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Mini Review

Polygonatum cyrtonema lectin, a potential antineoplastic drug targeting programmed cell death pathways

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ABSTRACT

Polygonatum cyrtonema lectin (PCL), a mannose/sialic acid-binding plant lectin, has recently drawn a rising attention for cancer biologists because PCL bears remarkable anti-tumor activities and thus inducing programmed cell death (PCD) including apoptosis and autophagy in cancer cells. In this review, we focus on exploring the precise molecular mechanisms by which PCL induces cancer cell apoptotic death such as the caspase-dependent pathway, mitochondria-mediated ROS–p38–p53 pathway, Ras–Raf and PI3K–Akt pathways. In addition, we further elucidate that PCL induces cancer cell autophagic death via activating mitochondrial ROS–p38–p53 pathway, as well as via blocking Ras–Raf and PI3K–Akt pathways, suggesting an intricate relationship between autophagic and apoptotic death in PCL-induced cancer cells. In conclusion, these findings may provide a new perspective of *Polygonatum cyrtonema* lectin (PCL) as a potential anti-tumor drug targeting PCD pathways for future cancer therapeutics.

1. Introduction

Plant lectins, a group of highly diverse non-immune origin proteins ubiquitously distributed in a variety of plant species, contain at least one non-catalytic domain which enables them to selectively recognize and reversibly bind to specific free sugars or glycans present on glycoproteins and glycolipids without altering the structure of carbohydrate [1,2]. According to their molecular structures and evolutionary relationships, plant lectins can be divided into 12 different families, such as *Agaricus bisporus* agglutinin (ABA), Amaranthin, chitinase-related agglutinin (CRA), Cyanovirin, *Euonymus europaeus* agglutinin (EEA), *Galanthus nivalis* agglutinin (GNA), Hevein, Jacalins, Legume lectin, Lysin motif (LysM), Nictaba and Ricin_B families [1,3]. Amongst the above-mentioned 12 families, *Galanthus nivalis* agglutinin (GNA)-related lectins have been reported to possess a broad range of biological functions including anti-tumor, anti-HIV and anti-fungal activities [4,5].

Polygonatum cyrtonema lectin (PCL), a mannose/sialic acid plant lectin belonging to GNA-related lectin family, was firstly isolated from the rhizomes of *Polygonatum cyrtonema* Hua, a traditional Chinese medicinal herb in the year of 1996 [6]. Subsequently, the nucleic acid sequence of PCL and its important physiological and biochemical characteristics were sequentially demonstrated [7]. In addition, PCL was also reported to be abundantly acquired from *in vitro* plantlet regeneration system from rhizomes of *Polygonatum cyrtonema* Hua [8]. Recently, *Polygonatum cyrtonema* lectin (PCL) has been reported to be synthesized as a 160-residue polypeptide with a 28-residue N-terminal signal sequence and a 22-residue C-terminal cleavage polypeptide. Thus, the amino acid sequence of PCL is the mature 110-residue polypeptide (Fig. 1A) [7]. And, the secondary structure of PCL is typically built from beta-sheets connected by turns and loops, creating a very tight structural scaffold (Fig. 1A). Recently, the crystal three-dimensional structures of ligand-free PCL have been reported (Fig. 1B) [9]. PCL subunit binds mannose by using a potential bivalent mode, in which sugar-binding site (SBS) I and adopts the conserved mannose-binding motif of QXDXNXVXY (X is one of any amino acid residues) as observed in other structurally characterized GNA-related lectins, while SBS II and III adopt the modified motifs with some replaced residues, respectively (Fig. 1B). As a result, SBS II and III are unfit for mannose-binding but may bind other types of sugars such as sialic acid as previously reported [7]. In addition, the ligand-free PCL is dimeric, with both subunits adopting the beta-prism II fold (Fig. 1C) [9]. Hitherto, PCL, a well-studied GNA-related lectin, has drawn much attention for its widely biological activities, especially the remarkable anti-tumor activities toward different types of cancer cells [5]. More importantly, accumulating evidence has demonstrated that PCL can induce cancer cell death targeting programmed cell death (PCD) pathways (hereafter referring to apoptosis and autophagy), such as the caspase-dependent pathways, mitochondrial ROS–p38–p53 pathway, Ras–Raf and PI3K–Akt pathways [10–14]. Therefore, the precise mechanisms of *Polygonatum cyrtonema* lectin (PCL)-induced cancer cell apoptotic and autophagic death should be discussed in details, and targeting

