Ribosome-Inactivating Proteins (RIPs) in Cancer Therapy: A Review of Reviews From Mechanisms to Medical Breakthroughs in Immunotoxin

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Abstract

Ribosome-inactivating proteins (RIPs) are plant-derived toxins that inhibit protein synthesis by removing adenine residues from eukaryotic ribosomal RNA. RIPs exhibit antiviral, antifungal, and insecticidal properties, and their expression in plants increases under stress. Over a century of research has uncovered their significant impact in cancer therapy and medical research. This review traces the evolution of RIPs, from their discovery to their current applications in oncology, highlighting their ability to selectively induce apoptosis in malignant cells. The mechanisms underlying RIP-induced cell death are examined, focusing on enzymatic activity, structural classification, and apoptosis-inducing pathways. Special emphasis is placed on RIPs' role as components of immunotoxins, where they are conjugated with tumor-specific targeting agents to enhance therapeutic precision. Recent advances in molecular biology and bioengineering have facilitated the development of recombinant immunotoxins, addressing challenges such as off-target toxicity and limited specificity. By synthesizing insights from diverse studies, this review underscores the transformative potential of RIPs in precision oncology while identifying the need for further research to optimize their clinical application.

Keywords: apoptosis, bioengineering, cancer therapy, immunotoxins, precision oncology, ricin, ribosome-inactivating proteins, saporin.

Structure of the paper:

I. Introduction

Ribosome-inactivating proteins (RIPs) are toxins predominantly found in plants, bacteria, and fungi, known for their ability to inhibit protein synthesis by permanently altering ribosomes (Ragucci et al., 2024). RIPs have been extensively studied for their therapeutic potential, particularly in cancer treatment, due to their capacity to selectively target malignant cells (Daniel Gillet (Ed.) & Julien Barbier (Ed.), 2019; Puri et al., 2012; Zeng et al., 2015). This has garnered significant attention, positioning RIPs as highly selective agents capable of delivering toxins specifically to cancer cells. Various RIPs, such as ricin and saporin, have shown promise in targeting cancer cells (P. Chen et al., 2024). Advances in molecular biology techniques, including bioinformatics and genetic engineering, have enabled scientists to gain deeper insights into the development of cancer cells (Lu et al., 2020). Furthermore, research into the general mechanisms and enzymatic activity of RIPs can enhance understanding of their function, opening new avenues for biotechnological applications (Fabbrini et al., 2017).

The rationale for this paper stems from the need to synthesize and expand on existing knowledge about ribosome-inactivating proteins (RIPs) and their therapeutic potential in cancer treatment. Early research demonstrated apoptosis pathways triggered by RIPs, highlighting their cytotoxic mechanisms (Sikriwal & Batra, 2010). However, the complexity of these mechanisms necessitated further investigation. A review on RIPs and their tumor-targeting potential revealed the evolution of RIPs from plant defense molecules to tumor-targeting agents, providing foundational insights into their anticancer applications (Virgilio et al., 2010). In 2016, another review presented a historical overview of RIPs' potential in agriculture, medicine, and drug development (Bolognesi et al., 2016).

More recent studies have focused on clinical applications and advanced therapeutic technologies. Research on RIP-based immunotoxins has addressed challenges such as specificity and toxicity while advocating for clinical refinement (Rust et al., 2017). Additionally, a 2024 review highlighted advancements in antibody-drug conjugates and other targeted therapies, showcasing RIPs' integration into modern oncology (Crescioli et al., 2024). By synthesizing these developments and addressing key gaps, this paper aims to advance RIP-based cancer therapies, with a focus on selective cytotoxicity and innovative delivery mechanisms.

This paper underscores the need for a comprehensive review of reviews to address critical gaps in the understanding of ribosome-inactivating proteins (RIPs) and their therapeutic applications in cancer treatment. By synthesizing insights from diverse studies, it highlights RIPs' selective cytotoxicity, apoptotic mechanisms, and potential in oncology while identifying challenges such as off-target toxicity and limited clinical specificity. Although previous reviews have explored general mechanisms of RIP action, significant gaps persist in understanding the complex

processes that trigger cell death in cancer cells. This paper aims to unify existing knowledge, emphasize recent discoveries, and propose innovative strategies to overcome current limitations, ultimately advancing the therapeutic potential of RIPs and guiding future research in precision oncology.

