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Harnessing of Programmed Necrosis for Fighting against Cancers

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Abstract

Chemotherapy has long been considered as one of useful strategies for cancer treatment. It is primarily based on the apoptosis that can selectively kill cancer cells. However, cancer cells can progressively develop an acquired resistance to apoptotic cell death, rendering refractory to chemo- and radiotherapies. Although the mechanism by which cells attained resistance to drug remains to be clarified, it might be caused by either pumping out of them or interfering with apoptotic signal cascades in response to cancer drugs. In case that cancer cells are defective in some part of apoptotic machinery by repeated exposure to anticancer drugs, alternative cell death mechanistically distinct from apoptosis could be adopted to remove cancer cells refractory to apoptosis-inducing agents. This review will mainly deal with harnessing of necrotic cell death, specifically, programmed necrosis and practical uses. Here, we begin with various defects of apoptotic death machinery in cancer cells, and then provide new perspective on programmed necrosis as an alternative anticancer approach.

Key Words: Necroptosis, Apoptosis, Autophagy, Programmed necrosis, Chemotherapy

OVERVIEW

Cells continuously strive to maintain the balance of survival and death from interior or exterior situations. Cells consume most energy to keep homeostasis, and its disturbance is more manifested in cancer cells (Buzea *et al*., 2007). Transformation of normal cell into cancer cells is characterized by loss of cell cycle and resistance to apoptosis as well as other phenotypic properties (Markert, 1968). These phenotypic alterations are related to malignant neoplasia. Therefore, the principal way for cancer treatment is to render cancer sensitive to chemotherapy, consequently causing programmed cell death apoptosis (Strasser *et al*., 2000; Dy and Adjei, 2002; Elmore, 2007). Apoptosis is a naturally occurring process in developing and adult tissues, but not for cancer. Moreover, dying cells by apoptosis are engulfed by neighboring cells or immune cells, leaving no danger debris that can provoke inflammation. Therefore, the apoptotic control of cancer cells with cancer drugs is considered as the most rational therapy. However, a large number of cancers have developed evasion mechanisms to chemotherapy (Gottesman, 1993, 2002). Since defects in cell death machinery limit the feasibility of clinical apoptosis inducer, novel regimens to improve clinical outcomes are being taken into consideration. Cell-based anticancer therapy has currently taken advantage of apoptosis, but another programmed cell death modes including autophagy and necroptosis have gained attention as potential therapeutic approaches. Since failures in the cell death process are frequently found in various cancers, key proteins governing cell death type could be used as therapeutic targets for a wide range of cancer. Therefore, molecular understanding of evasion mechanisms of cancer cells to death stresses could surely provide a customized strategy for choosing the cell death modality suitable for fighting cancer. This review will briefly begin with features of cell death types and crosstalk between cell death modes. Then, we particularly highlight state of the art reports on programmed necrosis, a specialized necrotic cell death and outlook of its clinical use for controlling cancers resistant to conventional chemotherapy.

CELL DEATH MODALITIES AND IMPAIRED CELL DEATH IN CANCERS

To begin with, general concepts and molecular machinery for cell death types were briefly described. Concomitantly, evasion mechanisms of cancer cells to cancer therapy based on cell death were dealt in this review. Regarding ineffective cancer treatment, defective death signals in various cancers were listed in Table 1.

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Table 1. Defective cell death found frequently in cancers

Defective proteins	Death type	Cells	References
Bcl-2, Bcl-xL, Survivin, Bax, APAF-1	Apoptosis	Various cancer cells	(Basu and Haldar, 1998; Lowe and Lin, 2000; Nelson *et al*., 2004; Strasser *et al*., 2000)
Caspase-9s, HSPs	Apoptosis	Non-small cell lung cancer/ many cancers	(Speirs *et al*., 2011)
Caspase-8	Apoptosis	Neuroblastoma/glioblastoma multiforme	(Hurst and Welch, 2011; Martinez *et al*., 2007)
Caspase	Apoptosis	CD34+ hematopoietic stem cells	(Mohr *et al*., 2005)
FLIP	Apoptosis	Jurkat lymphoma and MCF7 breast cancer cell lines	(Zobalova *et al*., 2008)
Beclin-1	Autophagy	Various cancer cells	(Gozuacik and Kimchi, 2004; Mizushima, 2007; Muzes and Sipos, 2012; Yang *et al*., 2011)
Atg7	Autophagy	A549-B480	(Shen *et al*., 2010)
PARP	Necroptosis	Various cancer cells	(Wang and Weaver, 2011)
HMGB1	Necroptosis	CT-26 MCA205	(Yamazaki *et al*., 2014)