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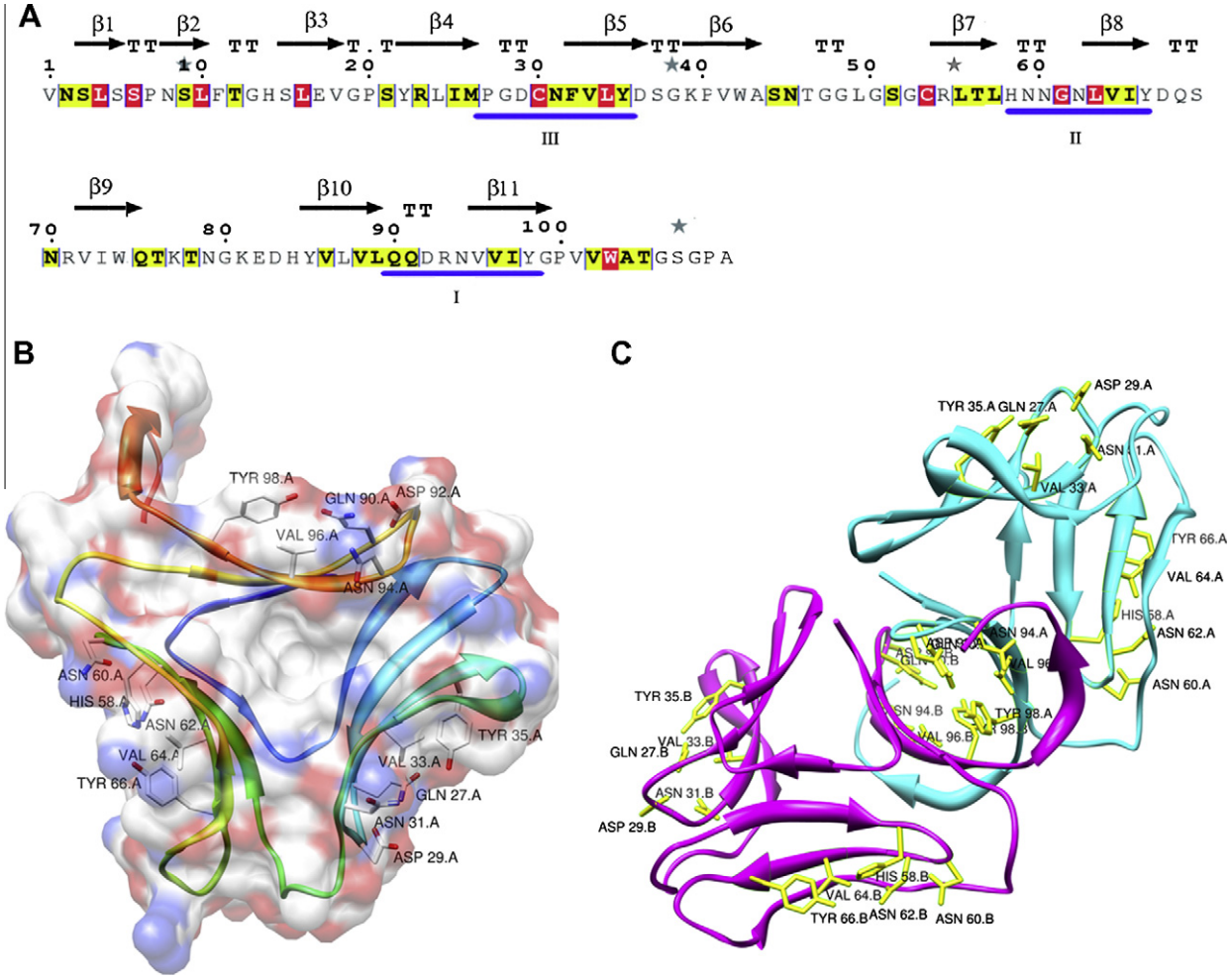

