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C-FLAR / C-FLICE: Thecaspase-8 modulator c-FLIP

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ABSTRACT
Cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP) is a major antiapoptotic protein and an important cytokine and chemotherapy resistance factor that suppresses cytokine- and chemotherapy induced apoptosis. c-FLIP is expressed as long (c-FLIPL), short (c-FLIPS), and c-FLIPR splice variants in human cells. c-FLIP binds to FADD and/or caspase-8 or -10 and TRAIL receptor 5 (DR5). This interaction in turn prevents Death-Inducing Signaling Complex (DISC) formation and subsequent activation of the caspase cascade. c-FLIPL and c-FLIPS are also known to have multifunctional roles in various signaling pathways, as well as activating and/ or upregulating several cytoprotective and pro-survival signaling proteins including Akt, ERK, and NF-κB. In addition to its role in apoptosis, c-FLIP is involved in programmed necroptosis (necrosis) and autophagy.

I. INTRODUCTION

The homeostasis of our tissues, organs,and whole body is maintained by theContinuous flow of birth, growth, differentiation, and death of cells. Cell death is therefore the indispensable component of our life, and several sophisticated cell death-inducing machineries are installed in the genomes of multicellular organisms.Various cell death signaling pathways have been described that are regulated at several levels. While the mitochondrion is the initiation point of these signals , there are several types of signaling platforms in cells that can initiate cell death. These complexes include the death-inducing signaling complex (DISC)], TNF complex, apoptosome , PIDDosome , and Ripoptosome . Interestingly, the c-FLIP isoforms, c-FLIPL, c-FLIPS, and c-FLIPR, regulate apoptosis [by interacting with the death signaling complex downstream of TNF-α receptors, Fas, and TRAIL receptors 1 (DR4) and 2 (DR5), necroptosis, and autophagy]. One physiological function of apoptosis is to kill and remove virus-infected cells in order to protect the hosts from viral propagation. To escape from the host's protective machinery, some viruses express anti-apoptotic proteins to prevent the host cells from apoptotic cell death. In 1997, Thome et al. identified viral FLICE-inhibitory proteins (vFLIPs), which contained two death effector domains (DEDs) and interfered with apoptosis signaling through death receptors. As vFLIPs were highly similar to the N-terminus of procaspase-8 (also known as FLICE, MACH or Mch-5), it was assumed that these viral genes might be derived from host gene. In this Review we, I discuss (1) apoptosis signaling pathways and the role of c-FLIP isoforms as critical anti-apoptotic and drug resistance factors,
(2) Regulation of c- FLIP Expression, and upregulation in human cancer (3) the potential for improving the outcome of cancer therapy by targeting c-FLIP isoforms.

II. APOPTOSIS SINGALLING PATHWAYS

Many elements influence whether a cell will undergo apoptosis. Four cellular receptors induce apoptosis after ligation; they are the Fas receptor,p55 tumor necrosis factor (TNF) receptor,and TRAIL/APO 2-L (TNF-related apoptosis-inducing ligand) receptors 1 andFas Ligand (FasL), TNF, and TRAIL/APO 2-L, respectively, bind these receptors to initiate apoptosis. In the case of FasL and TNF, membrane-associated proteins may be cleaved by the action of matrix metalloproteases to release soluble ligands that maintain their biologic activity. It is unknown whether TRAIL/APO 2-L exists as a soluble molecule. Ligation of these death receptors recruits the adaptor proteins FADD (Fas-associated death domain) TRADD (TNF receptor-associated death domain), or both, which sequentially activate a family of cysteine proteases that cleave at aspartate residues (cysteine-dependent, aspartate-specific protease), or caspases.Caspases are synthesised as inactive zymogens and become activated after proteolytic removal of a terminal prodomain.Fourteen mammalian caspase family members have been identified, each with varying involvements in the regulation of apoptosis. For example, caspase 8 (FLICE) and caspase 3

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(CPP32) are involved in apoptosis mediated by Fas, p55 TNF receptor, and TRAIL/APO 2-L receptor ligation. Activated caspasescatalyse the cleavage of other caspases, which, in turn, activate various cellular proteases and endonucleases that cleave host cell structural and regulatory proteins and host nuclear DNA, ultimately causing the cell to undergo the morphologic and biochemical changes that are characteristic of apoptosis.

2.1Cellular FLICE-like Inhibitory Protein (c-FLIP) and its Function

Human cellular FLICE inhibitory protein (c-FLIP) gene shares sequence homology with FADD, procaspase-8, and procaspase-10 and is located on chromosome 2q33–34. c-FLIP has eleven slice variants at the mRNA level. Two ofthese slice variants are considered major splicing variants detectable at the protein level in various types of cells and have been extensively studied thus far. One splice variant is designated the long isoform of c-FLIP (c-FLIPL), which is a 55 kDa protein consisting of 480 amino acids, and the other major splice variant is the short isoform of c-FLIP (c-FLIPS), which is a 26 kDa protein consisting of 221 amino acids. The third form of c-FLIP, designated c-FLIPR, which is a 23 kDaprotein.c-FLIP isoforms are Death Effector Domain (DED)-containing proteins that are recruited to the DISC and regulate activation of caspases-8 and -10 in the death receptor signaling pathways.