APOPTOSIS

Classical programmed cell death apoptosis has been well studied for four decades. Apoptosis can mainly be executed via intrinsic or extrinsic pathway according to the way that death signal initiates within or outside cell (Elmore, 2007; Jha *et al*., 2007). Intrinsic pathway is generally activated in response to diverse signals resulting from DNA damage, loss of cell-survival factors, or other types of severe cell stress. Normally, pro-apoptotic proteins are released from the mitochondria to activate a cascade of caspases, consequently leading to apoptosis. Its pathway is tightly regulated by a variety of other factors including Bcl-2 family proteins such as the mitochondrial anti-apoptotic proteins Bcl-2 and Bcl-xL, and pro-apoptotic proteins Bcl-2-associated X (Bax) and Bak. In various cancers, however, alteration and defects in the components of intrinsic pathway are identified (Elmore, 2007). Cancers themselves have developed refractory mechanisms to apoptotic stresses by overexpressing anti-apoptotic proteins or causing defects in pro-apoptotic proteins (Basu and Haldar, 1998; Strasser *et al*., 2000; Nelson *et al*., 2004). In fact, it has been reported that increased levels of Bcl-2, Bcl-xL or survivin correlate closely with drug resistance to chemotherapeutic agents in many cancer cell lines. Moreover, decreased expression or mutations of pro-apoptotic proteins such as Bax and APAF-1 contribute to chemotherapy resistance (Lowe and Lin, 2000). Besides, high expression of caspase-9S and heat shock proteins (HSPs), which can interfere with apoptotic signaling, has been observed in many malignant cancers (Speirs *et al*., 2011). Extrinsic pathway is triggered by ligand binding to death receptors, which can promote the formation of the death-inducing signaling complex (DISC). Sequentially, caspase-8 in turn activates effector caspases including caspase-3. Regulation of the extrinsic pathway can be achieved by cellular FADD-like interleukin-1 beta-converting enzyme (FLICE)-inhibitory protein (cFLIP), which prevents caspase-8 recruitment to the DISC. Upon DISC activation, the same effector caspases as used in the intrinsic pathway are activated for mediating apoptotic cell death. Additionally, inhibitors of apoptosis proteins (IAPs) and the second mitochondria-derived activator of caspases (Smac) affect negatively or positively apoptotic cell death (Deng *et al*., 2002). For instance, the x-linked IAP (XIAP) inhibits caspase-3 and -9 and is inversely inhibited by Smac. As deduced from extrinsic pathway, it is readily supposed that alterations of ligand binding confer the resistant mechanism to cells derived from multiple cancers. Both downregulation of receptor and the overexpression of decoy receptor can abolish the extrinsic signal pathway to apoptosis. Apart from aberrant expression of death receptor and decoy receptor, mutations and silencing in caspase-8 fail to convey death signals from ligation of death receptor to downstream signaling molecules. Such defects in caspase-8 are remarkable in neuroblastoma and glioblastoma multiforme (Martinez *et al*., 2007; Hurst and Welch, 2011). Also, deregulated apoptosis in hematopoietic system can cause the development of immune deficiencies and leukemia. CD34+ hematopoietic progenitor stem cells (HSCs) are constitutively refractory to CD95-mediated apoptosis. It is revealed that HSCs do not express caspase-8, a key player of the DISC of death receptors (Mohr *et al*., 2005). Moreover, it has been documented that cancer stem cells (CSCs) are resistant to apoptosis induction (Zobalova *et al*., 2008). Cell lines that express highly CD133 are more resistant than those harboring low CD133. It was concluded that FLIP conferred resistance to apoptosis mediated by the death ligand TRAIL.

AUTOPHAGY

Autophagy is regarded as the basic catabolic mechanism associated with cell degradation of unnecessary or dysfunctional cellular components through the lysosomal machinery. However, the physiological significances of autophagy present a wide range of spectra from cell survival, immunity to cell death. Largely, autophagy is classified into macroautophagy and compartment-specific autophagy (Amaravadi and Thompson, 2007; Yang and Klionsky, 2010). Macroautophagy is initiated by the formation of a double membrane to engulf the cytosol, followed by fusion with a lysosome to form an autophagolysosome (Burman and Ktistakis, 2010; Levine *et al*., 2011). Subsequently, the contents within resulting vesicles are enzymatically degraded. Compartment-specific autophagy in-

