Anti-tumor activities and apoptotic mechanism of ribosome-inactivating proteins

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Abstract

Ribosome-inactivating proteins (RIPs) belong to a family of enzymes that attack eukaryotic ribosomes and potently inhibit cellular protein synthesis. RIPs possess several biomedical properties, including anti-viral and anti-tumor activities. Multiple RIPs are known to inhibit tumor cell proliferation through inducing apoptosis in a variety of cancers, such as breast cancer, leukemia/lymphoma, and hepatoma. This review focuses on the anti-tumor activities of RIPs and their apoptotic effects through three closely related pathways: mitochondrial, death receptor, and endoplasmic reticulum pathways.

Keywords: Ribosome-inactivating protein (RIP), Anti-tumor, Apoptosis, Cancer

Introduction

Ribosome-inactivating proteins (RIPs) are a family of enzymes that inhibit the eukaryotic ribosome via N-glycosidase activity, by which they cleave a specific adenine residue from the 28S RNA within the 60S ribosomal subunit, therefore inhibiting protein synthesis [1, 2]. In addition to their effect on ribosomal RNA (rRNA), some RIPs display a variety of anti-microbial activities in vitro, including anti-fungal, anti-bacterial, and broad-spectrum anti-viral properties against both human and animal pathogens.

Ribosome-inactivating proteins were initially discovered in the castor oil plant Ricinus communis, from which ricin was isolated. RIPs are widely distributed among higher plants, and a few have been found in several fungi and bacteria. Plant RIPs are classified into three main categories based on their physical properties. Type I RIPs are single-chain proteins of approximately 30 kDa with N-glycosidase activity, including trichosanthin (TCS) and cucurmosin [3]. Type II RIPs, such as ricin and abrin, comprise two different domains: a 30-kDa enzymatic A-chain (similar to type I RIPs) linked to a slightly larger B-chain with lectin properties and specificity for sugars possessing galactose-like structures [3]. Thus far, type III RIPs, also considered atypical type I RIPs, have only been described in maize and barley, and the function of their extra domains remains unknown [4]. Therefore, the division of RIPs into types I and II RIP is now favored.

Over the past decade, RIPs appeared to be a great research interest due to their potential use in cancer therapy. Some RIPs exhibit strong toxicity towards cancer cells and low toxicity towards normal cells; they impede or inhibit tumor growth mostly via apoptosis, but the exact mechanism remains poorly understood. The aim of this study was to summarize the anti-tumor activities of RIPs and their possible apoptotic mechanisms to hopefully provide new insights for cancer research and treatment.

Anti-tumor activity

Effects of RIPs on breast cancer

The type I RIPs TCS, momordica anti-human immunodeficiency virus (HIV) protein of 30 kDa (MAP30), gelonium anti-HIV protein of 31 kDa (GAP31), gelonin, marmorin, and α-momorcharin (α-MMC) have been shown to negatively affect the growth of breast tumor cells in vitro and in vivo [5–7]. TCS inhibits cell viability, causes cell cycle arrest, and significantly reduces tumor volume and weight by inducing apoptosis through caspase-8 and caspase-9 in breast tumor cells [5]. MAP30, which shares 59% sequence similarity with...
TCS, effectively inhibits human breast cancer MDA-MB-231 cells through down-regulating the expression of human epidermal growth factor receptor-2 (HER2), similarly to GAP31 [6]. HER2 overexpression is observed in approximately 30% of all human breast cancers, and HER2-overexpressing tumor cells may be less sensitive to chemotherapy; in this case, the combination of MAP30 and GAP31 could represent a therapeutic strategy [7]. HER2 and fibroblast growth factor-inducible 14-kDa protein (Fn14) are frequently co-expressed in human breast tumors, and HER2 directly induces increase in Fn14 expression, therefore sensitizing tumor cells to an immunotoxin generated by fusing Fn14 antibodies to recombinant gelonin (designated hSGZ) [8]. Indeed, hSGZ can rapidly internalize and deliver recombinant gelonin (rGel) to the cytosol of tumor cells, where it enzymatically blocks protein synthesis. Because Fn14 enhances breast cancer cell migration and invasion, a question of whether there is a way to damage tumor cells while reducing Fn14 expression was raised. We assume that MAP30 and hSGZ used together might achieve a better outcome, and breast tumor cells can be sequentially treated with MAP30 and hSGZ; MAP30 would decrease HER2 expression and lead to reduced Fn14 expression, then hSGZ would target Fn14-positive cells and exert its function without increasing the invasive capacity of tumor cells. However, this hypothesis remains to be verified by appropriate experiments.

