Introduction

Cell survival must exist in equilibrium with cell death in order to maintain proper organ development and cellular and tissue homeostasis [1]. Several forms of cell death have been discovered and well characterized during the last years. One of the best studied form of cell death is apoptosis, which can be defined as an ordered and controlled way to program a cell to die. Apoptosis is essential for normal tissue turnover, development, differentiation and immune responses [2-4]. In apoptosis, a set of morphological features occur including chromatin condensation, nuclear fragmentation, cell shrinkage, plasma membrane blebbing and the formation of an apoptotic bodies. In contrast to the planned cell death in apoptosis, necrosis is another form of accidental cell death that results in a massive death of cells and disruption of normal homeostasis. It occurs upon exposure of cells to severe and overwhelming stresses like toxic compounds or high dose radiation. Recently, another form of ordered necrosis termed necroptosis has been described. This form of cell death serves central roles in development, cancer pathology, immunity and degenerative diseases [5-8]. It is regulated by receptor interacting protein kinase-1 (RIPK1), RIPK3, and mixed lineage kinase domain-like (MLKL) [9-11].

Mechanisms

Several death receptors can induce necroptosis: Fas, TRAIL1, TRAIL2 and TNFR-1. These receptors trigger specific pathogen recognition receptors like: TLR3 & 4 and DAI (the z-DNA sensor DNA-dependent activator of IFN-regulatory factors). Also they can activate T-cell receptors and IFNs type I and II. The binding of TNF to TNFR1 represents one of the best studied signaling pathways of necroptosis. This binding induces the formation of several protein complexes which involved in pro-inflammatory and survival signaling (complex I), apoptosis (complex IIa and
l1b) and necroptosis (complex IIC) [12-14]. Necroptosis induction by TNFR1 triggering is activated only when apoptosis signaling is blocked such as in down regulation or inhibition of caspase-8 or inhibitors of apoptosis [15]. Figure 1 [16].

For initiation of necroptosis, deubiquitylated RIPK1 binds to RIPK3 through RHIM domains (RIP homotypic interaction motif). This interaction leads to oligomerization of RIPK3 and its subsequent activation. Followed the activation of RIPK3 through phosphorylation, another effector protein called MLKL is activated and translocated to plasma membrane.

How MLKL is recruited into plasma membrane remains an enigma. Intense investigations carried out to explain the mechanisms by which MLKL is recruited to plasma membrane and executes death of the cell. Some studies showed that translocation of MLKL either to plasma membrane or to intracellular membranes involved with interaction PIPs (phosphatidylinositol phosphates) followed by oligomerization [17,18]. Shifting of MLKL from complex IIC to membranes employs lipids as essential components as PIPs, including PI(5)P and PI [4,5] P2, are required for MLKL membrane targeting. This was depicted as liposomes containing PI but not PIPs don’t result in MLKL-dependent leakage [18]. Moreover, interfering with the formation of PI(5)P or PI [4,5] P2 inhibits necroptosis but not apoptosis [17].

Natural products as pro-necroptotic agents in cancer therapy

A growing list of several natural products with distinct mechanisms of actions to employ necroptosis as a trigger of cell death were described. Of which, Shikonin, which is a naphthoquinone naturally occurring compound, triggers cell death with morphological features consistent with necroptosis that was inhibited in presence of Necrostatin-1 [19].

Shikonin exhibited a comparable potency toward drug-sensitive cancer cell lines as well as their drug-refractory variants with over expression of P-glycoprotein, MRP1, BCRP, Bcl-2, or Bcl-XL, suggesting that Shikonin can circumvent cancer drug resistance mediated by drug transporters or anti-apoptotic Bcl-2 proteins [19].

Another necroptosis-induced natural product is Obatoclax (GX15-070), which is a small molecule bipyrrrole compound that triggers necroptosis via formation of necrosome on autophagosomal membranes [20]. This complex assembly provide a connection between signaling pathways of necroptosis and Obatoclax-stimulated autophagy [20]. Obatoclax promote physical interaction between Atg5, a component of autophagosomal membranes with RIPK1 and RIPK3 (two key components of necrototic signaling) [20]. Additionally, autophagy-dependent necroptosis was found to overcome resistance toward glucocorticoids in childhood acute lymphoblastic leukemia (ALL) [21].

