Proceedings of ICBioMed 2024 Workshop: Computational Proteomics in Drug Discovery and Development from Medicinal Plants
DOI: 10.54254/2753-8818/71/2024.LA18824

Current knowledge of apoptosis and targeting apoptotic pathways for cancer therapy

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Abstract. The dual role of programmed cell death (PCD) has been detected in multicellular organisms, contributing to diseases such as cancer. This dissertation discusses current knowledge of apoptosis, the most studied form of PCD, and evaluates potential therapies targeting its pathways, which are crucial for maintaining tissue homeostasis and preventing irregular cell proliferation. In cancer, cells often evade apoptosis by inhibiting cascade activation, highlighting the importance of understanding the initiation phase of apoptosis. Based on the abnormalities in cancer pathogenesis along both intrinsic and extrinsic pathways, which include death receptors (DRs), B cell lymphoma (BCL)-2 family of proteins, and the Second Mitochondria-derived Activator of Caspases (SMAC), researchers have developed cancer therapies that promote apoptosis based on their underlying mechanism. However, recent studies reveal that apoptotic cells may influence the tumour microenvironment (TME), implying the possibility of apoptosis to promote tumour progression. These findings indicate the need for future studies to develop more effective therapeutic strategies that target other types of PCD other than apoptosis.

Keywords: Apoptosis, programmed cell death, cancer.

1. Introduction

Cancer, a group of genetic diseases, presents a worldwide public health challenge, with approximately 19 million diagnoses and 10 million deaths reported annually [1]. It is described as the rapid proliferation of tumour cells, resulting from excessive cell division and insufficient programmed cell death (PCD) [2]. PCD incorporates apoptosis, necroptosis, pyroptosis, and other less well-studied forms, such as ferroptosis, alkaliptosis, and lysosome-dependent cell death, which are triggered by specific toxins and distinguished by their molecular characteristics [2, 3]. PCD allows multicellular organisms to regulate the fate of individual cells, preventing abnormal cellular functions that could threaten organisms' internal environment, which is critical for organismal development and immune response [4, 5]. Therefore, PCD pathway-based cancer therapies have demonstrated the potential to promote novel treatment and clinical values by inducing various cell death modalities [6].

Among all forms of PCD, apoptosis is the earliest discovered and most studied one explored for removing surplus or damaged cells [7]. At the beginning of the 1980s, targeting the intrins pathway of apoptosis in neoplastic lymphoid cells through glucocorticoids to induce DNA fragmentation presented potential therapies for glucocorticoid-sensitive tumours, such as leukemia [8]. Over the past four decades, increasing attention has been given to chemotherapy, radiotherapy, and targeted therapy, all of

DOI: 10.54254/2753-8818/71/2024.LA18824

which incorporate the cytotoxic effects of apoptosis induction [9, 10]. Recent developments in novel cancer therapeutics, such as Venetoclax, which also targets the intrinsic apoptotic pathway, have shown its clinical benefits in leukemia therapy [11]. However, emerging challenges highlight the limitations of current therapies, including resistance to apoptosis-inducing therapies and their impact on tumour microenvironment [12].

Therefore, it becomes significant to gain more understanding of the mechanism of apoptosis which affects both cancer pathogenesis and treatments, and balance the effectiveness and limitations of current therapies. In sum, the objective of this article is to discuss current knowledge and provide future directions about targeting apoptosis pathways as a cancer treatment.

2. What is apoptosis

Apoptosis is a form of PCD that plays a critical role in tissue growth, repair, homeostasis, and defense mechanisms [13]. It involves specific changes in cellular morphology and biochemistry, regulated by a genetically controlled process involving a series of coordinated signaling pathways [14]. This highly specific mechanism targets stressed, damaged, or stimulated cells, ensuring controlled elimination with minimal inflammation and tissue destruction [5]. This process is vital during both development and aging, facilitating events like removing the webbing between fingers and the shaping of organs, both essential for embryogenesis and adult organ homeostasis [4].

