BIBLIOGRAPHIC INFORMATION SYSTEM

Journal Full Title: Journal of Biomedical Research & Environmental Sciences

Journal NLM Abbreviation: J Biomed Res Environ Sci

Journal Website Link: <https://www.jelsciences.com>

Journal ISSN: 2766-2276

Category: Multidisciplinary

Subject Areas: Medicine Group, Biology Group, General, Environmental Sciences

Topics Summation: 128

Issue Regularity: Monthly

Review Process: Double Blind

Time to Publication: 21 Days

Indexing catalog: [Visit here](#)

Publication fee catalog: [Visit here](#)

DOI: 10.37871 ([CrossRef](#))

Plagiarism detection software: iThenticate

Managing entity: USA

Language: English

Research work collecting capability: Worldwide

Organized by: [SciRes Literature LLC](#)

License: Open Access by Journal of Biomedical Research & Environmental Sciences is licensed under a Creative Commons Attribution 4.0 International License. Based on a work at SciRes Literature LLC.

Manuscript should be submitted in Word Document (.doc or .docx) through

Online Submission

form or can be mailed to support@jelsciences.com

**IndexCopernicus
ICV 2020:
53.77**

Vision: Journal of Biomedical Research & Environmental Sciences main aim is to enhance the importance of science and technology to the scientific community and also to provide an equal opportunity to seek and share ideas to all our researchers and scientists without any barriers to develop their career and helping in their development of discovering the world.

REVIEW ARTICLE

Therapeutic Application of Genetically Engineered Ribosome-Inactivating Toxin Proteins for Cancer

Safir Ullah Khan^{1*} and Munir Ullah Khan²

¹Hefei National Laboratory for Physical Sciences at the Microscale, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

²MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

ABSTRACT

Recently, Ribosome-Inactivating Proteins (RIPs) as a class of anticancer medicines have garnered considerable attention due to their novel anticancer mechanism. Although the medications are small, RIPs utilize the Large-Size Effect (LSE) to block the efflux procedure that are regulated through Drug Resistance Transporters (DRTs), and protect host cells from drug transfection. There are many significant challenges for their therapeutic applications that seriously restrict their usefulness, particularly their strategy towards tumor cells. The primary objective of this review is to emphasize Trichosanthin (TCS) along with Gelonin (Gel) and additional types of RIPs, particularly scorpion venom-derived RIPs, to demonstrate that they should be grappling through what kinds of bio-barriers to overcome in cancer therapeutic science. Next, we will emphasize the latest state-of-the-art in providing cancer treatment RIPs.

INTRODUCTION

Most molecular target-based therapeutic research facilities use two basic techniques: protein-kinase inhibitors and monoclonal antibodies. However, they continue to face numerous hurdles related to medication resistance and response rates. The continuous efforts to find clinically acceptable medicines have been aided by the fact that RIPs may have anticancer potential. RIPs are a special family of cytotoxic polypeptides that bind large ribosomal subunits. The principal effect of these interactions is irreversible inhibition of protein synthesis. RIPs were discovered in bacteria, fungi, and plants that functioned as N-glycosylases that removed an adenine residue from the sarcin-ricin loop. This structure serves a vital role as a core constituent through ribosome and elongation factor II interactions. RIPs have several intriguing qualities for cancer therapeutics, the first of which being their unrivalled efficacy in attacking tumor cells by inhibiting cell protein production pathways mechanistically even at picomolar levels [1]. Small molecule-based anticancer therapies could not obtain this advantage because they could reach malignant cells cytosolic ribosomes.