Fig. 1. Molecular structures of *Polygonatum cyrtonema* lectin (PCL). (A) The primary and secondary structures of PCL. (B) The crystal three-dimensional structure of PCL. (C) The quaternary structure of PCL.

the programmed cell death (PCD) pathways, PCL would be utilized as a potential antineoplastic drug for cancer therapy in the near future.

2. PCL induces cancer cell death targeting apoptotic pathways

2.1. Targeting the caspase-dependent apoptotic pathways

Apoptosis, a mechanism by which cells undergo death to control cell proliferation or in response to DNA damage, can provide novel potential drug targets that are able to induce death in cancer cells [15,16]. Caspase (cysteine-dependent aspartate specific protease), the members of a cysteine protease family, or structurally related group, are the central component of the apoptotic machinery that irreversibly commits a cell to die [17]. To study the roles of caspases in PCL-induced cancer cell apoptosis, a series of sequent studies were carried out as follows: PCL was firstly reported to possess remarkable anti-proliferative and apoptosis-inducing activities toward human cervical HeLa cells in a dose-dependent manner [13]. Then, PCL was found to induce human breast cancer MCF-7 cell apoptosis with caspase family participation [14]. To further confirm whether caspases play the key roles in PCL-induced cancer cell apoptosis, PCL was shown to induce apoptosis, accompanied with caspase-9, caspase-8 and caspase-3 activation in murine fibrosarcoma L929 cells, suggesting that the apoptotic pathway is a caspase-dependent manner [18]. These above-mentioned results demonstrate that PCL is able to induce the caspase-dependent apoptosis in cancer cells.

2.2. Targeting mitochondria-mediated ROS–p38–p53 apoptotic pathway

Of note, there are two major apoptotic pathways: extrinsic and intrinsic pathways [16,17]. The intrinsic pathway is also called the mitochondrial pathway owing to the essential involvement of mitochondria, which is not only the site where anti-apoptotic and pro-apoptotic proteins interact and determine cell fate, but the origin of signals that initiate the activation of caspases through various mechanisms [15,19]. Recent reports have shown that PCL induces apoptosis in human melanoma A375 cells. The mechanism of apoptosis following treatment with PCL involved regulation of Bax, Bcl-X_L and Bcl-2 proteins, which subsequently caused collapse of the mitochondrial membrane potential, leading to cytochrome *c* release and caspase activation [10,20]. The treatment with PCL also abrogated the glutathione antioxidant system, and induced mitochondria to generate massive reactive oxygen species (ROS) accumulation, which subsequently resulted in p38 and p53 activation [10,11,20]. Accordingly, these findings indicate that PCL induces apoptosis through a mitochondria-mediated ROS–p38–p53 pathway in cancer cells. However, these aforementioned studies focus on promoting several apoptotic signaling pathways to lead to cancer cell death. Next, we will discuss some recent studies that are

based upon blocking survival/anti-apoptotic pathways to induce cancer cell death.