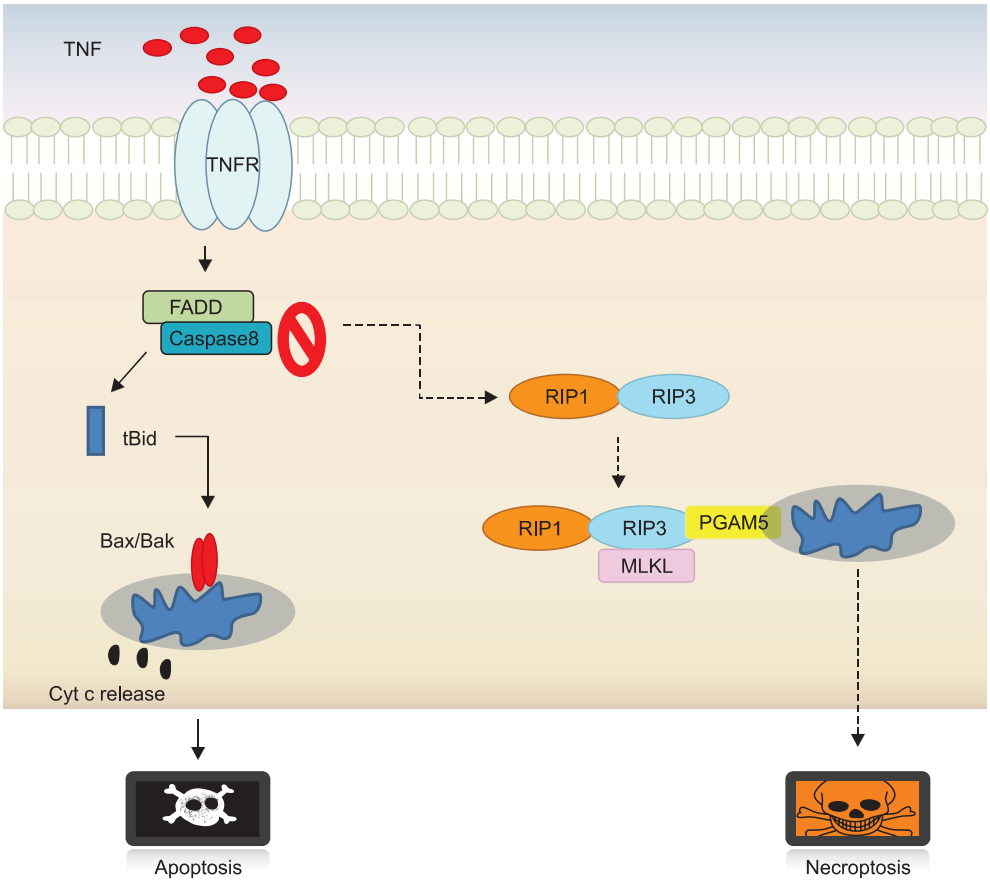

Fig. 1. Molecular switching from apoptosis to necroptosis. Once cells are stimulated with TNFα, FADD and caspase-8 dissociated from membrane form of TNF receptor reconstitutes a cytoplasmic complex and active caspase-8 within complex cleaves Bid that cause to transform Bax/Bak into multimers in mitochondria. Afterward, a series of downstream events result in default apoptotic death. In contrast, under the circumstances that apoptosis is blocked or hindered by chemical or biological factors, cells activate a back-up cell death programmed necrosis in an active and ordered fashion by a cascade of signaling pathway. In fact, RIP1 interacts physically with RIP3 to trigger consecutive downstream signaling events including MLKL and PGAM5 recruitments, which transmit cytosolic death signals to mitochondria.