Based on these facts and benefits, large-scale manufacture of RIPs for treating many types of tumors could be achievable using genetic recombination technologies that allow researchers to engineer the pharmacological ability of RIPs. Even though, RIPs may offer numerous advantages; despite this, the distribution of RIPs continues to struggle with various obstacles, including bio-barriers found within cells, tissue, and blood vessels. Various other delivery strategies have been suggested that are based on the RIPs. One challenge that vascular, intratumoral,

*Corresponding author

Safir Ullah Khan, Hefei National Laboratory for Physical Sciences at the Microscale, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

E-mail: safir@mail.ustc.edu.cn

DOI: 10.37871/jbres1375

Submitted: 10 December 2021

Accepted: 21 December 2021

Published: 22 December 2021

Copyright: © 2021 Khan SU, et al. Distributed under Creative Commons CC-BY 4.0

OPEN ACCESS

Keywords

- RIPs
- Proteins
- Cancer
- Scorpion venom
- Cytotoxicity
- Tumor drug delivery

MEDICINE GROUP

CANCER

VOLUME: 2 ISSUE: 12 - DECEMBER

How to cite this article: Khan SU, Khan MU. Therapeutic Application of Genetically Engineered Ribosome-Inactivating Toxin Proteins for Cancer. J Biomed Res Environ Sci. 2021 Dec 22; 2(12): 1216-1228. doi: 10.37871/jbres1375, Article ID: JBRES1375, Available at: <https://www.jelsciences.com/articles/jbres1375.pdf>

biobarriers and intracellular barriers all have in common is that they are all obstacles to the vascular system. For the time being, effective medication delivery methods have been utilized to overcome these issues and, more specifically, deal with permeability problems. The key challenge with this process is how well the protein permeates into the cells. Another technique is to inhibit protein function through the circulatory system to target cancer cells more effectively. The peptides deposited throughout the modification process that include introducing cell-penetrating peptides are becoming more specific and permeative for cancer cells.

Additionally, for effective intracellular transferring, the drug's ability to be released is a critical component that has proved a barrier for these techniques. The significant effects of this condition are cytotoxicity that cannot be controlled. Ensuring that the drug is delivered in a pharmacokinetically appropriate manner is another aspect of the problem that should be addressed concurrently. This review paper aims to highlight RIPs' structural and molecular characteristics and their potential as chemotherapy drugs.

TRICHOSANTHIN

TCS or Trichosanthin, derived from a Chinese herb, is classified as type I of RIP protein with a molecular weight of 27 kDa. This protein has seen considerable use in cancer treatment and the prevention of viral infections. An active type of TCS is produced when the 288 amino acid building block is combined with the 19 C- and 23 N-terminal building blocks. Determining the chain of amino acids in ricin TCS another has revealed five catalytic areas and two related domains in the same sequence [2]. TCS cytotoxicity is modulated by pH-dependent permeability during *in vitro* oligomerization. It is possible to test for the reduction in cytotoxicity by determining the capacity of the ribosomes to remain throughout the membrane structure following deactivation. The removal of 7 amino acid residues from the C-terminal region of TCS (C7-TCS) decreased its pH-dependent membrane penetration possibility; the removing of these amino acid residues resulted in complex formation and oligomerization, resulting in a decreased stage cytotoxicity (*in vivo*) and ribosome-inactivating property (*in vitro*) [3,4]. TCS is often used as a gynaecological medication to treat pregnancy complications and abortions in controlled doses.

ANTICANCER APPLICATION OF TCS

TCS exhibits anticancer characteristics during apoptosis by releasing reactive oxygen species (ROS) and altering the cancerous cells' mitochondrial system and endoplasmic reticulum [4]. Despite there being no association between N-glycosylase enzyme activity and apoptosis caused by TCS, there are a significant number of literature reports which describe the anticancer capabilities of TCS in both *in vivo* and *in vitro* situations (against carcinoma and hepatoma,

respectively) [5]. There have also been recorded cases where anticancer treatments employing TCS have been used to combat various malignancies, including melanoma, colon, prostatic, cervical, breast, and leukaemia/lymphoma [6]. The TCS has anticancer ability during apoptosis due to its inactivating ribosomes in cancer cells. In addition, it has been found to influence estrogen-dependent breast cancer cells (MCF-7 & MDA-MB-231) utilizing an ant proliferative and apoptotic influence on the caspase-8/-9 mechanisms⁴. TCS activates mitochondrial and ER pathways in HL-60 leukaemia cells. However, several findings propose that caspase-3 can activate the mitochondrial and endoplasmic reticulum pathways [7]. Thus, by regulating the protein kinase A, B & C pathways, the TCS can potentially decrease the proliferation processes of tumor cells⁸⁴. Ca²⁺ ions in extracellular media provide the inhibitory influence on adenylyl cyclase and cyclic adenosine monophosphate that serves to trigger apoptosis by causing activation of the caspase cascade [8].