Many cell membrane receptors are expressed at low levels in normal cells but are highly expressed in tumor cells. Estrogen receptor α (ERα) is expressed in approximately 75% of breast cancer tissues at higher levels compared with those in normal breast tissues ($P = 0.001$) [9]. In ERα-positive breast cancer cells, the ERα-mediated signaling pathway is involved in the inhibitory action of marmorin on proliferation; many drugs target ERα. Marmorin inhibits angiogenesis by lowering the viability of human umbilical vein endothelial cells in vitro; therefore, it was suggested that marmorin might starve tumors to death by reducing the amount of blood vessels in vivo [10]. Marmorin also induces DNA damage and endoplasmic reticulum stress, resulting in the induction of apoptosis in mice bearing MDA-MB-231 tumor xenografts [10].

Ribosome-inactivating proteins have the potential to become innovative anti-tumor agents, but they also possess toxic adverse effects, including severe systemic anaphylaxis, immunogenicity, and toxicity. To reduce the undesirable effects and achieve better therapeutic efficacy, Deng et al. [11] modified α-MMC with polyethylene glycol (PEG) to explore the anti-tumor efficacy on breast carcinoma; they demonstrated that α-MMC PEGylation extends the half-life of α-MMC and mitigates non-specific toxicity. Indeed, α-MMC-PEG exhibited improved anti-tumor efficacy with tolerable toxic reactions.

**Effects of RIPs on leukemia and lymphoma**

Trichosanthin significantly inhibits the proliferation of various leukemia and lymphoma cell lines [12]. Notably, TCS can damage leukemia and lymphoma cells through different mechanisms according to the cell type. TCS induces apoptosis in T-lymphocyte cell lines, but inhibits growth of B-lymphocyte cell lines via S-phase cell cycle arrest [12]. It has been suggested that cucurmosin is more potent than TCS in killing the chronic myelogenous leukemia K562 cells; both cucurmosin and TCS downregulate P210$^{bcr-abl}$ and inhibit tyrosine kinase, resulting in cell growth suppression [13]. Cucurmosin also inhibits proliferation and induces apoptosis in tumor cells; interestingly, cucurmosin combined with trans-retinoic acid or arsenic trioxide was shown to synergistically increase these effects on the human acute promyelocytic leukemia NB4 cell line [14].

Articulatin-D, the first cytotoxic RIP with a B-chain lacking sugar-binding activity, has been shown to highly inhibit leukemia and lymphoma cells in vitro; the highest toxicity was obtained with Jurkat cells, followed by Molt-4, U-937, HL-60, and Raji cells [15]. With its special physical properties, articulatin-D is a good candidate for the synthesis of immunotoxins capable of efficiently and specifically killing tumor cells.

Immunotoxins are emerging targeted agents composed of a toxin fragment and an antibody/cytokine. Saporin and rGel have been widely used to construct immunotoxins, which have been reported to be useful in cancer treatment by multiple studies [16–18]; several such molecules have been evaluated clinically [19, 20]. It is feasible to locate cancer cells through membrane proteins CD22, CD7, CD19, and CD38, and corresponding antibody HB22.7, HB2, BU12, and OKT10 are used to construct immunotoxins. HB22.7-saporin was cytotoxic against a panel of non-Hodgkin's lymphoma (NHL) cell lines and was shown to significantly prevent tumor development in a xenograft model of NHL [21]. HB2-saporin, BU12-saporin, and OKT10-saporin were shown to be selectively cytotoxic toward human acute lymphoblastic leukemia in vitro and in vivo [22–24].

Luster et al. [25] have reported that treatment with rGel-BLyS, rGel fused to a B-lymphocyte stimulator, rapidly reduced the tumor burden and markedly prolonged survival in xenograft mouse models of spread lymphoma or leukemia; in this setting, cell death was not induced by caspase activation but rather was partially mediated by the ribotoxic stress response. Furthermore, the rGel-BLyS fusion toxin combined with the proteasome
inhibitor bortezomib restrained lymphoma growth and down-regulated nuclear factor kappa B (NF-κB) activity, which is critical for cellular proliferation and survival [26].