The process of apoptosis can be categorized into two main stages: the initiation phase, represented by the activation of cysteine proteases known as caspases, which cleave corresponding target proteins at aspartic acid sites when triggered by initial stimuli, and the execution phase, driven by a downstream cascade that finally results in the ultimate cell death [15, 16]. Furthermore, the initiation phase of apoptosis is irreversible once activated, thereby inevitably preventing target cells' growth and spread [17]. For this reason, it is supposed to focus on targeting the initiation phase of apoptosis, which not only minimizes harm to normal cells but also provides treatment efficiency. It can be demonstrated mainly into two pathways below: (i) the intrinsic (mitochondria) pathway, which is the most common apoptotic mechanism in vertebrates, initiated upon the breakdown of the mitochondrial outer membrane's integrity, which cause pro-apoptotic factors to release into the cytosol for caspase-9 activation, and (ii) the extrinsic (death receptor) pathway, when the apoptosis is directly induced by the interaction of external death ligands and their specific receptors as caspase-activation platforms [14, 18].

2.1. Intrinsic pathway

The intrinsic pathway of apoptosis is primarily regulated by the multifunctional protein p53 and the B cell lymphoma (BCL)-2 protein family [19]. It is initiated from mitochondria-associated intracellular stimuli, including both positive stimuli, such as toxic substances, viral infections, radiation, and DNA damages, that directly activate apoptosis mediators, and negative stimuli, such as the deficiency of growth factors, certain hormones or cytokines, which downregulates anti-apoptotic factors within the cell [19]. Once the cell experiences severe stress, multiple genes are activated, among which the tumor suppressor gene p53 is the most broadly investigated [20]. p53, a significant regulator of apoptosis, arrests the cell cycle in response to DNA damage and induces the transcriptional activation of BH3-only proteins, which belong to the BCL-2 protein family mentioned above [21, 22].

BCL-2 family proteins contain up to four distinct regions known as BCL-2 homology (BH) domains: BH1, BH2, BH3, and BH4 [6]. These proteins are categorized into two main groups based on their function: pro-apoptotic and anti-apoptotic [23]. Pro-apoptotic proteins are further categorized into effector proteins, such as BCL-2 antagonist killer 1 (BAK) and BCL-2 associated x protein (BAX), which have BH1-4 domains and execute the apoptosis, and BH3-only proteins, which activate the effector proteins through either directly binding or neutralizing the anti-apoptotic proteins [23]. Anti-apoptotic members incorporate BCL-2, BCL-xL, and MCL-1, which also contain BH1-4 domains while playing the opposite role to effector proteins, maintaining the integrity of OMM by directly interacting and suppressing pro-apoptotic BCL-2 proteins [23].

DOI: 10.54254/2753-8818/71/2024.LA18824

As mentioned above, the pro-apoptotic effector proteins BAK and BAX are primarily activated by direct interactions with BH3-only proteins, which are produced in response to p53 activation. Upon activation, BAK and BAX promote caspase activation by forming proteolipid pores in the outer mitochondrial membrane, which further results in mitochondrial outer membrane permeabilization (MOMP), and subsequently leads to the release of pro-apoptosis factors including cytochrome c and Second Mitochondria-derived Activator of Caspases (SMAC) to cytosol [24]. Since then, in non-cancerous cells, the occurrence of MOMP is committed to irreversible apoptosis without caspase activation [25]. Therefore, targeting MOMP to manipulate apoptosis presents significant therapeutic potential for cancer.

Cytochrome c and dATP combine with apoptotic protease activating factor-1 to form the apoptosome [4]. At the same time, SMAC interacts with and neutralizes inhibitors of apoptosis proteins (IAPs) [4]. The apoptosome then recruits pro-caspase-9, which is processed into active initiator caspase-9, and then caspase-9 can further carry out the activation of the executioner caspases -3, -6, and -7, initiating the ultimate death of cells [4]. IAPs normally inhibit caspases by attaching to their N-terminal signal sequence known as the N-degron with IAPs' BIR domain to prevent apoptosis [7]. However, the release of SMAC, which also exposes an N-degron, competes with caspases for the IAPs' BIR domain, thereby promoting the cell death process [7].