2.3. Targeting Ras–Raf and PI3K–Akt anti-apoptotic pathways

It is well known that Ras–Raf and class I phosphatidylinositol 3-kinase (PI3K)–Akt pathways are two significant anti-apoptotic/survival signaling pathways in cancer cells [21,22]. Recent reports have shown that PCL can induce apoptosis in murine fibrosarcoma L929 cells. Subsequently, inhibition of Ras could promote L929 cell death, suggesting that Ras–Raf signaling pathway plays the key negative regulator in PCL-induced apoptosis. Furthermore, class I PI3K–Akt signaling pathway is further shown to play the negative regulator in PCL-induced apoptosis [12]. Taken together, these findings demonstrate that PCL induces apoptosis via blocking Ras–Raf and PI3K–Akt signaling pathways in murine fibrosarcoma L929 cells.

3. PCL induces cancer cell death targeting autophagic pathways

Autophagy, (from Greek auto-oneself, phagy-to eat), is independent of phagocytes and differs from apoptosis by the presence of autophagosomes, autolysosomes, and an intact nucleus in the cell. Autophagy is not only a survival response to either growth factor or nutrient deprivation but an important molecular mechanism for tumor cell suicide [22–24]. Recently, *Polygonatum cyrtonema* lectin (PCL) has been reported induce autophagic death via a mitochondria-mediated pathway in human melanoma A375 cells. Subsequently, PCL-induced autophagic death was further confirmed to be a mitochondrial-mediated ROS–p38–p53 pathway [5,11,20]. Furthermore, PCL has been shown to induce autophagy in murine fibrosarcoma L929 cells. Subsequently, inhibition of Ras could promote L929 cell death, suggesting that Ras–Raf signaling pathway plays the key negative regulator in PCL-induced autophagy. In addition, class I phosphatidylinositol 3-kinase (PI3K)–Akt signaling pathway is also reported to play the negative regulator in PCL-induced autophagy [22]. Thus, these aforementioned evidence demonstrates that PCL induces cancer cell autophagy via promoting ROS–p38–p53 pathway, as well as blocking Ras–Raf and PI3K–Akt signaling pathways.

As mentioned above, PCL-induced autophagy and apoptosis could connect with each other to participate in leading to cancer cell death via promoting a mitochondria-mediated ROS–p38–p53 pathway, as well as via blocking Ras–Raf and PI3K–Akt pathways. Additionally, the regulation of tumor cell death might be considered as a "dynamic balance" between autophagy and apoptosis [24–26]. It is a complex, and not fully defined relationship between autophagy and apoptosis that may vary depending on their biological context [23,25]. In our studies of PCL, the autophagic and apoptotic signaling pathways were simultaneously regulated by some common factors (such as ROS, p38, p53 or Ras, Raf, PI3K and Akt); thus, they share common components, they can exert overlapping and even same biological functions. A schematic model of PCL is shown as a potential anti-tumor drug targeting PCD pathways in cancer cells (Fig. 2).

4. *Polygonatum cyrtonema* lectin, a potential antineoplastic drug

Polygonatum cyrtonema lectin (PCL), a mannose/sialic acid-binding plant lectin belonging to GNA-related lectin family, has been reported to markedly inhibit the growth of various types of cancer