Effects of RIPs on hepatoma and other cancers
MAP30 was shown to display anti-tumor activity in cell cultures and mice. In HepG2 cells, for example, cell viability was inhibited by MAP30 in time- and dose-dependent manners, with S-phase arrest; moreover, apoptosis and necrosis induced by MAP30 resulted in tumor volume reduction in HepG2-bearing mice [27]. Cucurmosin induced G0/G1 arrest and apoptosis in HepG2 cells; these effects also translated into potent anti-tumor activities in vivo [28]. Abrus agglutinin not only activates the caspase cascade but also suppresses Akt phosphorylation and NF-κB expression in HepG2 cells [29]. The effects of RIPs on other cancers are summarized in Table 1.

Cellular mechanism of RIPs
Entry mechanism
Ribosome-inactivating proteins must enter cells to inactivate the eukaryotic ribosome via their RNA N-glycosidase activity. First, type II RIPs bind to glycoproteins and/or glycolipids on the cell membrane and enter the cell via endocytosis; then, RIPs undergo retrograde transport from the Golgi apparatus to the endoplasmic reticulum via an intracellular pathway [30]. The enzymatic moieties will not be released to cytosol and reach the ribosomes to exert their function until they exploit the endoplasmic reticulum-associated degradation pathway [3].

It is difficult for type I RIPs to enter cells because of their sugar-binding activity deficiency. They can enter cells to some extent, probably due to their interaction with phospholipids in the cell membrane; however, the exact entry mechanism remains unclear. To facilitate the entry of type I RIPs into cells, they can be linked to proper carriers such as monoclonal antibodies and other molecules. The resulting conjugates can be specifically toxic to target cells. Several immunotoxins have been well studied in experiment therapies against hematologic and solid tumors. The entry pathways of type I RIPs, type II RIPs, and immunotoxins are shown in Figure 1.

Induction of apoptosis in tumor cells
Caspases play an important role in apoptosis. They are classified into three types: initiator, executioner, and cytokine processor caspases. Great progress has been made in studying the three signaling pathways related to caspase activation, including mitochondrial, death receptor, and endoplasmic reticulum stress signaling pathways. The connections among these pathways are shown in Figure 2 [31]. Apoptosis also occurs through apoptosis-inducing factor (AIF), which is caspase-independent [32].

Mitochondria-mediated apoptosis
Recent studies have indicated that apoptosis-inducing substances can lead to excessive reactive oxygen species production, intracellular Ca2+ imbalance, and a series of pathologic changes, resulting in mitochondrial membrane potential and permeability changes. Then, the pro-apoptotic factors cytochrome c, AIF, second mitochondria-derived activator of caspases (Smac), and apoptotic protease-activating factor 1 (Apaf-1) are released from the mitochondria to participate in the process of apoptosis.

Mitochondrial membrane potential depolarization and caspase-9 activation were detected in MCF-7 cells and to a lesser extent in MDA-MB-231 cells after marimorin treatment [10]. Li et al. [33] reported the loss of mitochondrial membrane potential (the point of no return in apoptotic cascades) in HL-60 cells after apoptosis was induced by TCS. In addition, Orrenius et al. [34] noted that cytochrome c release is dominated by the Bcl-2 family of proteins. Furthermore, simultaneous Bax up-regulation, Bcl-2 down-regulation, and poly(ADP-ribose) polymerase (PARP) cleavage were noted in Abrus agglutinin-treated HepG2 cells, caspase-3/7 activity levels failed to increase after Bax knockout, and Bcl-2 overexpressing hepatocellular carcinoma cells were found to be ricin-resistant [29].

Several pumps, such as the Na+–K+ pump and the Ca2+ pump, maintain concentration gradients of various ions to achieve appropriate membrane potential. Alterations in the mitochondrial membrane potential after the induction of apoptosis lead to changes in membrane permeability. The results mentioned above suggest that changes in mitochondrial membrane permeability could cause apoptosis, which is induced by RIPs through decreasing the Bcl-2/Bax ratio (modifying the outer mitochondrial membrane permeability); this in turn enhances cytochrome c and Smac translocation into the cytoplasm and activates caspase-9 and the downstream executioner caspase-3, thereby increasing the production of cleaved PARP and resulting in DNA fragmentation and apoptosis [34–36].