2.2. Extrinsic pathway

Unlike the mitochondria pathway, the extrinsic pathway depends on a receptor signaling process, which involves the interaction between death receptors on cell surfaces and extracellular ligands. Those death ligands are members of a family of proteins named the tumour necrosis factor (TNF) family, while the corresponding death receptors are part of the TNF receptor (TNFR) superfamily, such as FasL/FasR, TNF/TNFR1, and TNF-related apoptosis-inducing ligand (TRAIL)/DR4 or DR5 [19, 26]. Once these death ligands bind to their receptors, they create a binding site for an adaptor protein and form a ligand-receptor-adaptor protein complex named the death-inducing signaling complex [27]. This complex then auto-catalytically assembles and activates pro-caspase 8 into caspase 8, which is also an initiator caspase similar to the caspase 9 in the mitochondria pathway and promotes apoptosis by activating downstream executioner caspases [28].

3. Apoptosis and cancer

As mentioned above, as a defense mechanism, the apoptosis pathway can be activated when DNA damages are detected to prevent uncontrolled cell growth, eliminating potentially cancerous cells. However, at the same time, except for maintaining physiological conditions, another important aspect of apoptosis in cancer, has been the focus of current studies in the field, with the resistance to apoptosis has been recognized as one of the hallmarks of tumour cells [29]. This resistance allows tumour progression and outgrowth and finally results in treatment failure [2]. Therefore, as both the cause and solution of the problem, understanding the dual roles of apoptosis in cancer not only reveals pathogenetic mechanisms but also provides potential therapeutic implications for cancer treatments [27].

Cancer cells avoid apoptosis mainly through two strategies: the upregulation of anti-apoptotic BCL-2 proteins and the downregulation of pro-apoptotic BCL-2 proteins [30]. The BCL-2 level has been observed to be increased in solid tumours and blood cancers, along with the overexpression of BCL-xL and MCL-1, contributing to tumour developments by preventing MOMP and the resistance to most cancer therapeutics [30, 31]. Also, the loss of BH3-only proteins, BAX, and BAK is discovered in various cancers, as well as the downregulation of the p53 tumour suppressor protein [32].

4. Therapeutic targeting of apoptosis

Apoptosis involves three primary types of biochemical changes: (i) activation of caspases (caspase cascade), (ii) DNA fragmentation and protein degradation, and (iii) plasma membrane rupture and recognition by phagocytic cells [27]. Although every abnormality along the apoptotic pathways could be targeted for cancer treatment, cancer cells evade cell death mainly in the initiation phase. Therefore,

DOI: 10.54254/2753-8818/71/2024.LA18824

recent discoveries about potential treatment strategies and drugs that target defects in the apoptosis pathway also primarily focus on the activation of the cascade, which will be discussed in two major pathways below.

4.1. Targeting the intrinsic pathway

4.1.1. Targeting the BCL-2 family of proteins. Mitochondrial outer membrane permeabilization (MOMP) is a crucial event in the mitochondria pathway of apoptosis, with both pro-apoptotic and anti-apoptotic BCL-2 family of proteins, identified by their BCL-2 homology (BH) domains, is highlighted in the MOMP process [33]. In addition, tumour cells commonly evade apoptosis by upregulating anti-apoptotic BCL-2 proteins, which block MOMP [34]. This understanding has resulted in the recent invention of BH3 mimetics, also known as BCL-2 inhibitors, a new category of anticancer treatments that block the activity of anti-apoptotic BCL-2 proteins (such as BCL-2, BCL-XL, and MCL-1) and promote cancer cell death by mimicking the BH3-only proteins [34]. Venetoclax (ABT-199/GDC-0199) is one of the most efficient and potent small molecule BCL-2 antagonists, which became the first FDA-approved BCL-2 antagonist in 2016 for the cancer therapy of blood cancers such as chronic lymphocytic leukemia and acute myeloid leukemia [35, 36].

