lipid rafts have been involved in IL-6-induced neuroendocrine differentiation of LNCaP prostate cancer cells [10]. IL-6Rα was found to localize to the lipid raft compartment in LNCaP cells, isolated by sucrose gradient centrifugation and identified by the raft-associated proteins flotillin-2 and $G_{i\alpha 2}$. Phosphorylated STAT3 was also found in the same fractions after IL-6 treatment, indicating that IL-6 signalling to STAT3 occurred through lipid rafts in prostate cancer cells [10].

In this issue, Woo *et al*. [11] found IL-6Rα and gp130 constitutively in lipid rafts, identified by the raft markers ganglioside GM1 and flotillin-1, after isopycnic sucrose gradient centrifugation in BV2 immortalized murine microglia cells. In addition to IL-6, which interacts with cells expressing IL-6Rα, activating the IL-6 classical signalling, they used hyper IL-6, a fusion protein of IL-6 and soluble IL-6Rα that mimics IL-6 *trans*-signalling, as a tool to analyse IL-6 *trans*-signalling. Incubation with IL-6 and hyper IL-6 induced a shift of IL-6Rα and gp130 to lipid rafts showing lower density and an increased level of cholesterol [11], suggesting the formation of raft platforms highly enriched in cholesterol. Lipid rafts were identified by super-resolution structured illumination microscopy (SIM), through the use of two fluorescently labelled probes, namely cholera toxin subunit B (CTB), a widely used raft probe binding to the ganglioside GM1 enriched in lipid rafts, and the D4 domain of perfringolysin O, which selectively binds to membranes with an elevated cholesterol content [11]. Perfringolysin O (theta toxin; *θ*-toxin) is a cytolysin protein secreted by the Gram-positive anaerobe *Clostridium perfringens* that binds to cholesterol in the plasma membrane, forming oligomers and transmembrane pores that lead to cell lysis [12]. Perfringolysin has four domains and, among them, domain 4 (D4) is responsible for the membrane cholesterol-binding activity with no cytotoxicity [13], and thus, fluorescent-tagged D4 can be used to label membranes with a high content of cholesterol.

Large raft platforms highly enriched in cholesterol as scaffolds in cell signalling

Incubation of BV cells and primary microglia with IL-6, and of hyper IL-6 with astrocytes, which, unlike microglia lack IL-6Rα, led to cholesterol-dependent STAT3 phosphorylation and to the formation of low-density raft fractions significantly enriched in cholesterol, both processes being blocked by cholesterol depletion. These cholesterol-rich raft platforms could be identified by SIM, using the D4 domain of perfringolysin O [11]. The size of the D4 rafts increased to the micrometre scale following cell treatment with IL-6 or hyper IL-6, as well as the size and co-localization of IL-6R and gp130 signals and STAT3 phosphorylation in the D4 rafts [11]. Taken together, these data strongly suggest that D4 rafts act as platforms for the assembly of functional IL-6 signalling.

A major inference of these studies is that cholesterol-rich raft platforms with restricted mobility of proteins serve as scaffolds to accrue the amount of proteins required for driving cell signalling, thus promoting their interaction and oligomerization. In this regard, cholesterol-rich raft platforms facilitate the recruitment and dimerization of gp130 proteins crucial for the triggering of IL-6 signalling, in both *cis*- and *trans*-signalling (Fig. 2). On the other hand, another example of the link between raft platforms, protein interaction and signaling includes that cholesterol-rich raft platforms facilitate the recruitment of procaspase-8, which interacts, through its two death effector domains (DEDs) in tandem with the corresponding homologous domains of the adaptor protein Fas-associated death domain protein (FADD) and other procaspase-8 molecules during the Fas/CD95 death receptor-mediated extrinsic pathway of apoptosis [9]. Cholesterol-rich raft platforms behave as raft-mediated supramolecular entities and efficient hubs for protein recruitment to facilitate and modulate signalling processes, including those closely related to inflammation and cancer [9]. Thus, cholesterol-rich raft platforms could be interesting targets in the inflammation–cancer interplay (Fig. 1). In this regard, IL-6, including both the cytokine itself and the ensuing IL-6 signalling, as well as inflammatory cells and cholesterol-rich raft platforms constitute major players in the interplay between inflammation and cancer, thus becoming promising therapeutic targets for the treatment of inflammatory and malignant diseases (Fig. 1).