Death receptor-mediated apoptosis
Death receptors, such as Fas, deliver apoptotic signals into the cytoplasm by binding to Fas ligand (FasL); the signals are then passed to downstream procaspase-8, the activation of which demands the cytoplasmic adaptor molecule, which is indispensable to the binding and proteolysis of procaspase-8 for activation. Once activated,
| RIP | Tumor type | Tested cell line(s) |
|-----|------------|--------------------|
| **Type I** | | |
| Trichosanthin | Breast cancer | MDA-MB-231<sup>a</sup> and MCF-7 [5] |
| Lymphoma | CEM, Hut-78, Raji, and Daudi [12] |
| Cervical cancer | HeLa [37], Caski [38] |
| Choriocarcinoma | JAR and BeWo [39] |
| Colon cancer | CT-26 [40], LoVo [41] |
| Hepatoma | HepG2 [42] |
| Leukemia | Mol-T-4 and Jurkat [12], K562 [43] |
| Lung cancer | 3LL<sup>a</sup> [44] |
| Melanoma | B16 [45] |
| Nasopharyngeal cancer | CNE1<sup>a</sup> and CNE2<sup>a</sup> [46], CNE2 [47] |
| Prostate cancer | RM-1 [48] |
| Gastric cancer | MCG803 [49] |
| α-Momorcharin | Breast cancer | MCF-7, EMT-6<sup>a</sup>, and MDA-MB-231<sup>a</sup> [11] |
| Colon cancer | SW480 and SW620 [50] |
| Epidermoid | A431 and Hep-2 [50] |
| Hepatoma | HepG2 and SMMC-7721 [50] |
| Lung cancer | NCI-H460 and A549 [50] |
| Melanoma | B16, M14, SK-MEL-28, and A2058 [50] |
| Nasopharyngeal cancer | CNE2 and HONE1 [51] |
| Momordica anti-HIV protein of 30 kDa | Bladder cancer | 5637 [52] |
| Breast cancer | MDA-MB-231<sup>a</sup> [6], BT20 [53], MCF-7 [54] |
| Epidermoid | A431 [53] |
| Glioma | U87MG [53] |
| Hepatoma | HepG2<sup>a</sup> [27], Hep-3B [53] |
| Melanoma | Malme-3M [53] |
| Myeloma | U266 [53] |
| Neuroblastoma | SK-N-SH [53] |
| Prostate cancer | DU145 [53] |
| Lung cancer | A549 [55] |
| Cucurmosin | Lung cancer | A549 [13] |
| Melanoma | B16 [13] |
| Hepatoma | HepG2<sup>a</sup> [28] |
| Leukemia | NB4 [14], K562<sup>a</sup> [56] |
| Myeloma | RPM18226 [57] |
| Pancreatic cancer | BxPC-3 [58], SW-1990 [59], PANC-1<sup>a</sup> [60], CFPAC-1 [61] |
| Saporin | Leukemia | NALM-6<sup>a</sup> [22], HSB-2<sup>a</sup> [23], CCRF CEM<sup>a</sup> [24] |
| Glioma | U87MG [62] |
| Lymphoma | Ramos, Raji<sup>a</sup>, Daudi, DOHH-2, and Granta S19, SUDHL-4 [21], HDLM2, KW/H2, and L428 [63] |
| Neuroblastoma | SK-N-MC<sup>a</sup> [64] |
| Ovarian cancer | PA-1<sup>a</sup> [64] |
| Melanoma | SK-Mel-1<sup>a</sup> [64], SK-Mel-28 [65] |
| Pancreatic cancer | BxPC-3<sup>a</sup> [66] |
| Prostate cancer | LNCaP<sup>a</sup>, CWR22Rv1, and DU145 [67], PC-3<sup>a</sup> [68] |
| Gelonin | Breast cancer | MDA-MB-231<sup>a</sup>, BT-474, SKBR3, MCF-7, and Eb1 [8] |
| Melanoma | MDA-MB-435<sup>a</sup>, WM35, WM46, WM3211, WM1346, WM1 361A, WM1366, WM793, WM983A, WM983B, MelWo, SB2, A375, A375S, SK-MEL-1, SK-MEL-3, SK-MEL-5, SK-MEL-24, SK-MEL-28, SK-MEL-32, WM35P2N1, AAB-527, and Sbc2 [18] |
| Cervical cancer | ME-180 [69] |
| RIP                        | Tumor type    | Tested cell line(s)                                                                 |
|----------------------------|---------------|------------------------------------------------------------------------------------|
| Marmorin                   | Breast cancer | MCF-7<sup>a</sup> and MDA-MB-231<sup>a</sup> [10]                                |
| α-Sarcin                   | Astrocytoma   | 251-MG [76]                                                                         |
|                            | Breast cancer | MCF-7 [76]                                                                          |
|                            | Glioma        | RuGli [76]                                                                           |
|                            | Pancreatic cancer | Patu II [76]                                                                 |
|                            | Bladder cancer | EJ [77]                                                                              |
|                            | Colon cancer | HT-29<sup>a</sup> and BCS-T22 [76]; SW1222 [78]                                     |
|                            | Sarcoma       | HT-1080 [76]; S-180 [79]; RD [80]                                                  |
| Curcin                     | Lung cancer   | NCL-H446 [81]                                                                        |
|                            | Gastric cancer | SGC-7901 [81]                                                                       |
|                            | Sarcoma       | S-180 [82]                                                                           |
| α-Luffin                   | Breast cancer | MCF-7 [83]                                                                           |
|                            | Choriocarcinoma | JEG-3 [83]                                                                            |
|                            | Hepatoma      | HepG2 [83]                                                                           |
| MCP30                      | Prostate cancer | LNCaP, PC-3, and PIN [84]                                                            |
| Gelonium anti-HIV protein of 31 kDa | Breast cancer | MDA-MB-231<sup>a</sup> [6]                                                            |
| Type II                    | RIProximin    | Breast cancer MCF-7 and MDA-MB-231 [62]                                              |
|                            | Larynx cancer | Hep2 [62]                                                                            |
|                            | Leukemia      | AR230, CML-T1, HL-60, LAMA84, SKW-3, KS62, and BV173 [62]                            |
|                            | Lung cancer   | NCI-H460 and Lewis [62]                                                              |
|                            | Pancreatic cancer | ASML [62]                                                                            |
|                            | Prostate cancer | PC-3 [62]                                                                            |
|                            | Sarcoma       | Saos-2 [62]                                                                          |
|                            | Cervical cancer | KB-3-1<sup>a</sup>; HeLa [85]                                                       |
|                            | Colon cancer  | HT-29, CC331<sup>a</sup>; and CT-26<sup>a</sup> [62]; HCT116 [86]                   |
| Abrus agglutinin           | Hepatoma      | HepG2<sup>a</sup> [29]                                                              |
| Momordica charantia lectin | Nasopharyngeal cancer | CNE1 and CNE2 [35]                                                                  |
| Articulatin-D              | Leukemia      | Jurkat, Molt-4, and HL-60 [15]                                                       |
|                            | Lymphoma      | U937 and Raji [15]                                                                   |
| Mistletoe lectin I         | Leukemia      | NALM-6 [87]                                                                          |
| Foetidissinin II           | Cervical cancer | HeLa [88]                                                                            |
|                            | Leukemia      | TF-1a [88]                                                                           |
| Ebulin I & Nigrin b        | Cervical cancer | HeLa [89]                                                                            |

