Drug Resistance Updates 12 (2009) 55–64

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Targeting apoptosis as an approach for gastrointestinal cancer therapy

Liang Qiao, Benjamin C.Y. Wong*

Department of Medicine and Centre for Cancer Research, The University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 5 January 2009
Received in revised form 9 February 2009
Accepted 10 February 2009

Keywords:
Apoptosis
Gastric cancer
Colon cancer
Therapy

ABSTRACT

Cancers in the gastrointestinal system account for a large proportion of malignancies and cancer-related deaths with gastric cancer and colorectal cancer being the most common ones. For those patients in whom surgical resection is not possible, other therapeutic approaches are necessary. Disordered apoptosis has been linked to cancer development and treatment resistance. Apoptosis occurs via extrinsic or intrinsic signaling each triggered and regulated by many different molecular pathways. In recent years, the selective induction of apoptosis in tumor cells has been increasingly recognized as a promising approach for cancer therapy. A detailed understanding of the molecular pathways involved in the regulation of apoptosis is essential for developing novel effective therapeutic approaches. Apoptosis can be induced by many different approaches including activating cell surface death receptors (for example, Fas, TRAIL and TNF receptors), inhibiting cell survival signaling (such as EGFR, MAPK and PI3K), altering apoptosis threshold by modulating pro-apoptotic and anti-apoptotic members of the Bcl-2 family, down-regulating anti-apoptosis proteins (such as XIAP, survivin and c-IAP2), and using other pro-apoptotic agents. In this review, the authors reviewed the currently reported apoptosis-targeting approaches in gastrointestinal cancers.

1. Introduction

Cancers of the gastrointestinal (GI) tract account for more than a third of total cancer incidence and nearly half of the cancer-related deaths in the world. Among GI cancers, colorectal cancer (CRC) and gastric cancers (GC) are most common, being the third and fourth most common malignancies worldwide with an estimated incidence of 1 million and 934,000 new cases per year in 2002, respectively (Jemal et al., 2005; Parkin et al., 2005). More than 42% of GC patients are found in China alone (Wang et al., 2007). It is estimated that by 2010, the yearly incidence of GC will reach more than 1.1 million (Parkin et al., 2005). Approximately 700,000 patients with GC and 529,000 patients with CRC die annually, making GC and CRC the second and third most common cause of cancer-related death worldwide, respectively (Parkin et al., 2005). Currently available therapeutic approaches for both cancers are less effective, and thus the prognosis is poor. The selectively targeting of cancer cells by induction of apoptosis is a major goal of current research.

2. A brief overview of molecular pathways involved in apoptosis

2.1. Apoptosis pathways

Apoptosis, also termed programmed cell death, is a highly organized cell death process. It is an evolutionarily conserved process and is essential for organ development, tissue remodeling, immune response, and tumor suppression. Thus, apoptosis is an important process required for homeostasis (Iannolo et al., 2008; Call et al., 2008). Aberrant apoptosis is believed to contribute to cancer initiation, progression and treatment failure. In addition, as apoptosis usually does not elicit host inflammatory or immune response, this type of cell death is the preferred way of cancer cell killing by various treatments. Accordingly, selectively inducing apoptosis in cancer cells has been increasingly recognized as a promising therapeutic approach for many cancers.

Apoptosis occurs through two broad pathways: the intrinsic pathway (also known as the mitochondrial pathway) and extrinsic pathway (also known as the death receptor pathway) (Lorenzo and Susin, 2007). Apoptosis via intrinsic pathway can be triggered by any stimuli that cause oxidative stress, mitochondrial disturbances and DNA damage, such as cancer therapeutic agents, hypoxia, ischemia-reperfusion injury, and ionizing irradiation. Mitochondrial damage can cause permeabilization of the outer mitochondrial membrane which facilitates Cytochrome *c* release into the cytoplasm. The released Cytochrome *c* then binds the caspase adaptor Apaf-1 (apoptotic protease-activating factor-1), thereby triggering

Abbreviations: CRC, colorectal cancer; GC, gastric cancer; IAP, inhibitor of apoptosis protein; PPARγ, peroxisome proliferator-activated receptor gamma; XIAP, X-linked inhibitor of apoptosis.