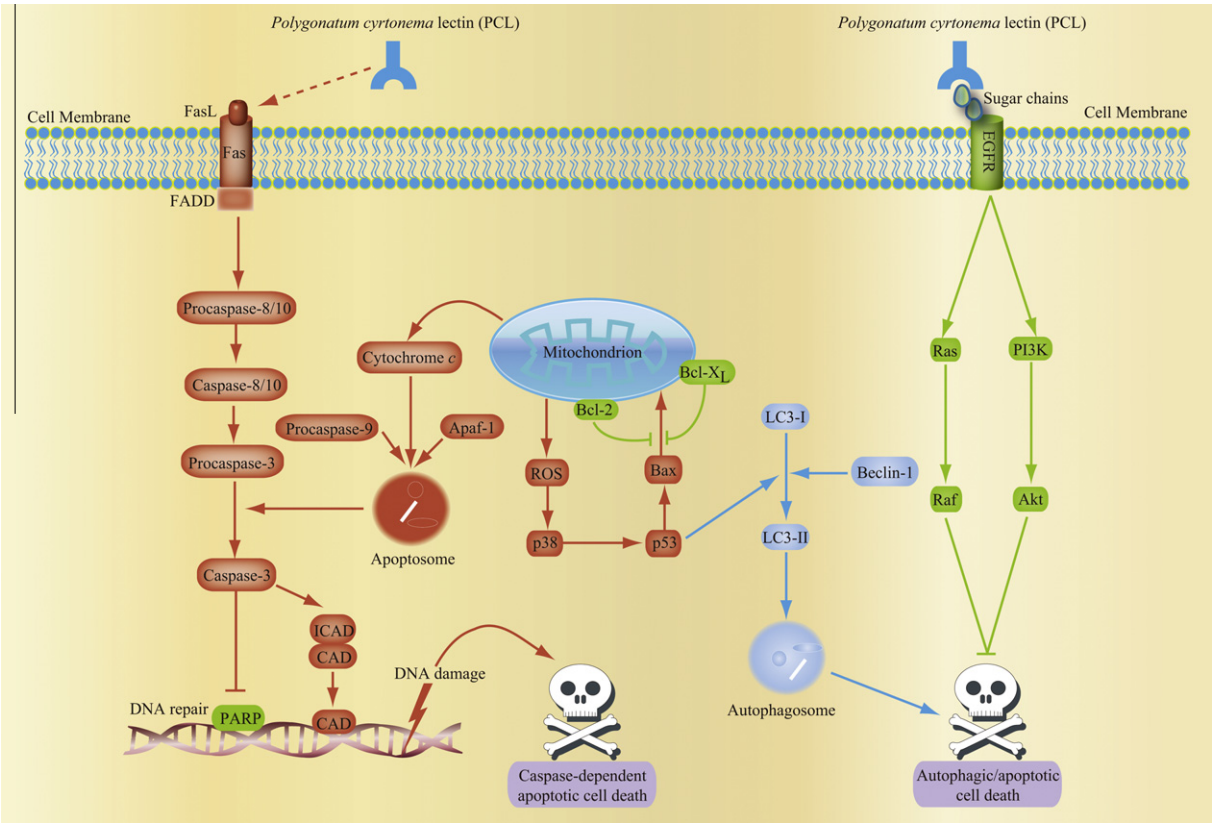

Fig. 2. *Polygonatum cyrtonema* lectin (PCL), is a potential anti-tumor drug targeting programmed cell death (PCD) pathways. Expression or function of some proteins with either pro-apoptotic (indicated in red) or anti-apoptotic (green) consequences have a causative or contributing role in PCL-induced cancer cell death. In addition, expression or function of other proteins with pro-autophagic (indicated in blue) consequence play an apoptosis-promoting role in PCL-induced cancer cell death. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells (e.g., HeLa, MCF-7, A375 and L929 cells), but with concomitant low toxicity to the normal cells such as melanocytes cells. Further studies demonstrate that PCL induces cancer cell apoptosis through activating the caspase-dependent pathway and mitochondrial ROS–p38–p53 pathway, as well as blocking Ras–Raf and PI3K–Akt pathways. In addition, PCL can also induce cancer cell autophagic death via promoting mitochondrial ROS–p38–p53 pathway, as well as via inhibiting Ras–Raf and PI3K–Akt pathways, suggesting an intricate interplay between autophagy and apoptosis in PCL-induced cancer cells. With the complex molecular mechanisms of PCL-induced apoptotic and autophagic death are becoming better understood, new therapeutic strategies would no doubt be developed and spark off other promising therapeutic strategies for promoting apoptotic and autophagic death pathways in cancer. Further research, including pre-clinical and clinical trials into the mechanisms of actions at molecular level, would help cancer biologists and clinicians to further understand the therapeutic effects of PCL and harness this plant lectin as a potential antineoplastic drug targeting programmed cell death (PCD) pathways for future cancer therapeutics.

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