<sup>a</sup> Cell lines that have been studied in mouse.

<sup>b</sup> Cell lines that have been studied in rat.
the initiator caspase-8 can activate caspase-3, eventually leading to cell apoptosis.

Marmorin was found to trigger the death receptor apoptotic pathway in MCF7 cells; this pathway is also preferentially activated in MDA-MB-231 cells [10]. Due to caspase-3 deficiency in MCF7 cells, caspase-8 amplifies the apoptotic signal through cleavage of the protein Bid, which punctures the mitochondria and causes mitochondrial collapse, thereby generating sufficient effector caspase levels. Conversely, TCS does not affect Fas or FasL levels, indicating that the Fas/FasL pathway is not involved in TCS-induced apoptosis [32].

**Endoplasmic reticulum stress-mediated apoptosis**

Endoplasmic reticulum stress is found in cells exposed to environmental toxins, hypoxia, viruses, ultraviolet light, and other stimuli. Its manifestations include misfolded and/or unfolded protein aggregation in the endoplasmic reticulum lumen as well as Ca\(^{2+}\) balance disorders. Endoplasmic reticulum stress can promote a series of physiologic changes in the endoplasmic reticulum. Accumulated misfolded and/or unfolded proteins are processed, allowing cells to maintain their normal functions and remain alive. However, excessive endoplasmic reticulum stress can cause apoptosis.