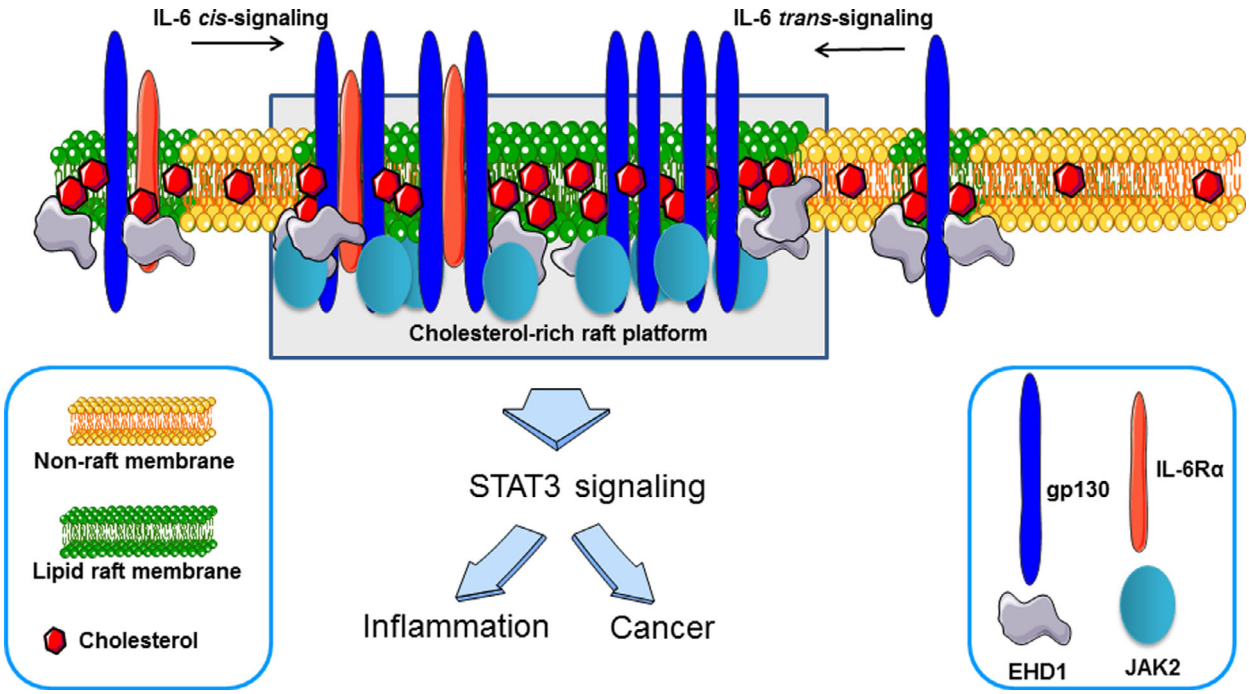

Fig. 2. Involvement of cholesterol-rich raft platforms in IL-6 signalling. Classical *cis*-signalling (triggered by IL-6) and *trans*-signalling (triggered by IL-6/sIL-6Rα) are mediated by the generation of cholesterol-rich raft platforms that act as scaffolds for the assembly of functional IL-6 signalling complex. EHD1 promotes and stabilizes cholesterol-rich raft platforms. See text for further details.

EHD1 in the formation of cholesterol-rich raft platforms

How do lipid rafts coalesce into larger domains? Initial assays based on the copatching of cross-linked raft components and its dependence on cholesterol led to the suggestion that raft clustering and partitioning could be modulated by protein oligomerization [14]. In the case of IL-6 signalling, Woo *et al.* [11] found that Eps15 homology domain-containing protein 1 (EHD1), a regulator of the vesiculation of cholesterol-rich membranes [15], was involved in D4 raft formation (Fig. 2), as EHD1 knockout and silencing inhibited STAT3 phosphorylation and recruitment of gp130 and JAK to D4 rafts in BV2 cells [11].

Conclusion

Lipid rafts are mobile membrane structures that can be clustered in response to different stimuli in order to form large signalling platforms, able to harbour temporal accrual of signalling proteins. An increasing number of signalling processes are being found to be dependent on their assembly in cholesterol-rich raft platforms for optimal activation. This suggests that different raft platforms can be generated for the triggering of distinct signalling pathways. Cholesterol is critical in forming and stabilizing these raft platforms. This highlights the importance of cholesterol metabolism and transport, as well as of cholesterol-interacting proteins in dissecting the biological and molecular underpinning of the formation of cholesterol-rich raft platforms. Because raft platforms serve as scaffolds for IL-6 signalling and other signalling processes modulating cell fate, new insights in understanding how these cholesterol-rich platforms are generated, and which proteins determine the size and type of these membrane domains, such as EHD1 in IL-6 signalling, could provide new therapeutic targets for a variety of pathological states, including inflammation and cancer.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

FM wrote the manuscript.

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