cludes chaperone-mediated autophagy (CMA) and mitophagy (Speirs *et al*., 2011; Nordgren *et al*., 2013). CMA is responsible for the degradation of approximately 30% of cytosolic proteins in tissues. It is a very complex and specific pathway by which heat shock cognate protein of 70 kDa (hsc70)-containing complex recognizes and binds to substrate proteins. This resulting complex moves then to the lysosomal membrane-bound protein, allowing the substrate proteins into the lysosomal matrix, where those proteins are completely degraded by the proteases. Mitophagy is referred to as the autophagy of damaged mitochondria that specifically caused by starvation. Its process begins with the release of mitochondrial intermembrane proteins. In general, autophagy indicates macroautophagy unless otherwise specified. As for regulation, autophagy-related proteins are representatively mammalian target of rapamycin (mTOR) and Beclin-1. Atg13/Ulk1/FIP200 complex is prerequisite for autophagosome formation and can be inhibited by a cascade of PI3K/Akt/mTOR pathway through Atg13 phosphorylation. Beclin-1 is a tumor suppressor that activates autophagy together with UV irradiation resistance-associated gene (UVRAG) and endophilin B1 (Bif1). Autophagy was initially regarded as a prosurvival function due to its compensatory tendency to maintain ATP deficiency. Recent studies support that autophagy also plays a significant role in promoting cell death. In fact, not only excessive autophagy leads to cell death, but also autophagy activates apoptosis during HIV infection. There is another report that autophagy selectively degrades survival factors, reducing cell viability. Autophagy-related genes have been suggested to act as tumor suppressors, so that autophagy defects are found in a variety of tumors (Gozuacik and Kimchi, 2004; Mizushima, 2007; Yang *et al*., 2011; Muzes and Sipos, 2012). Deletions and mutations in Beclin-1 gene as well as its binding molecules were frequent identified in cancer patients. Meanwhile, once Bcl-2 bound to Beclin-1, autophagy is inhibited to increase cell survival concomitantly. Therefore, facilitating dissociation between Bcl-2 and Beclin-1 could be an attractive approach for inducing autophagic cell death in cancer patients. Additionally, it was documented that defective LC3 distribution was closely correlated with the capability of cells to be refractory to microtubule-stabilizing agent epothilone B. This consequence was attributed to complete loss of Atg7, essential for autophagy (Shen *et al*., 2010).

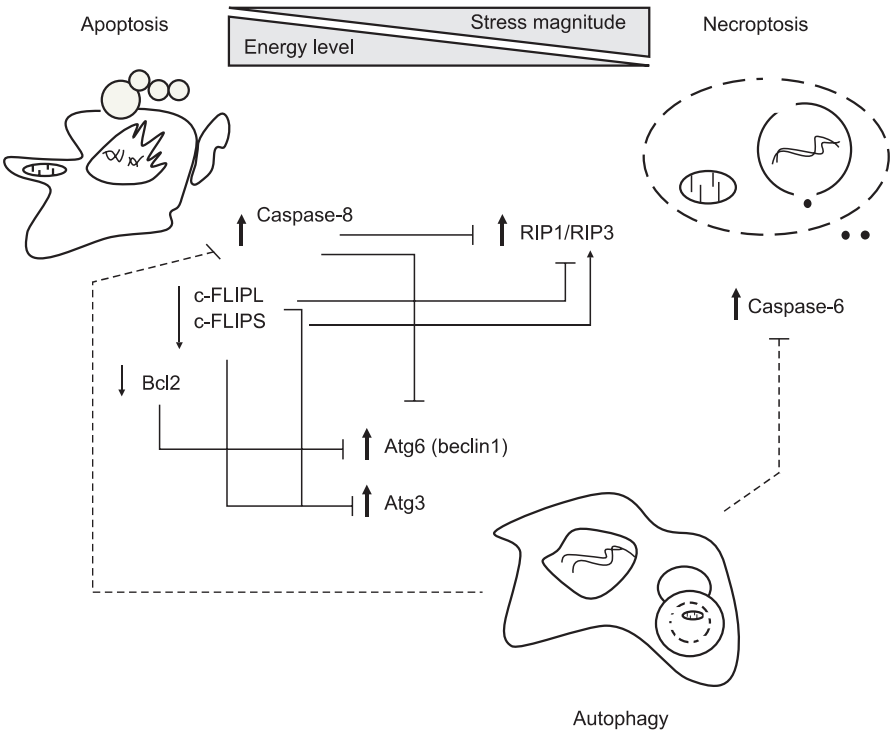

Fig. 2. Crosstalk between apoptosis, necroptosis and autophagy. Cells determines actively cell death modes to external stresses through integrated decision of various factors including stress amplitude, cellular energy levels and induction of survival signals. Generally, controlled stimuli trigger default cell death apoptosis in a well-organized way while a specialized cell death necroptosis is activated under the condition when some death signals can induce apoptosis but a part of apoptotic machinery is defective. In aspects of energy crisis, apoptotic cell death occurs in the energy state sufficient to fuel caspase activity while necroptotic cell death prevails in the energy-deficient conditions, and autophagic suppression facilitates necroptosis. Occasionally, autophagy can be induced when default apoptosis is suppressed, but consequent effects depends on death contexts, leading to counteracting or promoting necroptosis. As for molecular regulation, apoptosis-associated proteins caspase-8 cleaves both RIP1 and RIP3 to abolish necroptotic downstream signals. Another antiapoptotic regulator c-FLIPL prevents formation of ripoptosome complex consisting of RIP, FADD and caspase-8/10 whereas c-FLIPS facilitates its assembly. Meanwhile, autophagy can also degrade caspase-8 to impede apoptosis although apoptosis reciprocally inhibits autophagy through Bcl-2 mediated sequestration and caspase-dependent degradation of Beclin-1. Autophagy can suppress necroptotic cell death by downregulation of C-6. Therefore, interplay between cell death modalities is so complicated that death fate will be determined depending on the different circumstances. Solid and dotted lines represent if associated proteins are identified or not. Also, bold and plain arrows indicate that specific proteins function positively and negatively on associated cell death, respectively.