Additionally, Due to the anticancer effects of TCS through apoptotic and telomerase suppressing mechanisms, these suppressive capacities require protein kinases C/A activity. They are dependent on such antitumor influences of TCS [9,10]. Notably, in the JAR/BeWo choriocarcinoma cell, low-density lipoproteins that act as phagocytosis receptors participate in the endocytosis pathway [11]. The purpose of which is most likely to include carrying the JAR/BeWo choriocarcinoma cells for endocytosis, transferring JAR/BeWo choriocarcinoma cells to endocytosis advancing phagocytosis of the cells [12]. Using results from the cDNA array analysis, a significant increase in the expression of Smac mitochondrial proteins, which operate as activators of cytochrome C caspase, was detected in human CaSki cells. Furthermore, a TCS resistance was developed in the stimulation of the CaSki cells, which required additional treatments [13].

ANTIVIRAL ACTIVITY OF TCS

Eventually, reputable publications demonstrated the TCS's robust antiviral effectiveness against many viruses. A substantial number of scientific research support the role of TCS in antiviral treatments for infections of the brain, which includes HSV-1 (the herpes simplex virus) [14]. Additionally, it offers excellent protection against the Herpes Simplex Virus (HSV) and HIV/AIDS [15]. Another example is the effect of TCS on HIV, where the organism's ribosome has been reported to be inactivated, and also p38 (a viral protein kinase) is lowered, limiting viral reproduction [16].

GELONIN (GEL)

A structure-based characterization would classify Genuine as having the molecular properties of RIP type I isolated from Gelonium multiflorum seeds containing 258 amino acids with MW of 28 kDa [17] homologies exists

between ricin and TCS, the former with an N-glycosylase and the latter with depurination adenine-4324 helps 28S RNA from α -sarcin/ricin (28S RNA from α -sarcin-ricin has a structural resemblance with TCS, having N-glycosylase with depurinates adenine-4324) gain significant consideration for anticancer as well as antiviral applications [18,19]. Low permeability into cells may result in poor therapeutic effect if a carrier⁵³ does not carry the Gel [20]. In the subsequent sentences, it will be demonstrated that during the 1980s, numerous efficient delivery methods have been developed, which can be characterized as relying on genetic engineering sciences' novel classes of substantially modified recombinant Gel that had more outstanding anticancer capabilities [21,22].

GEL FUSION PROTEINS AS ANTICANCER AGENTS

Genetic engineering has produced various Gel-based proteins via a chemical conjugation approach. One is rGel conjugation to anti-CD33 M195 antibodies with disulfide bond to boost tumor-targeted distribution efficiency [23]. The combination, as mentioned earlier, demonstrated increased cytotoxicity against CD33 cell lines expressing HL60, OCI/AML5, and OCIJAML2 [23]. Vegf121-rGel is another example. It has an endothelial signalling pathway attached to it via a G4S linker. It helps improve selectivity and efficacy against endothelial cells. The significant inhibitory effect was seen in prostate and melanoma animal models that used human prostate xenografts with VEGF121/rGel [24].

Some research projects were obtained, such as the B-lymphocyte stimulator and Gela selective binding, which utilized appropriate cells that expressed the B-lymphocyte stimulation sites. The specificity results have also been successful in B-cells, mantle-cell lymphomas have been associated with Mino, JeKo-1, and SP53, and broad-based lymphoma types B consist of OCI-Ly3 and SUDHL-6 [25]. As long as the receptors are bound to the cell, they bind to the NF- κ B molecules and stimulate their NF- κ B activity. Once bound to the cell, the NF- κ B molecules will induce their NF- κ B activity. Targets of NF-B such as Mcl-1, Bcl-Xl and survivin were shown to be knockdown after therapy [26].