II. Evolution of research on RIPs ever since its discovery

Currently, the term “ribosome-inactivating protein” yields approximately 106,000 results on Google Scholar (including patents) and about 11,034 results on PubMed, reflecting the extensive body of research on these proteins. The discovery of RIPs dates back to the late 19th century, primarily from plant-derived sources (Daniel Gillet (Ed.) & Julien Barbier (Ed.), 2019). Plant toxins were identified in the seeds of *Ricinus communis L.* (castor bean) and *Abrus precatorius L.* (jequirity bean), the respective sources of the RIPs ricin and abrin (Walsh et al., 2013). These toxins were initially recognized as proteins capable of agglutinating red blood cells (Polito et al., 2019). Early studies erroneously attributed their toxicity to this ability, but the identification of ricin marked a pivotal moment in biochemistry, demonstrating a distinct biological function for a plant protein. Similarly, abrin proved instrumental in advancing early immunological research.

A significant breakthrough came in 1891 through Paul Ehrlich’s work at the Berlin Institute of Infectious Diseases, where he purified ricin and proposed the revolutionary “magic bullet” concept (Summers, 2024). This idea laid the foundation for targeted therapy by demonstrating how specific molecules could selectively attack and destroy target cells (Bolognesi et al., 2016). Ehrlich’s experiments showed that mice exposed to small, non-lethal doses of ricin or abrin developed immunity to the specific toxin. Notably, immunity to one toxin did not confer protection against the other, underscoring the specificity of immune responses (Summers, 2024). In 1898, Elfstrand introduced the term “agglutinin” to describe proteins capable of causing red blood cells to clump (Van Damme et al., 2008). Subsequent research revealed that the hemagglutinating properties of plant proteins varied significantly depending on the type of blood cells tested, offering new insights into protein-blood cell interactions.

RIPs have also played a prominent role in traditional Chinese medicine for centuries. For instance, Trichosanthin has shown remarkable potential in cancer treatment, particularly against hepatocellular carcinoma (F. Fang et al., 2011). Studies have demonstrated its efficacy in killing liver cancer cells both in vitro and in vivo using murine xenograft models (Zhang et al., 2024). This therapeutic concept was further advanced in 1970 when Moolten and Cooperband successfully conjugated a protein synthesis-inhibiting toxin with a targeting antibody, creating the first immunotoxin for cancer treatment (Rust et al., 2017).

While most RIPs are plant-derived, some are produced by bacteria, such as Shiga toxins. These bacterial RIPs are believed to aid in the survival and replication of pathogens within host

organisms (Walsh et al., 2013). From their initial discovery to their contemporary applications in cancer therapy and immunology, RIPs have undergone a remarkable evolution in understanding and utilization, paving the way for innovative therapeutic strategies.

III. Mechanism of action of RIPs

Ribosome-inactivating proteins (RIPs) are protein toxins that irreversibly inhibit protein synthesis by interacting with or inactivating ribosomes or disrupting the translation process through modifications in the elongation phase. These proteins induce cell death, commonly referred to as apoptosis. RIPs are classified into several types based on their structure and function.

Type I RIPs consist of a single toxic subunit (A-chain), whereas Type II RIPs are composed of two polypeptide chains: a toxic A-chain and a galactose-specific lectin subunit (B-chain), connected via a disulfide bond as presented in Figure 1 (Sharma et al., 2023). Type II RIPs are heterodimeric proteins, with an enzymatic A-chain (approximately 30–35 kDa) and a lectin-like B-chain that facilitates cell binding (Wang et al., 2016).