NECROPTOSIS

With typical features of necrotic cell death, cells are committed to die in an ordered and orchestrated manner when challenged with cytokines, infectious agents and chemicals. Such specialized cell death is referred to as type III programmed cell death, programmed necrosis or necroptosis. Necroptosis can be discriminated from other death types by caspase activation-defective and lysosome-independent characteristics (Kepp *et al*., 2011; Kaczmarek *et al*., 2013). Subcellular organelles including endosome, Golgi body and mitochondria are swollen at an early stage of necroptosis, later leading to functional loss of cell membrane. Receptor-mediated necroptosis and posttraumatic necrosis occur through a series of events: mitochondrial dysfunction, reactive oxygen species (ROS) generation, ATP depletion, calpains- and cathepsins-directed protein degradation. Finally, nuclear disintegration is accompanied by release of high mobility group box 1 (HMGB1), which invokes inflammation by recruiting inflammatory cells to damaged tissues. Necroptosis has been activated in an orchestrated manner via a cascade of signaling pathways (Fig. 1). Key mediators such as RIP1, RIP3, a mitochondrial phosphoglycerate mutase family member 5 (PGAM5) and mixed lineage kinase domain-like (MLKL) proteins for executing necroptosis have been identified through a genome-wide RNA interference screen. Necrotic cell death is commonly suppressed in cancer patients subjected to chemotherapy. Alkylating agent-induced poly (ADP-ribose) polymerase-1 (PARP-1) hyperactivation induces necrosis, although PARP-1 is ordinarily involved in repairing DNA damage. Accordingly, PARP inhibition in combination with standard chemotherapy can potentiate apoptotic cell death (Gaymes *et al*., 2009; Koster *et al*., 2011). By contrast, excessive activity of PARP-1 may be useful in the defective condition of apoptotic cell death machinery. In fact, patients with inactive retinoblastoma protein do not achieve clinical improvement from PARP inhibition. The primary screening for PARP mutations would be essential for decision-making of cancer therapy (Wang and Weaver, 2011). HMGB1 is released during necroptosis and at the late stage of other cell death. It can provoke an anticancer immune response as well, resulting in improvement of chemotherapy or radiotherapy. HMGB1-deficient tumors

compromise chemotherapy-induced immunogenic cell death. To strengthen chemosensitivity, therefore, their immunogenicity could be successfully altered by providing artificial TLR4 ligands (Yamazaki *et al*., 2014). Ligation of death receptor with TNFα also induces necrosis through complex formation and activation of RIPs. More interestingly, it has been reported that RIP1 or RIP3 mediates necroptosis independently (Cho *et al*., 2009b; Galluzzi *et al*., 2009; Christofferson and Yuan, 2010; Vandenabeele *et al*., 2010; Linkermann *et al*., 2012). Therefore, mutational profiles in RIP1 and RIP3 may be the key issues for harnessing necrosis as the alternative therapeutic force to defective cell death machinery.

