Editorial

The plasma membrane: a catalyst in the decision to die or not to die?

Patrick Legembre

Faustino Mollinedo

Death receptor-mediated cell death

Induction of cell death plays a crucial role in morphogenesis, homeostasis, immune tolerance and surveillance and chemotherapy. Supernumerary, damaged, transformed, or infected cells can be eliminated through intrinsic or extrinsic cell death programs. The induction of the extrinsic signal occurs when cytokines (*i.e.*, CD95L, TRAIL, TNF-α) present in serum or anchored in immune cells (*e.g.*, T-lymphocytes, natural killer cells) bind to their respective death receptors, namely CD95 (also called APO-1 or Fas), DR4 and DR5 or TNF-R1. Interaction with the ligand orchestrates aggregation and conformational alteration of the death receptors, whose intracellular domains recruit adaptor proteins (*i.e.*, TRADD, FADD), which in turn drive, through protein/protein interactions, the induction of caspases evoking the death program.

Plasma membrane and "death receptor" signaling pathways

The extensive characterization of the protein/protein interactions promoting the ignition of the apoptotic signal, led to underestimate the role played by the lipid bilayer plasma membrane in the signaling induced by death receptors. In this regard, death receptors are anchored into a 3-D support, the plasma membrane, which exhibits a complex structure and exerts constraints affecting receptor motility, aggregation, conformation and consequently signaling. Recent evidences established that not only the lipid composition of the membrane bilayer, but also the partition of the death receptors into subdomains designated lipid rafts, detergent-resistant

membranes (DRMs), or merely microdomains may affect the transmission of the apoptotic pathway. For instance, distribution of CD95 into lipid rafts dramatically enhances the induction of the signal [1-6]. More strikingly, not only the ligand fixation is able to redistribute CD95 into lipid rafts, but also different anti-tumoral agents have been reported to achieve the partition of CD95 into large platforms constituted of aggregated lipid rafts [2, 7-12]. It is noteworthy that the reorganization of CD95 into DRMs can occur independently of its ligand upon addition of certain chemotherapeutic drugs (*e.g.*, rituximab [13], resveratrol [9, 14], edelfosine [7, 11], aplidin [15, 16], perifosine [15, 16], cisplatin [12]). The molecular mechanisms that underlie this process remain to be elucidated. Nevertheless, the current evidence let us to envision that intracellular signal(s) or modulation of the plasma membrane biophysical properties mimic the death cytokine-driven initial events [17].

It is noteworthy that designation of these receptors as "death receptors" originated from initial studies that were seeking for apoptotic inducers [18, 19]. However, this appellation leads to a misunderstanding since all death receptors are able to induce non-apoptotic signals that, in certain context, promote carcinogenesis [20-23]. While theoretically speaking, decrease in the apoptotic threshold has been reported to switch the CD95 signal from a non-apoptotic to an apoptotic signaling pathway [24-26], it remains to identify the molecular mechanism(s) underlying this phenomenon. Endocytosis of CD95 may reduce the apoptotic threshold and thereby, to discriminate between apoptosis and non-apoptosis signaling. Indeed, cells harboring a mutation in the AP-2-binding motif of CD95 (*e.g*, Y291F) were not only unable to internalize the death receptor and to transmit the apoptotic signal, but they continued to induce non-apoptotic signals in the presence of CD95L [27]. In this topic issue, Milosavljenic and colleagues reported that endocytosis relies on the forces applied on the membrane and thus, on the composition of the membrane itself. Consequently, we may envision that the plasma membrane composition, which diverges between normal and tumor

cells, controls the fate of the CD95 signal, even if the role of endocytosis in death receptor signaling remains controversial [28, 29]. Likewise, the partition of CD95 into aggregated lipid rafts, whose micrometer-sized structure accumulates and/or excludes critical death modulators, may alternatively contribute to modulating the apoptotic threshold and thus, the cell fate. The partition of death receptors together with downstream apoptotic signaling molecules in aggregated DRMs [15, 16, 30-32] has led to the emerging concept of "liquid-ordered" plasma membrane platform designated as "cluster of apoptotic signaling molecule-enriched rafts" (CASMER) [33]. These CASMERs may reduce the apoptotic signal threshold by stabilizing protein/protein interactions and thereby, catalyze the transmission of the apoptotic signal [33].

Biophysical properties of the plasma membrane and "death receptor" signaling pathways

Another parameter regulating the biophysical properties of plasma membrane and its composition is the intracellular pH, which in turn alters the induction of the apoptotic signal induced upon death receptor engagement. Indeed, reduction of the intracellular pH not only modulates the plasma membrane composition by activating acidic sphingomyelinase, which in turn generates ceramides, but also promotes protonation of the lipid polar heads present in the inner leaflet. This latter effect reduces membrane packing and tension, which both influence drug permeation and membrane endocytosis, affecting the amounts of chemotherapeutic drugs retained in tumor cells and the death receptor signaling, respectively. In addition, numerous pollutants alter the biophysical properties of the plasma membrane through the production of reactive oxygen species (ROS), which in turn enhance lipid peroxidation [34], activate acid sphingomyelinase [35], and modulate gene expression involved in lipid metabolism, and thus modify plasma membrane composition.

In agreement with these notions, the fine regulation of ceramide synthesis (balance between hydrolysis of sphingomyelin and *de novo* biosynthesis) should be an essential player in promoting or impeding both immune response (death receptor signaling) and chemotherapy outcome (death receptor signaling and drug retention). In this regard, numerous patents cover the association of the apoptotic cytokines (*i.e.*, CD95L, TRAIL, TNF) or chemotherapeutic regimens with ceramide or its derivatives in order to re-sensitize malignant cells to death [36-43]. Nevertheless, it remains to define the appropriate length of the fatty acid linked to the sphingosine backbone, that will structurally impact the plasma membrane conformation in order to enhance endocytosis, accumulate drugs inside tumor cells, and/or catalyze the apoptotic signal triggered by death receptors.

This thematic issue tempts to provide some new insight into the potential chemotherapeutical roles of CD95 and TRAIL-R signaling pathways and their modulation by the biophysical properties of the plasma membrane.