In contrast to Bax and programmed cell death, such downregulation was also observed for IL-6R and STAT3 activity. While a rigorous and specific attachment of BAFF-R (BAFF-R Selective Binding), the B-lymphocyte stimulator/Gel, can successfully mutate CD19 + B-CCL lymphocytes, the downregulation of protein formation with inhibition and downregulation of BAFF-R from even 1800-fold greater efficiency can be obtained using a protein synthesis inhibition strategy with BAFF-R downregulation (a BAFF-R Inhibition Strategy) [27]. In one experiment, an Fdt linker, a fusion protein, was explored with an anti-HER2 human

antibody (C6.5) alongside an L linker, Fpe and Fdt, a linker that has AGNRVRRSVG, GGGGS, and Furin cleavage sites, respectively. In the case of Fdt, the cleavage efficiency increased by 18.5% when the pH was adjusted to 7.2, but only for Fpe was there any notable improvement. In addition, incubation at 37°C yielded the lowest anticancer activity for the Fdt linker [28]. Different forms of structurally engineered fusion proteins with peptide carriers such as melittin, chlorotoxin, F3 peptide, and anti-IGF-1R a body have been created recently [29]. For example, Cell membranes contain melittin, a toxin that can generate pores.

This toxin in the cell membranes might potentially disrupt the membranes' structure, making it easier for drugs to get into the cell membranes and specifically killing cancer and tumor cells. Melittin's drawback is its high dosage requirement for tumor treatment, limiting its therapeutic applicability. One technique for resolving that difficulty is fusing with the Gel that significantly improves cytotoxicity in HeLa, U87 MG, 9L, CT26, and LS174T cell lines compared to essential cancer treatments [30]. The capacity to perform such work could be attributed to the efficient uptake of Gel that has been improved by melittin. However, another example is a peptide as chlorotoxin, derived from scorpion venom, which could treat brain tumors due to its selective binding capabilities. Gel/chlorotoxin transgenic fusion hybrid increased the permeability of the cell membrane for U87 MG human glioma cells by four times that reported for rGel. For non-tumorigenic 293 HEK cells, this transfected potential produces 25-fold greater cytotoxicity than naked one [31]. F3 has the exclusive ability to reveal the angiogenesis endothelium, and malignant cells surround a peptide that binds to nucleolin. A collection of fusion F3/rGel hybrids was created utilizing cell-specific genetically engineered methods and showed an adequate intake and cytotoxicity for the carcinoma cells, including U87 MG, 9L LnCaP, and HeLa cell lines. After testing, the study found that Gel peptide and F3 peptide had a strong connection, serving an essential role in absorption and cytotoxicity [31]. These findings are consistent with previous findings in a mouse model of LNCaP s.c. Xenograft malignant, showing more tumor growth rate inhibition [31].

RIPS IN THE DELIVERY PROCESS

RIPs bypass the biobarriers to reach the tumor. Blood artery networks, the interstitial stress of the tumor, and the reticuloendothelial system are all components of the tumor's microenvironment, and an out-of-control blood flow rate is present in all cancers [32]. Another important consideration is that tumor cell membranes act as the last barrier to RIP transfection into the cells. These barriers must be eliminated to overcome the obstacles to an efficient delivery technique in cancer treatment [33]. Other cancer therapies, such as nanoflares, have additional scientific hurdles that have not been conclusively settled [34].