SWITCHING AND REGULATION BETWEEN CELL DEATH MODES

When cells are challenged by various death stimuli, they are typically doomed to undergo one form of 3 different death modalities such as apoptosis, necrosis and autophagy. Cell death modes are believed to cross-talk each other via a web of signaling networks in a tightly regulated way (Orrenius *et al*., 2011). Over the last 30 years, a cascade of signaling pathways leading to apoptosis has been well documented when compared with other death types like autophagy and necroptosis. Recently, proposed is a new notion that a specialized back-up cell death necroptosis is activated under pharmacological inhibition of apoptosis or defective apoptotic machinery in response to TNFα stimulation. This is a typical example of cell death switching between apoptosis and necroptosis. Regulatory crosstalk between apoptosis, necrosis and autophagy was schematically depicted in Fig. 2. Notably, novel necroptosis regulators RIP1 and RIP3 were identified, and their interacting complex RIP1-RIP3 was proposed to cause cells to die in a necroptotic way. Once caspase 8 activated, however, necroptosis is suppressed due to the cleavage of RIP1 and RIP3. c-FLIP functions as a master antiapoptotic regulator by binding to FADD and/or caspase-8 and caspase-10 (Nakajima *et al*., 2006; Safa, 2012). It is expressed as splice variants c-FLIPL and c-FLIPS, which are known to have multifunctional roles in various signaling pathways (Safa and Pollok, 2011; He and He, 2013). Interestingly, it is also involved in modulating necroptosis positively or negatively. When cells are killed by genotoxic stress, a large cell death-inducing signaling platform (~2 MDa) referred to as ripoptosome is assembled. It consists of the core components such as RIP1, FADD and caspase-8/10. Specifically, c-FLIPL prevents formation of ripoptosome complex whereas c-FLIPS facilitates its assembly (Feoktistova *et al*., 2011). Moreover, the cellular inhibitor of apoptosis proteins (cIAPs) blocks ripoptosome formation by direct ubiquitination of its components (Feoktistova *et al*., 2011; Imre *et al*., 2011).

Although autophagy is initially activated as a protective responses to stimuli, persistent autophagy can result in cell death by excessive degradation of essential cellular proteins. Therefore, autophagy and apoptosis play opposing or accompanying roles in cancers. The cytoprotective role of autophagy was supported by the finding that inhibition of autophagy by chloroquine sensitized apoptosis-resistant tumor cells to cancer therapy in chronic myelogenous leukemia (CML) cell lines (Carew *et al*., 2007). Another evidence was suggested by the report that overexpression of Beclin-1 diminished TNF-related apoptosis-inducing ligand (TRAIL)-mediated death (Cho *et al*., 2009a). Mechanism study revealed that active caspase-8 in apoptosis-resistant tumor cells are targeted to autophagosomes to be degraded in lysosomes, nullifying activation of downstream apoptotic signals. Since autophagy inhibition has been generally believed to promote cancer survival, a combined treatment of autophagy inhibitor and chemotherapy directs cell death toward apoptosis (Hou *et al*., 2011; Rikiishi, 2012). Clearance of ER stress-misfolded proteins by autophagy limited the induction of apoptosis. Evidently, crosstalk between apoptosis and autophagy is suggested from the report that caspase-8 degrades Atg6 during receptor activated cell death (Kang *et al*., 2011). Conversely, autophagic degradation of caspase-8 disconnects death signals to the downstream apoptotic machinery (Rikiishi, 2012). Accordingly, autophagic cell death become manifested under conditions that apoptosis is inhibited (Yu *et al*., 2004; Boya *et al*., 2005). cFLIP and Bcl-2 can physically interact with Atg3 and Atg6, respectively (Gordy and He, 2012). Notably, both isoforms of c-FLIP interfere with autophagic process by preventing Atg3 from binding LC3 while antiapoptotic protein Bcl-2 inhibits Atg6-dependent autophagy. On the contrary, autophagy can augment apoptosis by replenishing ATP levels which can drive ATP-dependent apoptotic processes. In contrast, autophagy induction leads to apoptotic cell death. Rottlerin causes autophagic and apoptotic cell death in breast and prostate cancers via AMP-activated protein kinase (AMPK) activation, suppression of Akt/mTOR and activation of caspases (Kumar *et al*., 2013). Additionally, compound K, an active metabolite of ginsenosides, induces apoptosis and autophagy via ROS generation and JNK activation in colon cancer cells (Kim *et al*., 2013). It was demonstrated that its dual roles in apoptosis and autophagy were caused from disruption of interaction between Bcl-2 and Atg6. Interconnections between apoptosis and autophagy are corroborated by the finding that both death modes can cooperatively induce cell death in parallel or in sequence (Wang *et al*., 2013). Not only apoptosis but also autophagy is induced in arsenic trioxide-treated T-lymphocytic leukemia and imatinib-treated Kaposi's sarcoma, resulting in tumor remission (Qian *et al*., 2007; Jain *et al*., 2013). Under any circumstances, autophagy is thought to act upstream of apoptosis since cell death with autophagic features is found even in the inhibition of apoptosis. Suppression of autophagy by chemical inhibitors or siRNA knockdown of Beclin1 and Atg7 in turn leads to inhibition of apoptosis (Espert *et al*., 2006).