Staurosporine triggers necroptosis in leukemia cells when caspase activation is inhibited [22]. This natural product is an inhibitor of protein kinases and the Staurosporine dependent necroptosis is blocked by Necrostatin-1 (RIP1 inhibitor) and necrosulfonamide (an inhibitor of MLKL) [22]. In contrast, the enzymatic role of PARP1 was found to be dispensable for staurosporine-induced necrototic cell death [22].

BI2536 is another cancer specific necroptotic-trigger natural product. It is a small-molecule inhibitor of the mitotic kinase polo-like kinase 1 (Plk1). It initiates cell death by employing necroptosis in androgen-resistant prostate cancer cells [23]. This was concluded from experiments that showed: (1) Necrostatin-1 (an inhibitor of RIP1 kinase) attenuates cell death induced either by the Plk1 inhibitor BI2536 or by Plk1 silencing, (2) in cells where BI2536 induced cell death, there was no caspase activation, and (3) Using live cell imaging techniques, the morphological features of cell death was consistent with necrototic cell death [23]. So, using BI2536 as an inhibitor of Plk1 during mitotic progression results in mitotic catastrophe that ends with cell death by necroptosis.

FTY720 is a sphingolipid analog that mimics ceramide. It induces necroptotic cell death by causing changes in lipid signaling [64]. FTY720 binds to inhibitor 2 of PP2A (I2PP2A/SET) in the nucleus. I2PP2A is an oncoprotein that inhibits PP2A (tumor suppressor enzyme protein phosphatase 2A) [24]. Upon binding of FTY720 to I2PP2A/SET, it reactivates PP2A and results in RIPK1-dependent necroptosis and suppression of lung cancer [24]. To confirm if the cell death was due to necroptosis, the authors used Necrostatin-1 to inhibit RIP1 as well as knockdown of RIPK1 or PARP in this type of cell death [24].

In addition to previously mentioned compounds, there are some apoptotic-induced agents that have been shown to induce necroptosis under certain conditions. For example, at acidic extracellular pH, TRAIL (the death receptor ligand) was shown to induce necroptosis in colon and hepatocellular carcinoma cells [25]. RIPK1 or PARP1 pharmacological inhibitors as well as genetic blockage of RIPK1 or RIPK3 results in reduction of TRAIL-mediated necroptosis. Moreover, these interventions reduced intracellular PARP-dependent ATP depletion, indicating that both RIPK1 and 3 act upstream of PARP in this type of cell death [25]. In a mouse model of concanavalian A (Con A)-caused murine hepatitis (depend on TRAIL and Natural Killer (NK) T-cells for death of mouse hepatocytes), the liver injury was shown to be correlated positively with PARP1 activity. These effects was rescued by the inhibition of RIP1 or PARP1 [25].

Conclusion

In conclusion, necroptosis can be triggered in drug-resistant cancers that fail to induce apoptosis. Therefore, induction of
necroptosis confers benefits in killing cancer cells and in improving immune responses to molecules released from dying cells even if these molecules can also promote neoplasia. Rhabdomyosarcoma exposed to Obatoclax results in autophagy that results, in turn, in activation of RIP3 and necroptotic cell death [26]. Of particular importance, in these cells caspase-8 didn’t inhibit RIP3 activation in autophagosome driven necroptotic process, unlike receptor mediated necroptosis [26]. These data highlight that understanding the cross talk between necroptosis and other cell death types is a prerequisite for selecting optimal treatments customized to specific types of cancer and necroptosis associated diseases. Finally, a better understanding of the molecular events that regulate necroptotic cell death in different types of human cancers is expected to provide exiting novel opportunities in the coming years for therapeutic exploitation of cell death programs for the treatment of cancer.

References