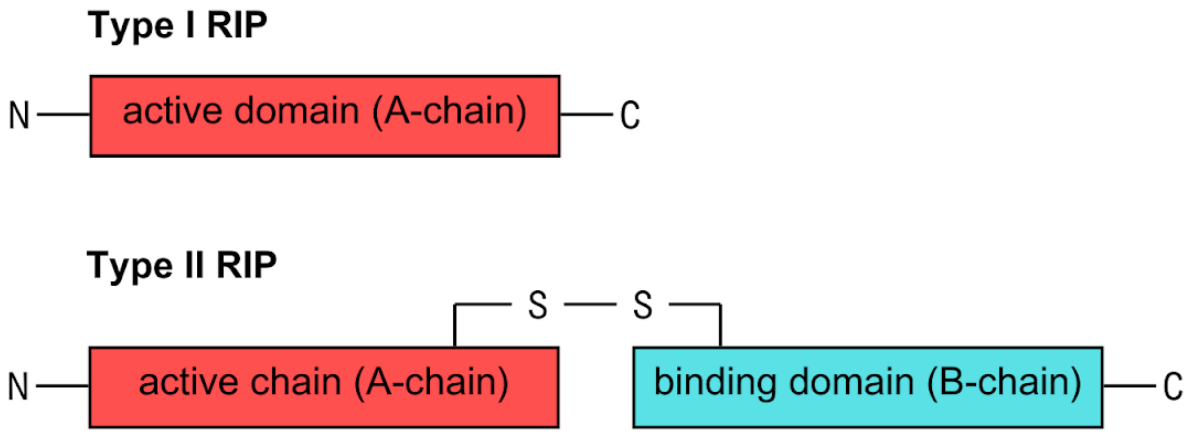

Figure 1. A schematic representation of the molecular structures of type 1 and type 2 RIPs.

Research on the endocytic mechanism of Type II RIPs has been primarily conducted on ricin. Ricin targets the large 60S ribosomal subunit of eukaryotic ribosomes (Sharma et al., 2023) and modifies elongation factors dependent on GTPase activity. It enzymatically depurinates adenine at position 4324 of the 28S rRNA (Figure 2), disrupting the stem-loop structure necessary for elongation factor binding during translocation (X.-Y. Chen et al., 1998). This action results in the complete inhibition of translation within the cell (Narayanan et al., 2005).

The B-chain of Type II RIPs binds to galactose-containing glycoproteins or glycolipids on the plasma membrane, facilitating cellular uptake via endocytosis. Once internalized, RIPs are

transported from the Golgi apparatus to the endoplasmic reticulum (ER), where the A-chain is separated from the B-chain. The A-chain mimics ER-associated degradation substrates, avoiding degradation due to its low lysine content, and enters the cytosol to inactivate ribosomes, ultimately inducing apoptosis (Ruschig & Marschall, 2023).