VASCULAR BIOBARRIERS

Because of their bioavailability, protein-based intravenous medicines have a big advantage over other treatments, making them sufficient compared to oral delivery. Involving the circulatory system is easier with intravenous administration. However, delivering the RIPs medicines to pathological locations must confront other problems [35]. Endothelial cells serve the vascular system by identifying and then blocking previously unknown spices (RIPs) agents because these are unrecognized external spices. Another vascular barrier that regulates the selective transit of necessary oxygen and nutrients into the brain is the blood-brain barrier—primarily regulated by a receptor-mediated transcytosis system via an endothelium-mediated transport mechanism. The drug delivery method described here could be employed for many therapies for cancer, including the administration of medicines directly into the brain [36]. This method may be used to administer medications to reversibly open the firm connections among endothelial cells in the blood-brain barrier. For this purpose, it is vital to consider bioavailability and degradability while creating drug carriers [37]. This idea may work because Endothelial sphingosine-1 phosphate-1 may be a receptor for size transport, especially if it occurs in conjunction with their size. Its receptor facilitates the movement of low-molecular-weight proteins that provide a pathway for transporting RIPs across the blood-brain barrier as drug delivery vehicles. Another potential side effect is the prolonged time for the medicine to reach various organs, resulting in lower drug efficiency [38]. When there are circulating proteins and immunocytes in the plasma, it causes medication molecules to be captured and bound when being phagocytosed, which results in decreased efficacy and terminated treatment. In this situation, macrophages are treated with IL-4/-13, which afterwards inhibits the INF- γ , which means that these macrophages can now capture protein medicines (substrates for macrophages) [39,40]. For in vivo pharmacokinetic studies, it must be kept in mind that the cardiovascular system could interact with the RIP medicines, resulting in differences in vivo pharmacokinetic data [36].

TUMORAL BIOBARRIERS

High interstitial fluid pressure and thick extracellular matrix are obstacles to RIP medicines getting to tumors. It has been well accepted that the endothelial walls play a key role in maintaining homeostasis [41]. Endothelial barriers that are imperfectly integrated cause an inadequate vessel, while leaky networks are disordered and physically faulty. It has led to a net loss of control over the inflating process within inflammatory cells and an unregulated increase in angiogenesis and extravasations of tumor cells, as there is an increased fluid volume pressure [42]. Therefore, increased interstitial fluid pressure coupled with a low blood flow causes therapeutic medicines to be under-delivered.

Regarding over-pressure, we can say that the symptom appears in resistance to nutrient transition, which generates an undesirable aggravation and an aberrant vascular system. These anomalies involve too much tortuosity in the arteries, which allows the growing tumor capillary sheaths to be completely unstressed [43]. The events mentioned above could harm the delivery of drugs to tumors. Although the intercellular barriers must also be considered, the extracellular matrix also plays a vital role in physiological homeostasis. They are tissue-specific and can poorly regulate dynamics leading to considerable cancer development. When it comes to cancer, bigger tumors are denser and stronger than smaller ones. It is because of the extracellular matrix and the surrounding fibroblasts [44]. These occurrences interfere with the delivery of the treatment and significantly raise the risk of cancer spread and cell proliferation.

Further, the remodelling of new tumors could cause metastasis, which could be due to the release of growth factors by the tumors [45]. It must be said that all of these extracellular variables influence the delivery efficiency within the tumor to a substantial degree. Thus, because of their big and high molecular size, the RIPs class of anticancer medications struggle with these barriers, preventing them from entering tumor tissue [46].

CELLULAR BIOBARRIERS

The next obstacle that anticancer drugs must overcome is the intracellular barrier, which involves binding to membranes. It is considered that electrostatic interactions between membrane and receptors play a significant role in this scenario. Interactions strength mediates binding and internalization. An example of this is the experiments, which found that nanoparticles having a positive charge are more likely to bind to cancer cell membranes than other types [47]. It could be read as cancer cells having a negative charge on their surface. Endocytosis is the process of taking in large molecules like RIPs, nanoparticles, proteins [47]. While endocytosis occurs in two primary pathways, the size-dependent absorption happens during the process [48]. The first is that Clathrin-mediated endocytosis takes up large and non-permeable particles such as those larger than about 200 nanometers. Secondly, caveolae mediate endocytosis for particles of approximately $> 5 \mu\text{m}$ [46]. This endosomal barrier within the intracellular medium might thus be considered the final step since the RIPs molecules might have been degraded due to the harsh proteolytic and acidic lysosomal cellular environment. They probably underwent a harsh lysosomal environment [19]. A careful understanding of how the cancer cells get to where they need to be in the cytoplasm is necessary for the efficient destruction of the tumor cells.