* Corresponding author at: Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong. Tel.: +852 28554049; fax: +852 29049443.
E-mail address: bcywong@hku.hk (B.C.Y. Wong).

1368-7646/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.drup.2009.02.002

the apoptotic cascade by activating procaspase 9 and forming a complex termed the "apoptosome". This complex in turn activates several downstream effector caspases, such as caspases 3, 6 and 7, leading to DNA fragmentation and cell death (Oliver and Vallette, 2005; Iannolo et al., 2008). Thus, caspases are key players in the intrinsic apoptotic pathway. Another group of key players in the mitochondrial pathway of apoptosis is the Bcl-2 (B-cell leukemia/lymphoma 2) family, which consists of more than 20 members of pro-apoptotic proteins (including Bax, Bak, Bok, Bad, Bid, Bik, Bim, Bcl-Xs, Krk, Mtd, Nip3, Nix, Noxa, and Bcl-B), and anti-apoptotic proteins (including Bcl-2, Bcl-X_L, Mcl-1, Bfl-1/A1, Bcl-W, and Bcl-G) (Guo et al., 2001; Antonsson and Martinou, 2000). Members of the Bcl-2 family can form homo- or heterodimers, thereby functioning as agonists or antagonists of each other (Antonsson and Martinou, 2000). Pro-apoptotic members of the Bcl-2 family such as Bax, Bak, Bim, Bid, and Bim induce the release of Cytochrome *c* from mitochondria, whereas anti-apoptotic members such as Bcl-XL can bind and inactivate Apaf-1. In addition, pro-apoptotic members can dissociate the Bcl-XL-Apaf-1 complex, allowing Apaf-1 to activate caspase 9 which leads to subsequent apoptotic process (Reed, 1999). Bcl-2 family members are determinants of cellular drug sensitivity of many cancers. Increased levels of anti-apoptosis protein such as Bcl-2 and Bcl-XL, or reduced expression of pro-apoptosis members such as Bax and Bak are associated with increased resistance of cancer cells to chemotherapeutic agents (Zhang et al., 2007; Eberle et al., 2007). In addition, Bax and Bak can promote apoptosis by stimulating the release of Smac/DIABLO protein from mitochondria, thereby inactivating inhibitors of apoptosis proteins (IAPs, such as XIAP; cIAP1; cIAP2; and survivin).

In contrast to intrinsic pathway, extrinsic pathway of apoptosis is induced by ligand binding of death receptors. The most important ligand-death receptor system include tumor necrosis factor (TNF)-tumor necrosis factor receptor 1 (TNFR1), Fas ligand-Fas (also known as CD95 or Apo1) (Mollinedo and Gajate, 2006), TRAIL-TRAIL receptors (including TRAIL-R1, also termed DR4, and TRAIL-R2, also termed DR5). Binding of the receptors by their respective ligands leads to receptor oligomerization and recruitment of death signal adaptor proteins. For example, binding of Fas ligand (Fas-L) to Fas, or TRAIL to TRAIL-R1 (Thorburn et al., 2008) leads to recruitment of FADD (Fas-associated death domain), and binding of TNF to TNFR1 leads to recruitment of TRADD (TNFR-associated death domain) (Iannolo et al., 2008). The oligomerized receptors and recruited FADD or TRADD form a complex termed DISC (death-inducing signaling complex), which can bind to initiator caspases (caspase 8 and 10), thereby triggering the caspase cascade such as activation of caspases 3, 7, and 9, and leading to apoptotic events.

Despite the above arbitrary classification of intrinsic and extrinsic apoptosis pathways, some signals can activate both pathways, thus an extensive crosstalk exists between these two apoptosis pathways. In either pathway, activation of key caspases is the critical component in the death process. Activation of caspases eventually leads to typical apoptotic features such as characterized by DNA fragmentation, chromatin condensation, cell shrinkage and membrane blebbing.