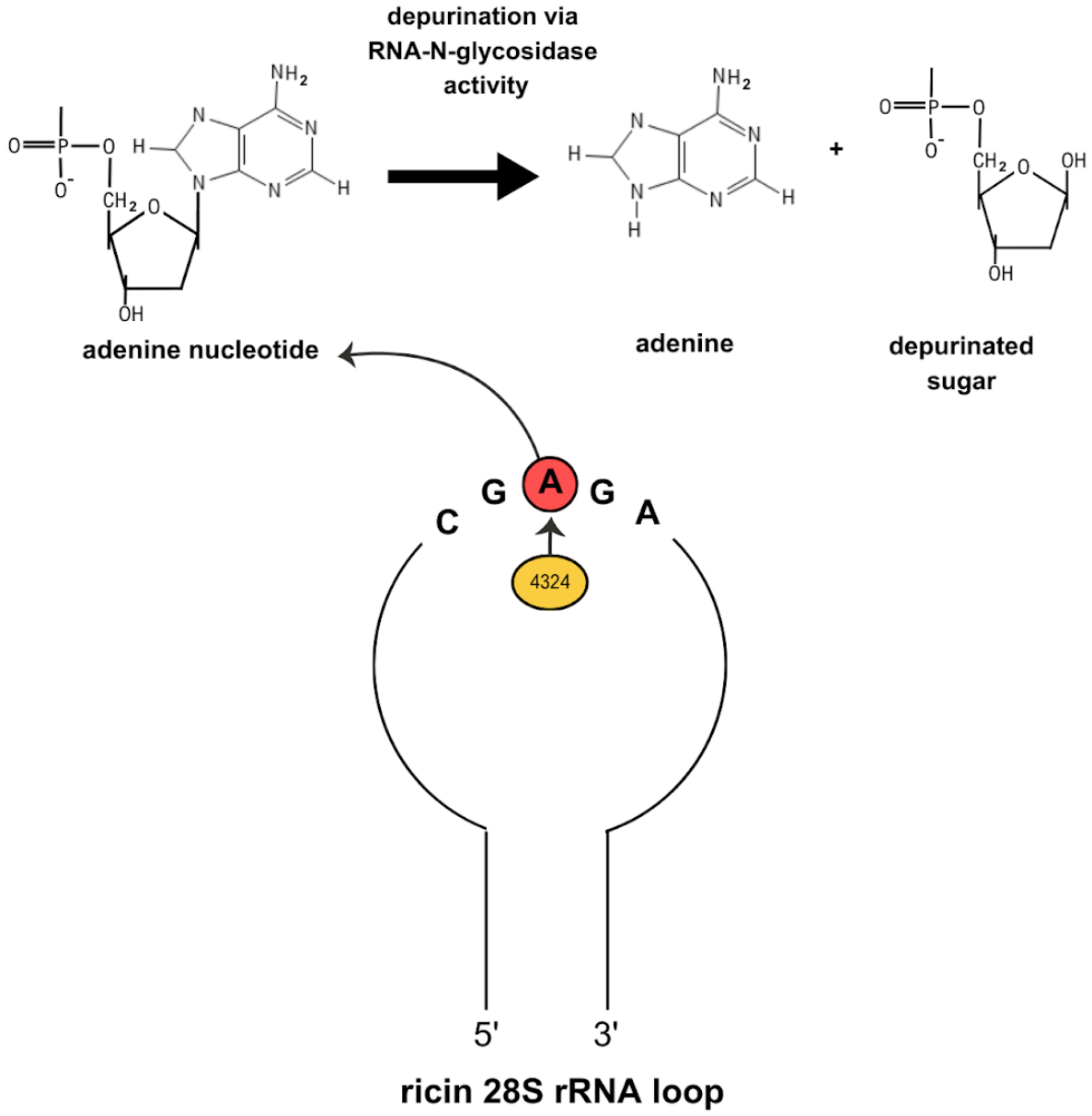

Figure 2. Schematic representation of ricin disrupting the stem-loop structure, leading to complete inhibition of cellular translation.

When comparing cytotoxicity, Type I RIPs are generally less potent than Type II RIPs due to the absence of the cell-binding B-chain (Bolognesi et al., 2016). Type II RIPs are more cytotoxic because their lectin-like B-chain enhances cell binding (Rust et al., 2017). However, binding

alone is insufficient to determine potency, as cytotoxicity levels vary among RIPs, even those derived from the same source. For instance, ricin is 68 times more potent than ricinus agglutinin (RCA), likely due to RCA's reduced ability to translocate into the cytoplasm (Stirpe & Battelli, 2006). Differences in cytotoxicity may also arise from variations in intracellular trafficking. For example, type II RIPs such as nigrins and ebulins exhibit low toxicity in vitro and in vivo but show high toxicity in acellular systems due to distinct membrane trafficking pathways (DiMaro et al., 2015).

Initially, RIPs were believed to induce cell death solely by inhibiting protein synthesis, leading to necrosis. However, recent studies indicate they can also trigger apoptosis through various mechanisms, including mitochondrial membrane potential loss, caspase activation, and the regulation of apoptotic proteins (Narayanan et al., 2005). Although all RIPs share the ability to target 28S rRNA, they do not follow a single pathway to induce apoptosis. Instead, multiple mechanisms are likely at play, including ribotoxic stress responses, ER stress, activation of unfolded protein response (UPR) genes, interactions with antioxidant proteins, and the production of reactive oxygen species. These processes may act synergistically, with their intensity and manner varying across cell types (Mercatelli, 2015)

RIP-induced apoptosis can occur via multiple pathways (Rust et al., 2017). While protein synthesis inhibition plays a critical role, it is not always essential. For instance, ricin has been shown to cause early nuclear DNA damage independently of protein synthesis inhibition (Lord et al., 2003). Similarly, saporin isoform S6 triggers apoptosis through the mitochondrial cascade before affecting protein synthesis (Giansanti et al., 2018).