CELL-PENETRATING APPROACHES OF DELIVERIES

Various delivery mechanisms have been created to overcome these issues because hurdles such as Internet congestion could interrupt RIP delivery. Various delivery mechanisms have been created to overcome these issues [44]. The first choice is TAT peptides when looking for a RIP carrier due to their high potential for application. Until now, reports of their capability to deliver genes, small molecules, and certain types of drugs have been made. However, there is a continuing challenge in controlling the RIPs' activating mechanism, which allows them nonselective during the cancer treatment.

Furthermore, to address these issues, several smart methods have been found to deal with these problems. For instance, they involve the electrically modified antibodies called "Antibody Targeted Triggered Electrically Modified Prodrug Type Strategy (ATTEMPTS)." Additionally, they use methods triggered by enzymes or stimuli-responsive methods [49].

THE ANTIBODY TARGETED TRIGGERED ELECTRICALLY MODIFIED TYPES STRATEGY

ATTEMPTS, a novel approach to medication delivery based on the introduction of RIP to tumor cells, was created by Chen, et al. [50]. Mechanistically, two key aspects were followed throughout this technique: first, the conjugation of ligands and heparin, which provide a negative force; secondly, the construction of the hybrid cell penetration peptides and RIPs. Electrostatic forces between components are crucial for the stated delivery system, resulting in a long-lasting, stable structure. The internalization process happens during conjugation between a cell-penetrating peptide and an antibody; both boost delivery efficiency. Cell penetration peptides must have a low molecular mass to prevent the reversible and prolonged electrostatic attraction of heparin and protamine peptides. These interactions contribute to the inactivation of RIPs in the conjugated structure during blood circulation [51].

In summary, ATTEMPTS has strong tumor-targeting efficiency, protein delivery efficiency, cell internalization, and delivery efficiency [48]. While this research was difficult, new technology has made it a lot easier, as the study described finding colon cancer cells targeted with carcinoembryonic antigen utilizing the ATTEMPTS technique. TAT peptide has been attached to the structurally modified Gel to conjugate it via the YGRKKRRQRRR sequence. The results showed that 48 of the TAT-Gel test samples have anticancer qualities due to their cytotoxic characteristics. T84.66-heparin conjugated with TAT-Gel hybrid were more selective and more toxic colon cancer cells in vivo in vitro shows that conjugation increases selectivity and efficacy [21,52,53].

Furthermore, when compared to raw Gels, the genetically modified Gels fusion protein demonstrates superior internalizing capabilities. For example, the modified Gels cytotoxicity is stronger than that of unmodified Gels, approximately 93, 229, 391, and 108-fold more functional against the MDCK, HCT116, LS174T, and 293HEK cell, respectively (Figure S1). Some findings indicated that adding heparin into polyanionic formulation can reduce the heparin/TAT-Gel internalization capability. These findings illustrate the mechanics of charge transfer in ATTEMPTS approach RIP release mechanisms (Figure S1) [54,55].

ENZYME-TRIGGERED DELIVERY APPROACH

The potential benefit is the ability to attack tumor cells with cell-penetrating peptides, which are altered by RIPs. Regrettably, this technology is nonselective and inactive in terms of medication delivery. When using four techniques to treat glioma, scientists were able to compare their therapies by employing cell-penetrating peptides, peptide-linked matrix metalloproteinase 2 (enzyme), lactoferrin (carrier), and TCS (therapeutic agent) [55,56]. However, only one among the various aspects of the TCS-cell penetrating peptide project was made public; the researchers had demonstrated their antitumor effects. RIP delivery has also been claimed to be an intein-based approach of site-selective PEGylation. This process involved cell-penetrating sequence, matrix metalloproteinase 2, and TCS-fused proteins. A site-specific conjugation was generated using intein-based PEGylation technology, which showed prodrug-like characteristics and stability in the circulatory flow and large absorption into tumor cells (Figure S2) [57]. Animals who were provided with only a small amount of HT1080 (an experimental cancer treatment) had more systemic toxicity and worse tumoricidal abilities than animals who were given HT1080 with their normal food. Synergistic antitumoral effects could be obtained if PEGylated TCS is combined with paclitaxel, assuming that it has been combined with paclitaxel and tested in vivo and in vitro. The inhibitory induction effect of paclitaxelcaspase-9 phosphorylation and apoptotic processes might be associated with the mechanistic view [58].