The interplay between autophagy and necroptosis appeared to be rather intricate and controversial. Its molecular interpretation remains elusive y*et al*though autophagy modulate necroptosis positively or negatively (Hammerova *et al*., 2012). That is, autophagy is required for necrotic cell death of acute neurodegenerative disorder such as ischemic stroke. By contrast, autophagy has been documented to antagonize TNFα-induced programmed necrosis (Tsuda *et al*., 2012). Moreover, zVAD mediates necroptosis via dual-inhibition of caspase and lysosomal cathepsins, which play key roles in undergoing apoptosis and autophagy, respectively (Wu *et al*., 2011). Inhibition of autophagy via the mTOR signaling pathway enhances zVAD-induced necroptosis while autophagic induction by starvation and chemical treatment protects cells from necroptosis (Chen *et al*., 2011). More recently, autophagy was reported to interplay with necroptosis via caspase-6 (Ye *et al*., 2013). Caspase-6 is required for TNFα-derived necroptosis,

Table 2. Harnessing of necroptosis for controlling cancers

Chemicals/Therapy	Target/Pathway	Stages	Comments	References
Imatinib	BCR-ABL	Clinical trial	Leukemia cell	(Okada *et al*., 2004)
PDT (photodynamic therapy)	ROS generation	Clinical application	Cancer cell defective of apoptosis	(Bown *et al*., 2002; Castano *et al*., 2006)
DNA alkylating agents	PARP-1	Proof of concept	Cells deficient in p53 or Bax/Bak	(Zong *et al*., 2004)
Deoxynyboquinone	NAD(P)H:quinone oxidoreductase 1 (NQO1)/PARP-1	Proof of concept	Pancreatic and lung cancers	(Huang *et al*., 2012)
Shikonin	Multitargets/RIP1-dependent	Proof of concept	Glioma	(Huang *et al*., 2013)

and autophagic inducer downregulates substantially caspase 6, resulting in suppression of necroptosis.

PRECLINICAL TEST OF NECROPTOSIS AGAINST CANCER

Most human cancers have mutations that disarm default cell death apoptosis to promise cancer survival. Accordingly, alternative cell death should be considered in order to effectively control apoptosis-resistant cancer cells. Various biological tests or preclinical trials of necroptosis for anticancer therapy were presented in Table 2. Okada group reported that leukemia cells with BCR-ABL positive cells were induced to cell death in a caspase-independent pathway when treated with an Abl kinase inhibitor Glivec (imatinib) (Okada *et al*., 2004). Imatinib-mediated necrosis is closely linked to release of HtrA2/Omi (serine protease) from mitochondria. Necrotic approaches have not been widely used to treat cancer cells due to inflammatory response. However, its clinical significance is increasing as a new emerging tool to overcome cancer with acquired anticancer drug-resistance. Harnessing of ordered necrotic death in clinical cancer therapy includes photodynamic treatment (PDT) and alkylating DNA-damaging agents. Primarily, selective localization of sensitization molecules to tumor sites is prerequisite for PDT. Upon illumination with light possessing specific wavelength spectra, ROS are generated by the photosensitizer, resulting in necrotic cell death. Therefore, its efficacy is dependent upon preferential accumulation of photosensitizer in tumor over normal and treatment of defined tumor area. PDT has the advantage that can selectively target the cancer cells resistant to apoptosis. In fact, apoptosis-resistant cells that overexpressing Bcl-2/Bcl-xL or xenografts of a breast cancer deficient in caspase-3 readily succumb to PDT (Bown *et al*., 2002; Castano *et al*., 2006). Also, given that PARP is suppressed, DNA damage-induced chemotherapy exhibits more outstanding efficacy. It could be inferred from the fact that PAPR is involved in repairing low levels of DNA damage. However, excessive activity of PARP-1 induces cell death through necrosis, so that it could be applicable when other cell death types are not activated properly. In fact, Treatment of cells with DNA alkylating agent causes PARP hyperactivation, leading to depletion of cellular NAD and ATP, and finally cell death with necroptotic characteristics (Zong *et al*., 2004). It was consequently concluded that DNA damage induced necroptosis in a PARP-dependent way distinct from the mitochondrial apoptotic pathway. Mechanistically, cleavage of PARP-1 induces JNK activation through RIP1 and TRAF2 and further depolarizes mitochondrial potential, eventually leading to cell death. Interestingly, patients with inactivated retinoblastoma protein were not treated if PARP was suppressed. It indicates that activation in PARP-1 signal transduction can affect feasibility of cell death. Accordingly, it is essential to detect mutations in PARP of cancer patients for anticancer control. Either inflammatory cytokine TNFα or other agonists mediate cell necrosis through activation of RIP1 phosphorylation (Christofferson *et al*., 2012; Moquin *et al*., 2013). RIP1 is a necroptosis mediator, but it not only activates NF-κB, but also induces apoptotic cell death. Physical interaction of RIP1 with RIP3 is prerequisite in determining a cell death route, but RIP3 overexpression is sufficient for inducing necroptosis (Moujalled *et al*., 2013). On the contrary, RIP3 deficiency facilitates RIP1-mediated apoptosis (Moriwaki and Chan, 2013). A recent in vivo research demonstrates that RIP3 potentiates necrotic cell death when infected with virus that is able to inhibit the apoptotic machinery, implying that RIP3 can play a key role in cancer with resistance to apoptosis-based therapy. Accordingly, mutation profiles in RIP1 and RIP3 could provide basis of decision-making that is able to take advantage of necroptosis for cancer control. Besides TNFα, some chemicals or agents such as lapachone, apoptolidin and honokil have been reported to kill cells through necrosis although their precise mechanisms remain elusive (Li *et al*., 1999; Salomon *et al*., 2000; Tagliarino *et al*., 2001; Bai *et al*., 2003; Sun *et al*., 2006). Furthermore, deoxynyboquinone, a NAD(P)H:quinone oxidoreductase 1(NQO1) substrate, exerts a potent antitumor activity with selectivity by PARP1 hyperactivation and concomitant ATP loss (Huang *et al*., 2012). More lately, shikonin induced necroptotic cell death of glioma cells by a dose-dependent upregulation of RIP1 (Huang *et al*., 2013). Perspective understanding of molecular mechanisms of necroptotic cell death caused from various sources could make it possible to adopt a strategy suitable for the tumor specific responses to necrotic stimuli.