Trichosanthin treatment was shown to up-regulate the endoplasmic reticulum stress-related proteins Bip (immunoglobulin-binding protein) and CHOP (C/EBP homologous protein) in HL-60 cells, thereby activating caspase-4, which is involved in caspase-3 activation [89]. Endoplasmic reticulum stress was also described in marmorin-treated MCF7 and MDA-MB-231 cells, as evidenced by CHOP up-regulation and caspase-12 cleavage [10].

Horrix et al. [86] identified activation of the unfolded protein response (UPR) in response to endoplasmic reticulum stress. UPR includes three main pathways: the PERK-IRE1\(\alpha\)-XBP1 pathway, the ATF6 pathway, and the IRE1\(\beta\)-XBP1 pathway. These pathways are critical in mediating the survival of cells under stress conditions. The PERK-IRE1\(\alpha\)-XBP1 pathway is activated by phosphorylation of the eukaryotic translation initiation factor 2 (eIF2\(\alpha\)), leading to the inhibition of translation initiation and the activation of transcription factors such as ATF-4 and CHOP. The ATF6 pathway is activated by the release of calcium from the endoplasmic reticulum, leading to the cleavage and activation of the transcription factor ATF-6. The IRE1\(\beta\)-XBP1 pathway is activated by the binding of XBP-1 to the IRE1\(\beta\) protein, leading to the spliced form of XBP-1, which is then translocated to the nucleus and regulates the expression of genes involved in endoplasmic reticulum stress response.

**Figure 1** Cell entry mechanism of ribosome-inactivating proteins (RIPs). Different types of RIPs enter the cell through endocytosis and are subsequently degraded in the endoplasmic reticulum. They inactivate ribosomes through cleavage of the A\(_{4324}\) N-glycosidic bond, resulting in protein synthesis blockade.
reticulum stress; the UPR is induced in MDA-MB-231 cells exposed to low concentrations of the type II RIP riproximin. As many cancer cells activate the UPR to cope with stressors, α-MMC was shown to down-regulate the UPR in NPC cells; however, substantial apoptosis was not observed until the α-MMC dosage reached a certain threshold, indicating that α-MMC at low concentrations probably inhibit increased cell generation via down-regulation of the UPR [51]. There are two conceivable strategies to initiate apoptosis through endoplasmic reticulum stress: (1) prolonging the UPR to induce apoptosis, which likely occurs in riproximin-induced apoptosis; and (2) blocking the UPR so that tumors are vulnerable to stressors, as with α-MMC.

Future research emphasis

Conventional cancer drugs that are currently in use often lack tumor specificity, which greatly limits the therapeutic dose and curative effect. A feasible way to overcome this issue is the use of targeted therapy, as follows: (1) suitable targeted delivery such as with the immunotoxins mentioned above or with bi-specific antibodies (containing two different specific antigen recognition Fab fragments); (2) a tumor-specific expression strategy, in which the cDNA of RIP is synthesized and cloned into a plasmid vector controlled by a cancer-specific promoter, eventually producing RIP in the cell cytoplasm. These strategies must be investigated in a series of pre-clinical studies before assays can be conducted in human...
subjects. Several saporin-containing immunotoxins in clinical trials have exhibited promising results [20], whereas other RIP-containing immunotoxins have barely been studied. A few factors must be considered when translating preclinical data into the clinic: the risk of immunogenicity and toxicity in patients should be minimized; the minimum effective dose and maximum tolerated dose should be determined; and possible adverse effects during treatment should be predicted. Tumor-specific expression strategies are rarely reported; therefore, this idea remains to be explored.

Conclusions

Abundant evidence indicates that RIPs exert their cell-killing abilities through a variety of mechanisms, many of which are caspase-dependent. Although several mechanisms involved in RIP-induced apoptosis have been elucidated, more studies are required to reveal the precise mechanism. Considering the potential use of RIPs in important diseases and their effectiveness as immunotoxins for targeted therapy, RIPs are worthy of further exploration.

Authors’ contribution

MZ conceived the topic and drafted the manuscript. MZ helped in the manuscript preparation. DL and JW participated in the data collection. WJ and OS helped to conceive the topic and contributed significantly to the manuscript revision. All authors read and approved the final manuscript.

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Acknowledgements

We thank the Basic Research Program of Shenzhen (JCYJ20120613113228732) and the University Innovation Program of Guangdong Province (201410590040).

Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

Received: 9 February 2015 Accepted: 14 April 2015

Published online: 17 July 2015

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