Instead of using drugs or therapies to block anti-apoptotic BCL-2 proteins, it has been proved that directly silencing the genes that encode those anti-apoptotic proteins can also increase cancer cell apoptosis [27]. For example, BCL-xL specific siRNA knockdown can enhance apoptosis of chemoresistant chondrosarcoma cells effectively, providing pro-apoptotic effects in vitro [37].

4.1.2. SMAC mimetics. IAPs are observed to be overexpressed in solid tumours or blood cancers, which reveals its potential role as a treatment target [38]. As mentioned above, MOMP will lead to the release of SMAC and cytochrome c from the mitochondria to the cytosol. Due to its ability to bind IAPs to free downstream caspases, SMAC is the most well-known antagonist to IAPs [39]. SMAC mimetics (SMs), a kind of small synthetic molecular inhibitors used as anti-cancer drugs, mimic the N-degron of SMAC which competes with caspases for binding to the BIR domain of IAPs [39, 40].

4.2. Targeting the extrinsic pathway

Among all death ligands, although the apoptosis-inducing ability of both FasL and TNF offers the potential as agents for cancer treatment, their significant side effects due to the lack of selectivity restrict their therapeutic utility [41]. However, TRAIL/Apo2L, a trimeric glycoprotein, as the extracellular ligand of DR4 or DR5, can induce apoptosis specifically in cancer cells without harming surrounding cells [6]. Therefore, first-generation recombinant human TRAIL (rhTRAIL/Dulanermin) therapeutics were developed by researchers and evaluated in clinical trials in the early 2000s [16, 38]. They did not cause significant negative effects in patients with solid tumours or blood cancers without observing clinically meaningful activity [42, 43]. This is because the half-life of Dulanermin is limited in blood, from 0.56 to 1.02 hours, which results in a reduced ability to induce high-order conformational changes of DR receptors and then generates weak apoptotic signals [42, 43].

TLY012, the second generation rhTRAIL therapeutics, utilizes polyethylene glycol (PEG) to rhTRAIL to increase its size to prevent it from being rapidly cleared through renal filtration and thus increase its half-life to twelve to eighteen hours and antitumour activity [44]. Nevertheless, it does not tackle the root of problems, because high-order receptor clustering requires more receptor binding sites, while both rhTRAIL therapies are bivallent [6].

Later, the invention of APG350 solved this problem and achieved full antitumour activity by the design of two TRAIL receptor-binding domains, each has three receptor-binding sites, which has been regarded as a current clinical development candidate that causes efficient apoptosis induction in vivo [45].

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5. Future direction

Although targeting defects in the apoptosis pathway in cancer cells to promote selective cell death is the primary focus of current development of cancer therapies, recent advancement in the interaction of apoptosis and cancer progression demonstrates that although apoptosis is normally tumour-suppressive, its influence on tumour microenvironment (TME) such as induce angiogenesis and pro-growth signals promote cancer cell growth [2]. Also, except for apoptosis, other types of PCD such as regulatory pathways of necroptosis, pyroptosis, ferroptosis, and others also provide therapeutic potential for cancer therapies which can avoid risks of resistance emerging in previous therapies targets on apoptosis pathways [46].

6. Conclusion

Targeting apoptotic pathways in cancer cells has shown promising effects in clinical treatments. A deeper understanding of apoptotic factors and potential defects associated with them in the process of apoptosis activation provides novel therapeutic agents for cancer treatment. However, emerging research reveals that apoptotic cells may influence the TME, potentially promoting the growth of surrounding cancer cells. Also, the invention of apoptosis-based cancer therapies still struggles with challenges due to tumour cell resistance. Therefore, further studies are required to promote the understanding of the apoptosis pathway and the development of cancer therapies.

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