IV. Emerging trends of therapeutic application of RIPs in cancer treatment

Ribosome-inactivating proteins (RIPs) are highly sought after in cancer therapy due to their potent ability to induce apoptosis with minimal required dosages. This high potency means that only a few RIP molecules need to enter the cytosol of a cancer cell to trigger cell death. However, this same toxicity poses a significant challenge, as RIPs can affect both malignant and healthy cells (Pizzo & Di Maro, 2016). To enhance specificity and reduce off-target effects, RIPs are often conjugated with targeting ligands, such as antibodies, growth factors, or cytokines, to form immunotoxins. These immunotoxins leverage the targeting capabilities of antibodies to deliver the cytotoxic RIP directly to cancer cells, thereby increasing selectivity and therapeutic efficacy (Akbari et al., 2017).

The most promising applications of RIPs in experimental medicine involve the use of advanced conjugation techniques to create targeted recombinant molecules (Crescioli et al., 2024). Despite the potential, unmodified Type I RIPs have shown limited clinical success. For instance, studies investigating their effectiveness in slowing HIV progression in AIDS patients yielded

poor results (Stirpe, 2013). Similarly, Type II RIPs face challenges due to the non-specific binding of their B-chains to various cell types, raising safety concerns for clinical applications.

To address these issues, research efforts have focused on harnessing the cytotoxic power of RIPs specifically against harmful cells, such as cancer cells, virus-infected cells, or autoreactive cells. By linking RIPs to targeting molecules like monoclonal antibodies (mAbs), lectins, hormones, and growth factors via disulfide bonds, scientists have developed immunotoxins (ITs) and other targeted compounds that selectively eliminate unwanted cells. These RIP-based immunotoxins are now utilized in cancer treatment and are also being explored for managing certain autoimmune disorders (DiMaro et al., 2015).

Despite these advancements, immunotoxin development is currently limited to a few types of cancers, primarily due to the difficulty in identifying specific antigens that are highly expressed on cancer cells but restricted in healthy tissues. Effective targets for immunotoxins must be abundantly present on the surface of cancer cells while being minimally expressed in normal cells to minimize off-target toxicity (Alewine et al., 2015). Nonetheless, immunotoxin development has made significant progress in treating cancers such as glioblastoma, leukemia, lymphoma, and breast cancer.

By 2010, antibody-based cancer therapies had gained substantial prominence, with 28 FDA-approved drugs for oncologic indications. Notably, eight of these therapies achieved global revenues exceeding US $1 billion, contributing to a total market value of over US $50 billion. The market for antibody-based cancer therapies has continued to grow, driven by increased research and development efforts. As of 2023, the field saw 16 new approvals and over 130 candidates in late-stage trials, highlighting significant advancements in precise, targeted therapies for complex diseases (Crescioli et al., 2024).

The most commonly used plant-based RIPs in immunotoxin development include the A-chain of ricin and Type I RIPs such as saporin and gelonin, known for their potency and stability. Recently, Type II RIPs with lower in vitro and in vivo toxicity but strong ribosomal RNA N-glycosidase activity, such as the A-chains of ebulins and nigrins from *Sambucus* species, have been proposed as alternatives for creating highly cytotoxic immunotoxins (Jiménez et al., 2015).

During the development of second-generation immunotoxins, significant progress has been made in addressing off-target toxicity and lethality at low doses. One strategy involves the removal of the toxin's cell-binding domain, allowing for higher dosages and reduced non-specific internalization. This is achieved by isolating the A-chain through purification and removing the B-chain via reduction of disulfide bonds. Isolating the A-chain minimizes residual non-specific toxicity and enhances blood circulation (Bolognesi et al., 2016).

The first Type I RIP immunotoxin was developed in 1980, consisting of gelonin conjugated to a thymus cell antigen (Thy 1.1) (Rust et al., 2017). Similarly, the Pokeweed Antiviral Protein (PAP), a Type I RIP, was utilized to create immunotoxins that demonstrated comparable potency against Thy 1.1. Saporin, another Type I RIP, has become a preferred choice for immunotoxin development due to its high thermodynamic stability (Bolognesi et al., 2016).