2.2. Important regulators for apoptosis

An essential role of apoptosis in living organisms is the maintenance of a dynamic balance between cell survival and cell death signaling. For instance, survival signaling via the epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K) pathways is counterbalanced by downstream signaling pathways leading to apoptosis. Members of the inhibitor of apoptosis proteins (IAP) family such as X-linked inhibitor of apoptosis protein (XIAP) and survivin can directly bind and inhibit key effector caspases such as caspases 3, 7, and 9, thereby inhibiting apoptosis, but are on their turn counteracted by Smac/DIABLO (second mitochondrial activator of caspases/direct IAP binding protein with low pI) or Omi/HtrA2, therefore maintaining a dynamic balance between cell survival and cell death (Verhagen et al., 2000, 2002). Flice inhibitory protein (FLIP) is another apoptosis inhibitory protein acting mainly on the extrinsic pathway (Dutton et al., 2006). Also, the transcription factor NF-κB is a widely recognized inhibitor for apoptosis. It activates the transcription of some anti-apoptotic genes such as FLIP, Bcl-XL, XIAP and cIAP1, but on the other hand NF-κB enhances the expression of apoptosis-inducing genes such as Fas, Fas-L, TRAIL-R1 and TRAIL-R2 (Kucharczak et al., 2003). Thus, the role of NF-κB in apoptosis regulation is complex, and perhaps depends heavily on cellular context (Lam et al., 2008; McConkey and Zhu, 2008).

3. Targeting apoptosis as an approach for CRC and GC therapy

As discussed above, apoptosis plays an important role in cancer development and therapy and is regulated by many different signaling pathways. Thus, any agent that can *selectively* induce apoptosis in cancer cells is potentially useful in cancer therapy. Approaches which are relevant for CRC or GC therapy will be discussed here in more detail and are broadly categorized in five approaches:

1. Activation of the cell surface death receptors Fas, TRAIL and TNF receptors.
2. Inhibition of cell survival signaling via EGFR, MAPK and PI3K.
3. Altering the balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family.
4. Down-regulating anti-apoptosis proteins such as XIAP, survivin and c-IAP2.
5. Other approaches.

Fig. 1 shows the commonly recognized apoptosis signaling pathways and potential apoptosis-targeting approaches that might be of therapeutic value in cancer treatment.

3.1. Activation of cell surface death receptors

3.1.1. Fas/FasL

Fas and Fas-L are constitutively expressed in the gastrointestinal tract (Sträter and Möller, 2003) as well as in gastric cancer (Nagashima et al., 2001; Osaki et al., 2001; Lim, 2003) and colon cancer (Korkolopoulou et al., 2007; Zhu et al., 2005). Thus, Fas/Fas-L system can be an important target for apoptosis induction in GC and CRC. Tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) induced cytotoxicity in colon cancer cells has been shown to be mediated by Fas-L (Ebert and Groh, 2008). In support of the role of Fas-L/Fas interaction in apoptosis, siRNA mediated Fas knock-down reduced the sensitivity of colon cancer cells to 5-FU induced apoptosis (Borralho et al., 2007). Recently, a recombinant form of human Fas-L, APO010 and Fasaret (a recombinant adenovirus encoding Fas-L) are currently tested in several solid tumors in a phase I clinical trial (Call et al., 2008).

3.1.2. TNFα/TNF receptor

High-dose recombinant TNF-α has been approved in the treatment of regionally advanced melanomas and soft tissue sarcomas in Europe, but short-term and systemic use of TNF-α as an anticancer agent was hampered by its severe toxicity such as hypotension and organ failure (Lejeune et al., 2006), whereas low-dose long-term administration of TNF-α was associated with cachexia and possibly carcinogenesis (Lejeune et al., 2006; Call et al., 2008). The use of recombinant TNF-α in gastrointestinal cancers has been