III. REGULATION OF C-FLIP EXPRESSION

c-FLIP is regulated both at the transcriptional and posttranscriptional levels by various stimuli.A diverse group of transcription factors are known to transcriptionally regulate the c-FLIP gene.The transcriptional control of c-FLIP isoforms is quite complex and differentially regulated in various cells in a signals specific manner. While numerous transcription factors trigger c-FLIP transcription including NF-κB, AP-1 (cFos/c-Jun), P53, FoxO, CREB, NFATc2, EGR1, AR, SP1 [19,34,36,39-43], and p63 (NP63) [40], others induce repression of c-FLIP expression such as c-Fos, c-myc, FoxO3a, IRF5.

IV. UPREGULATION OF C-FLIP IN HUMAN CANCERS

c-FLIP has been found to be overexpressed in several types of malignancies and could be associated with cancer progression due to its ability to inhibit the apoptotic process. Elevated expression levels of c-FLIP have been reported in colorectal cancer , bladder urothelial cancer , cervical cancer , Burkitt's lymphoma , non-Hodgkin's lymphoma , Head and Neck Squamous Cell Carcinoma (HNSCC) , hepatocellular cancers , and high-grade prostatic intraepithelial neoplasia (HGPIN) with maximal c-FLIP expression detected in Castrate-Resistant Prostate Cancer (CRPC) . c-FLIPupregulation is also seenin gastric cancer and plays an important role in lymph node metastasis, which ultimately contributes to tumor progression.c-FLIP expression may serve as a potential cervical cancer progression marker. Recent results demonstrated that c-FLIP may play an important role in the potential of osteosarcoma to metastasize to the lung.evidence that c-FLIP may also serve as a prognostic biomarker in Acute Myeloid Leukaemia (AML). While overexpression of the c-FLIPS variant has been reported in human lung adenocarcinomas with low levels of E2F1, c-FLIPL protein expression was not altered.

V. C-FLIP AND CANCER TREATMENT

The roles of c-FLIP isoforms as proteins that cause resistance to pro-cell death signals, cytokines, and anticancer drugs have been established . Moreover, small interfering RNAs (siRNAs) that specifically silence the expression of c-FLIPL in diverse human cancer cell lines have been shown to enhance TRAIL-induced DISC recruitment and increase the efficacy of chemotherapeutic agents, thereby elevating apoptosis. Furthermore, small molecules causing degradation of c-FLIP as well as decreasing mRNA and protein levels of c-FLIPL and c-FLIPS splice variants have been found, and much effort is focused on developing other c-FLIP-targeted cancer therapies. c-FLIP therapeutic intervention aimed at inhibiting its transcription and post transcriptional changes is critical for developing anticancer agents . Because of significant resemblance to caspase-8 , c-FLIP protein is a very difficult target for drugs that inhibit its function, since small molecules capable of blocking its recruitment to the DISC would also likely inhibit recruitment of caspase-8 to this complex, thereby inhibiting apoptosis. Therefore, to reduce or inhibit c-FLIP expression, small molecules which target c-FLIP without inhibiting caspases-8 and -10 are needed. Diverse classes of agents that decrease c-FLIP expression and sensitize cancer cells to TRAIL or anticancer drugs have been reviewed . These include some conventional anticancer drugs includingcisplatin, doxorubicin, actinomycin D,

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cycloheximide, camptothecin, 9-NC, and topotecan; histone deacetylase (HDAC) inhibitors; the inhibitors of MEK1/2, PKC and PI3K, and numerous other compounds . These agents affect c-FLIP transcription, trigger c-FLIP degradation through the ubiquitin-proteasome system, or decrease c-FLIP translation. Moreover, DNA damaging agents are promising drugs with regard to downregulating levels of c-FLIP variants .Downregulating c-FLIP variants by small molecule therapeutics may be a potential strategy for developing agents to treat diseases in which this protein plays a role in preventing apoptosis, necroptosis, and autophagy. However, downregulation of c-FLIP in various cell types has induced significant cell death in healthy cells.

VI. CONCLUSIONS

c-FLIP is involved in inhibiting apoptosis, programmed necroptosis (necrosis) and autophagy. c-FLIP variants induce resistance to death receptor ligands and chemotherapeutic agents in various cancer cells. Moreover, c-FLIP upregulation has been correlated with a poor clinical outcome. Therefore, c-FLIP isoforms may serve as targets for counteracting therapy-resistant human malignancies. Hence, c-FLIP may be a clinically relevant biomarker and a molecular target for developing therapeutics for various diseases. Various classes of agents can downregulate c-FLIP expression. To reduce or inhibit c-FLIP expression, small molecules which target c-FLIP without inhibiting caspases-8 and -10 are needed. Compounds that inhibit or downregulate c-FLIP mRNA expression or cause degradation of c-FLIP at the protein level through proteasome degradation will be of particular interest.