Some clinical trials have demonstrated promise for RIP-based immunotoxins. VB6-845, a recombinant protein comprising a modified bouganin targeting epithelial tumors, and recombinant gelonin linked to an anti-CD33 antibody for refractory myeloid leukemia, have been tested in Phase I trials (J. G. Brown et al., 2006). Although published results are limited, both immunotoxins exhibited low immunogenicity and no instances of vascular leak syndrome. Additionally, recombinant saporin has proven effective in treating bone cancer pain in older dogs, demonstrating the potential of Type I RIPs in veterinary oncology (D. C. Brown & Agnello, 2013).

V. Conclusion

Ribosome-inactivating proteins (RIPs) have emerged as a promising frontier in precision oncology, demonstrating exceptional potential for targeting malignant cells with minimal doses. Despite notable progress in elucidating their mechanisms and therapeutic applications, challenges persist, including off-target toxicity, limited antigen specificity, and difficulties in clinical scalability. Advances in bioengineering and molecular biology have facilitated the development of more selective and effective RIP-based treatments, such as recombinant immunotoxins and antibody-drug conjugates. Nevertheless, further research is imperative to unlock their full therapeutic potential. Future efforts should prioritize enhancing targeting specificity, reducing immunogenicity, and deepening our understanding of their diverse mechanisms of apoptosis induction. By addressing these challenges, RIPs can be seamlessly integrated into cancer therapies, offering safer and more efficient options for combating malignancies. Continued exploration of RIP evolution, combined with innovative therapeutic strategies, will be pivotal in advancing these molecules from experimental frameworks to clinical practice.

Several limitations are acknowledged in this study. The scope of reviewed publications spans an extensive timeline, starting from the discovery of RIPs in 1887 to the present day, without a detailed segmentation by decade or specific years. This broad range may obscure nuanced trends in research development. Furthermore, the analysis does not systematically explore annual variations in keyword usage or thematic shifts. To address these gaps, a systematic literature review focusing on specific periods or themes is recommended to better elucidate emerging trends and refine the understanding of RIP applications in cancer therapy.

VI. Future Directions

Future research should leverage advancements in artificial intelligence (AI) and bioinformatics to overcome the existing challenges in RIP-based therapeutics. The increasing interest in antibody and immunotoxin applications, as evidenced by a surge in related publications in 2024, underscores the relevance of RIPs in contemporary medicine. AI-driven methodologies can facilitate the identification of unique antigens on cancer cell surfaces, enabling the design of highly specific targeting ligands for immunotoxins. Additionally, computational modeling can optimize RIP structures, enhancing their efficacy while minimizing off-target effects. Advanced omics approaches, including proteomics and transcriptomics, can provide valuable insights into the diverse apoptosis pathways activated by RIPs, potentially uncovering novel therapeutic mechanisms. Collaboration among molecular biologists, bioengineers, and computational scientists will be critical for refining RIP-based therapies and advancing them to clinical applications.

References

Akbari, B., Farajnia, S., Ahdi Khosroshahi, S., Safari, F., Yousefi, M., Dariushnejad, H., & Rahbarnia, L. (2017). Immunotoxins in cancer therapy: Review and update. *International Reviews of Immunology*, *36*(4), 207–219. https://doi.org/10.1080/08830185.2017.1284211

Alewine, C., Hassan, R., & Pastan, I. (2015). Advances in Anticancer Immunotoxin Therapy. *The Oncologist*, *20*(2), 176–185. https://doi.org/10.1634/theoncologist.2014-0358

Bolognesi, A., Bortolotti, M., Maiello, S., Battelli, M., & Polito, L. (2016). Ribosome-Inactivating Proteins from Plants: A Historical Overview. *Molecules*, *21*(12), 1627. https://doi.org/10.3390/molecules21121627

Brown, D. C., & Agnello, K. (2013). Intrathecal Substance P-Saporin in the Dog: Efficacy in Bone Cancer Pain. *Anesthesiology*, *119*(5), 1178–1185. https://doi.org/10.1097/ALN.0b013e3182a95188

Brown, J. G., Cizeau, J., Bosc, D., Rasamoelisolo, M., Entwistle, J., Glover, N., & MacDonald, G. (2006). A preclinical profile of VB6-845, a recombinant immunotoxin for targeting ovarian cancer. *Cancer Research*, *66*(8_Supplement), 278–278.