PH-TRIGGERED DELIVERY APPROACHES

The principal reason for metastasis is the accumulation and production of lactate in a low pH-compatible, extracellular tumor micro-environment [59,60]. The researchers focused on RIPs since only a pH-responsive delivery mechanism has a molecular structural change in the acidic tumor microenvironment [61]. The application of pH-responsive materials has recently been broadened, and pH-sensitive polymers, such as polyethylene glycol stearoyl phosphatidylserine (stearoyl-PEG-PS), may achieve these different apparent pH values, are being employed as modifiers for liposomes with protein-loaded goals. They

are excellent for the conductivity of electrostatic interaction and liposomal aggregations because their surface is negatively charged. It might be considered a benefit as liposomal medicinal products are provided in the tumor's microenvironment (Figure S3) [62]. We may add the toxins based on pH-sensitive proteins, which can be produced through genetic techniques as a further example. One of these is Listeriolysin O, a cytolysin. This protein is important in the pore structure creation of membranes, as the structure has a pH-responsive section in its composition [63].

NANOMATERIAL-BASED DELIVERY APPROACH

In industrial applications, nanoparticles have a high potency in energy sciences [64-68] and can find applications in biomedical engineering [69-71] and drug delivery due to their significant advantages of plasma medication stability, tumor microenvironment regulation, and utilization in codelivery [72,73]. Even if they give a good condition to safeguard the structural integrity of protein-based medications, a significant number of supporting data exist that show the connection between nanoparticle-

based drug absorption and skipping endosomal barriers by internalization. Pizzo and colleagues have extensively published on sustainable nanomaterials such as polymer-lipid nanostructures, gold, and nanocapsules on a vast scale [74]. About RIP bioconjugation employing nanomaterials. Another way to look at it is that low cellular absorption reduces gel protein's anticancer potency. A pH-sensitive delivery strategy founded on Listeriolysin O encapsulation was developed to address this issue. This liposomal Gel showed immediate cytotoxic effects towards B16 cell lines in vivo investigation in cancer mice [75]. Nanoparticle-based platforms that provide codelivery of many medications offer new avenues for combination therapies. The distribution of TCS/albendazole is one example of this case. Silver nanoparticles were used to treat an A549/T tumor of the lungs, together with self-assembled albumin, to produce a negative charge when contact with cell-penetrating peptides, which have a positive charge because of an electrostatic connection (Figure S4) [76].

LIGHT-INDUCED DELIVERY METHOD

A RIPs issue (like TCS and Gel) has inadequate cellular

Figure 1 Targeted tumor delivery to light-induced tumors utilizing VEGF121/rGel, regardless of light exposure. With authorization from reference [84], the figures have been republished.

Figure 2 Electrostatic connections for r-tam with SK2 (dark blue) and SK3 (cyan) channels. The r-tam impact on mutants (E25K/K27E). The figures in this publication have been republished with approval from the source reference [95].

absorption and endosomal translocation. Moreover, it might be considered a serious unsolved problem. When the number of endosomal escape events increases, it favors better translocation in the cytosol [69,70]. This goal is likely to be achieved by using light-induced delivery techniques. It is based on a photochemical process mediated by a photosensitizer, which plays a crucial role. Numerous sensitizers have been reported to date, including chlorins, porphyrins, and phthalocyanines [77-80]. Light-induced processes in photographic production rely on the ability to convert water molecules into Reactive Oxygen (ROS) to attack cancer cells. In the molecular sense, the ROS as damaging species could lead to the leaking of endosomal membranes and the destruction of the endosomal membranes. A similar technique could administer medicinal drugs like RIPs [81]. In this approach, one study found that treating cervical cancer cells with Gel/photosensitizer photoinduced cytotoxicity 200-fold more than bare Gel therapy. The elimination of sarcoma in 50% of the Gel-PC [82,83] was additionally noted. According to some research findings, treatment with the HER2-MH3-B1/Gel proteins successfully targeted cancerous ovarian cells. As noted for trastuzumab

and MH3-B1/Gel and SK-BR-3 cells, the cells' resistance to the medicines were compared and more pronounced in the SKOV-3 cells than in the other cell types. An effective delivery method was discovered in which photoinduced treatment caused the selective release of Gel/EGF through a cytosolic progression. In recent research, Gel/EGF protein delivery exhibited better efficacy for selectively releasing Gel/EGF from A431 tumor cell lines (Figure 1) [84].