OUTLOOKS AND CONCLUSIONS

Necroptosis, a form of backup cell death to apoptosis, is basically taken as the immunological concept of first defense mechanism against infection of virus with apoptosis evasion apparatus. Similar to virus' survival tactic against host cell's suicidal pressure, cancer cells have developed various ways to acquire resistance to anticancer drugs, perpetuating cancer

growth and proliferation. Moreover, many cancers hold genetic aberration and deletion in specific genes that could be targeted by cancer drugs. The default programmed cell death apoptosis would not be generally activated in cancer cells refractory to chemotherapy. Therefore, alternative cell death modalities to fight cancers with defective apoptotic machinery should be pursued. Since there have been little information on interplay and regulation between cell death modes yet, however, other cell death modes such as autophagy and necroptosis have still limitation for clinical uses when compared with apoptosis.

Necroptosis has significant implications in the biological systems subjected to death stimuli. Principally, cells cope actively with a failure in conducting TNFα-mediated apoptosis, and consequently divert potential apoptotic force into alternative one. Switching of death modes like this has differential effects on physiological outcomes, depending on stimuli and tissue niche. Therefore, extensive studies on its underlying mechanism by which cell death switching determines cancer fate could provide treatment regimens suitable for chemotherapy-resistant cancers derived from diverse circumstances. Meanwhile, necroptosis itself is pathologically associated with a variety of diseases including inflammation and ischemic brain injury. Therefore, most studies have been focused on the development of pharmacological inhibitors targeting necroptosis-relevant proteins. Up till now, many efforts have made to discover necroptosis inhibitors, since the first identification of RIP1, a necroptosis regulator. As a result, Nec-1 is the first small molecule targeting a specific molecule RIP1. Thereafter, some necrotic proteins have been extensively explored and protein-signaling networks have been partly unveiled. Therefore, a few chemicals have been currently identified as chemical inhibitors of necroptosis. Nonetheless, little attention on chemicals to promote necroptosis has been gained yet in an attempt to make them feasible for anticancer therapy. Only a few chemicals have been studied as a means of understanding mechanisms of toxicity induced by chemicals. The combined treatment of TNFα with Hsp90 inhibitor geldanamycin (GA) has been reported to augment considerably cell death caused by TNFα stimulation alone, providing basis that small molecules be pursued for taking advantage of them to treat cancers (Vanden Berghe *et al*., 2003). Taken together, required are still comprehensive identification of necroptosis target molecules and construction of a network of signaling molecules associated with necroptosis. Optimistically, the pharmacological modulation of cell death by design will be feasible by customizing death therapy to cancer types.

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