Chen, P., Cao, X.-W., Dong, J.-W., Zhao, J., & Wang, F.-J. (2024). Saponin and Ribosome-Inactivating Protein Synergistically Trigger Lysosome-Dependent Apoptosis by Inhibiting Lysophagy: Potential to Become a New Antitumor Strategy. *Molecular Pharmaceutics*, *21*(6), 2993–3005. https://doi.org/10.1021/acs.molpharmaceut.4c00140

Chen, X.-Y., Link, T. M., & Schramm, V. L. (1998). Ricin A-Chain: Kinetics, Mechanism, and RNA Stem−Loop Inhibitors. *Biochemistry*, *37*(33), 11605–11613. https://doi.org/10.1021/bi980990p

Crescioli, S., Kaplon, H., Chenoweth, A., Wang, L., Visweswaraiah, J., & Reichert, J. M. (2024).

Antibodies to watch in 2024. *mAbs*, *16*(1), 2297450. https://doi.org/10.1080/19420862.2023.2297450

Daniel Gillet (Ed.), & Julien Barbier (Ed.). (2019). *Ribosome Inactivating Toxins*. MDPI - Multidisciplinary Digital Publishing Institute.

DiMaro, A., Pizzo, E., & Girbes, T. (2015). Biotechnological Potential of Ribosome Inactivating Proteins (RIPs). In P. Gopalakrishnakone, C. R. Carlini, & R. Ligabue-Braun (Eds.), *Plant Toxins* (pp. 1–19). Springer Netherlands. https://doi.org/10.1007/978-94-007-6728-7_14-2

F. Fang, E., B. Ng, T., C. Shaw, P., & N.S. Wong, R. (2011). Recent Progress in Medicinal Investigations on Trichosanthin and other Ribosome Inactivating Proteins from the Plant Genus Trichosanthes. *Current Medicinal Chemistry*, *18*(28), 4410–4417. https://doi.org/10.2174/092986711797200499

Fabbrini, M., Katayama, M., Nakase, I., & Vago, R. (2017). Plant Ribosome-Inactivating Proteins: Progesses, Challenges and Biotechnological Applications (and a Few Digressions). *Toxins*, *9*(10), 314. https://doi.org/10.3390/toxins9100314

Giansanti, F., Flavell, D., Angelucci, F., Fabbrini, M., & Ippoliti, R. (2018). Strategies to Improve the Clinical Utility of Saporin-Based Targeted Toxins. *Toxins*, *10*(2), 82. https://doi.org/10.3390/toxins10020082

Jiménez, P., Tejero, J., Cordoba-Diaz, D., Quinto, E., Garrosa, M., Gayoso, M., & Girbés, T. (2015). Ebulin from Dwarf Elder (Sambucus ebulus L.): A Mini-Review. *Toxins*, *7*(3), 648–658. https://doi.org/10.3390/toxins7030648

Lord, M. J., Jolliffe, N. A., Marsden, C. J., Pateman, C. S. C., Smith, D. C., Spooner, R. A., Watson, P. D., & Roberts, L. M. (2003). Ricin: Mechanisms of Cytotoxicity. *Toxicological Reviews*, *22*(1), 53–64. https://doi.org/10.2165/00139709-200322010-00006

Lu, J.-Q., Zhu, Z.-N., Zheng, Y.-T., & Shaw, P.-C. (2020). Engineering of Ribosome-inactivating Proteins for Improving Pharmacological Properties. *Toxins*, *12*(3), 167. https://doi.org/10.3390/toxins12030167

Mercatelli, D. <1982>. (2015). *Ribosome-inactivating proteins and their immunotoxins for cancer therapy: Insights into the mechanism of cell death* [Application/pdf]. https://doi.org/10.6092/UNIBO/AMSDOTTORATO/6791

Narayanan, S., Surendranath, K., Bora, N., Surolia, A., & Karande, A. A. (2005). Ribosome inactivating proteins and apoptosis. *FEBS Letters*, *579*(6), 1324–1331. https://doi.org/10.1016/j.febslet.2005.01.038

Pizzo, E., & Di Maro, A. (2016). A new age for biomedical applications of Ribosome Inactivating Proteins (RIPs): From bioconjugate to nanoconstructs. *Journal of Biomedical Science*, *23*(1), 54. https://doi.org/10.1186/s12929-016-0272-1

