The Role of the Ripoptosome

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Recent research has led to the discovery of a protein complex capable of inducing cell death in cancer cells. The circumstances under which the complex forms, its physical structure, the mode of action of the complex, and the effects of regulatory proteins, as well as the implication of this discovery in terms of the future treatment of cancers are of particular interest.

Introduction
One of the defining characteristics of cancer cells that make them especially difficult to treat is their resistance to normal programmed cell death. In addition, cells have the potential to utilize multiple cell death pathways depending on the circumstance (Long and Ryan, 2012). This makes understanding the mechanisms by which cancer cells evade these cell death pathways of critical importance in the development of more effective therapies in treating cancers. A large cell death inducing complex named the ripoptosome was recently discovered. It consists of three main components (introduced below): RIP1, FADD, and caspase-8 and assembles spontaneously in response due to depletion in IAPs (Darding and Meier, 2012). The formation of the ripoptosome occurs independently of other common regulatory events and is regulated by cFLIP (Feoktistova et al, 2011).

Recent Progress
In studying cell death pathways, a new cytosolic signaling complex was discovered that ultimately causes cell death. This complex was determined to have unique qualities that differentiate it from other similar, previously identified complexes and was named the ripoptosome due to the essential role of one of the major components of the complex, the Receptor Interacting Protein kinase 1 (RIP1). RIP1 is a critical mediator of cellular stress and causes the aggregation of signaling complexes in response to the activation of cell-surface receptors. After the assembly of these signaling complexes, RIP1 acts by producing signals that promote either the survival or death of the cell, depending on various cellular conditions (Feoktistova et al, 2011; Tenev et al., 2011).
In addition to RIP1, ripoptosome contains two other major components: (i) the Fas Associated protein with Death Domain (FADD), an intermediate adaptor molecule that transmits signals from several death receptors, and (ii) caspase-8, a member of the family of proteins that serve roles in the execution phase of cell apoptosis (Tenev et al., 2011).

The importance of the roles of both caspase-8 and RIP1 in the effectiveness of the ripoptosome in stimulating cell death (Feoktistova et al, 2011; Tenev et al., 2011) was illustrated by separate experiments in which RNA interference (RNAi) techniques were utilized in order to silence the expression of the genes that encode these proteins (also called RNAi-mediated knockdown). In both cases, knockdown of these genes suppressed cell death by etoposide. In addition, it was determined that the formation of the ripoptosome and the subsequent induction of cell death occurs independently of other...
common regulatory mechanisms (Tenev et al., 2011). Because cancer cells that are sensitive to SM lead to the production of autocrine Tumor Necrosis Factor (TNF), a cytokine capable of inducing apoptosis by way of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) protein complex upon depletion of cIAPs, it was necessary to determine whether cell death was due to the activity of TNF or to the effects of the ripoptosome. Therefore, a dominant-negative form of the gene that blocks induction of TNF was expressed and this did not suppress cell death, suggesting that cell death was a result of the effects of the ripoptosome (Tenev et al., 2011). In addition, cell death was not suppressed when antibodies that block TNF, TNF-related apoptosis-inducing ligand (TRAIL), and cluster of differentiation 95 ligand (CD95L) are present, even in high concentrations, emphasizing that cell death is not dependent on these death ligands. Additionally, as the overexpression of the apoptosis regulator gene B-cl2 did not negatively affect the formation of the ripoptosome, the ripoptosome likely forms independently of the mitochondrial pathway (Tenev et al., 2011).

The identification of RIP1, FADD, and caspase-8 as a complex was substantiated using a reciprocal pull-down assay, an in vitro method that is often used to determine the relationship between two or more proteins. Mass spectrometry was also used to determine binding sites and immunoprecipitation (Tenev et al., 2011).

It was determined that the kinase activity of RIP1 is crucial to the assembly of the ripoptosome complex, as well as the cell death (Feoktistova et al., 2011; Tenev et al., 2011). This conclusion was reached after implementation of an inhibitor of RIP1 kinase called Necrostatin-1 (Nec-1) prevented the formation of the ripoptosome. RIP1 has the ability to cause cell death by way of caspase activation or necroptosis, so it was necessary to determine the specific mechanism by which cell death occurred. In order to accomplish this, a Fluorescence-Activated Cell Sorter was utilized in order to measure the permeability of the cell membrane, which in the process of necroptosis becomes increasingly permeable before bursting (Tenev et al., 2011). It was discovered that the pathway of cell death was dependent upon the cell type in question.

As previously mentioned, cIAP1, cIAP2, and XIAP work in conjunction to suppress formation of the ripoptosome (Darding and Meier, 2012), as evidenced by the experiment which showed that knockdown of each of these three proteins was required in order to produce the maximum formation of the ripoptosome complex. Furthermore, inhibitors of apoptosis proteins (IAPs) inactivate the ripoptosome by way of ubiquitylating the RIP1 and caspase-8 components of the ripoptosome (Tenev et al., 2011).

Cellular FLICE (FADD-like IL-1beta-converting enzyme)-inhibitory protein (cFLIP), which inhibits the apoptosis action of death ligands, was found to be a significant regulator of ripoptosome formation. Knockdown of this protein drastically increased the effectiveness of cell death signals even in the presence of cIAPs, though their effect increased still more upon depletion of the cIAPs (Feoktistova et al, 2011). Notably, two different isoforms of cFLIP (cFLIP<sub>L</sub> and cFLIP<sub>S</sub>) regulate cell death by the ripoptosome in contrasting ways, with cFLIP<sub>L</sub> suppressing cell death and cFLIP<sub>S</sub> promoting cell death by the ripoptosome (Feoktistova et al, 2011).

Discussion
As evasion of cell death is one of the characteristics that drastically complicates the treatment of cancers, a thorough understanding of the methods by which cancer cells bypass death and the modes of action of effective drugs in treating cancers are of great importance in the formulation of future cancer therapies. The potential of using joint therapies to treat cancers remains a promising prospect. While researchers have gained insightful knowledge on the formation and action of the ripoptosome complex, there are still many questions that remain unanswered. Among the most pressing is the determination of the exact action of the cytotoxic agent etoposide and its relationship with cell death inducing platforms such as the ripoptosome (Tenev et al., 2011). The interactions and associations between the ripoptosome and other cell death signaling complexes also remain unclear (Feoktistova et al, 2011).

References