Polito, L., Bortolotti, M., Battelli, M., Calafato, G., & Bolognesi, A. (2019). Ricin: An Ancient Story for a Timeless Plant Toxin. *Toxins*, *11*(6), 324. https://doi.org/10.3390/toxins11060324

Puri, M., Kaur, I., Perugini, M. A., & Gupta, R. C. (2012). Ribosome-inactivating proteins: Current status and biomedical applications. *Drug Discovery Today*, *17*(13–14), 774–783.

https://doi.org/10.1016/j.drudis.2012.03.007
Ragucci, S., Landi, N., & Di Maro, A. (2024). Ribosome-inactivating proteins from edible plants: Isolation, characterization and possible biotechnological applications. In *Defense-Related Proteins in Plants* (pp. 333–368). Elsevier. https://doi.org/10.1016/B978-0-443-13236-0.00013-0
Ruschig, M., & Marschall, A. L. J. (2023). Targeting the Inside of Cells with Biologicals: Toxin Routes in a Therapeutic Context. *BioDrugs*, *37*(2), 181–203. https://doi.org/10.1007/s40259-023-00580-y
Rust, A., Partridge, L., Davletov, B., & Hautbergue, G. (2017). The Use of Plant-Derived Ribosome Inactivating Proteins in Immunotoxin Development: Past, Present and Future Generations. *Toxins*, *9*(11), 344. https://doi.org/10.3390/toxins9110344
Sharma, A., Gupta, S., Sharma, N. R., & Paul, K. (2023). Expanding role of ribosome-inactivating proteins: From toxins to therapeutics. *IUBMB Life*, *75*(2), 82–96. https://doi.org/10.1002/iub.2675
Sikriwal, D., & Batra, J. K. (2010). Ribosome Inactivating Proteins and Apoptosis. In J. M. Lord & M. R. Hartley (Eds.), *Toxic Plant Proteins* (Vol. 18, pp. 167–189). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-12176-0_9
Stirpe, F. (2013). Ribosome-inactivating proteins: From toxins to useful proteins. *Toxicon*, *67*, 12–16. https://doi.org/10.1016/j.toxicon.2013.02.005
Stirpe, F., & Battelli, M. G. (2006). Ribosome-inactivating proteins: Progress and problems. *Cellular and Molecular Life Sciences*, *63*(16), 1850–1866. https://doi.org/10.1007/s00018-006-6078-7
Summers, W. C. (2024). *Magic Bullets, Miracle Drugs, and Microbiologists: A History of the Microbiome and Metagenomics* (1st ed). ASM Press.
Van Damme, E. J. M., Lannoo, N., & Peumans, W. J. (2008). Plant Lectins. In *Advances in Botanical Research* (Vol. 48, pp. 107–209). Elsevier. https://doi.org/10.1016/S0065-2296(08)00403-5
Virgilio, M. D., Lombardi, A., Caliandro, R., & Fabbrini, M. S. (2010). Ribosome-Inactivating Proteins: From Plant Defense to Tumor Attack. *Toxins*, *2*(11), 2699–2737. https://doi.org/10.3390/toxins2112699
Walsh, M. J., Dodd, J. E., & Hautbergue, G. M. (2013). Ribosome-inactivating proteins: Potent poisons and molecular tools. *Virulence*, *4*(8), 774–784. https://doi.org/10.4161/viru.26399
Wang, S., Li, Z., Li, S., Di, R., Ho, C.-T., & Yang, G. (2016). Ribosome-inactivating proteins (RIPs) and their important health promoting property. *RSC Advances*, *6*(52), 46794–46805. https://doi.org/10.1039/C6RA02946A
Zeng, M., Zheng, M., Lu, D., Wang, J., Jiang, W., & Sha, O. (2015). Anti-tumor activities and apoptotic mechanism of ribosome-inactivating proteins. *Chinese Journal of Cancer*, *34*(3), 30. https://doi.org/10.1186/s40880-015-0030-x
Zhang, Y., Ding, X., Zhang, Q., Zeng, C., Chen, H., & Lu, L. (2024). Trichosanthin elicits antitumor activity via MICU3 mediated mitochondria calcium influx. *Journal of Advanced*

Research, S2090123224004934. https://doi.org/10.1016/j.jare.2024.11.001