VOLTAGE-GATED CHANNEL AS A TARGET OF DELIVERY

Transmembrane proteins that function as voltage-gated sodium channels transfer sodium ions (Na^+) in the cytosol [85]. They are protected α -subunits in structure. The α one has four domains and six α -helices [86,87]. These studies, conducted by numerous research groups over many years, reveal that voltage-gated sodium channels may be blocked utilizing poisons that bind a radioligand, electrophysiological approaches, and mutations. Electrophysiological classification based on the effects on pores of pore-blocking toxins, voltage-regulating toxins, and extracellular toxin

Figure 3 Synthesized chlorotoxin peptide structures. The produced peptide and chlorotoxin have similar molecular structures but different RH chemical shifts. The figures have been republished for screening of the CTX/Cy5.5 bioconjugates with biophotonics with consent from ref [97].

inactivators includes, for example, TTX, μ -conotoxins, STX, and TTX-like compounds; batrachotoxin, grayanotoxin, brevetoxin, ciguatoxin, veratridine, and other toxic compounds found in the sea anemone; and α -scorpion toxin. Toxins can also be categorized according to their method of action, and neurotoxic locations on the body that they could attach as β -scorpion and spider toxins are voltage regulator types capable of de/hyper-polarizing their respective

neurotoxin sites. It causes inhibition of voltage regulator four, and α -scorpion toxins can neutralize channels, just like the deactivation of channel site through the effects of δ -conotoxin on neurotoxin site [88]. Animal venoms offer many types of peptide toxins that can target ion channels, which regulate cardiac pulse and blood pressure and are also relevant to the excitability of neurons. They offer exceptional selectivity when it comes to which they can target, which

Figure 4 Views of the scorpion venom from the side and the top AgTX structure and potassium channel. AgTX2-channel structure optimized. Toxin log K_d versus electrostatic interaction The figures have been republished with a request from reference [98].

makes them more intriguing to clinical applications. They are classified into nine categories based on their structure [89]. The $Na^+/Ca^{2+}/K^+/Cl^-$ channels are blocked by several peptides found in scorpion venoms, making them very active in binding neurotoxins. Oncogenes and ion channels have long played important roles in cancer development [90]. Scorpion toxins target K^+ and Ca^{2+} varied channels, including SK1, SK2, and SK3. The influence of SK3 on breast and melanoma cancer cell migration has been reported [91].

Moreover, greater and malignant tumors exhibit an overexpression in SK3 activity. Using small heterocyclic molecules, venoms, and amphiphilic chemicals [92], researchers have devised three major techniques for targeting SK3 channels in cancer cells [93]. For example, bee poison contains apamin, which blocks the SK channel. For example, tamapin toxin from red scorpion (*Mesobuthus*

stimulus) could be an acceptable specificity for SK2. As a result of these findings, It has recently been proposed that SK3 be used as an ion channel inhibitor to treat cancer cells. One study recommended drugs that block both SK2 and SK3. The effects of SK3 and SK2 channel mutants on migration were examined in HEK cell lines. The results showed that these blockers could stop cancerous cells proliferating (Figure 2) [94,95]. It is possible that overexpression of MDMX and MDM2 as tumor suppression proteins could prolong the survival of tumor cells. Several oncoproteins and associated interactions are already targeted for cancer cell treatment. Various chemicals have been discovered to block oncoprotein relationships. Small proteins like thioredoxin regulate MDMX and MDM2 interaction. Stoppin, an inhibitor derived from scorpion toxin, kills cancers by controlling MDM2 and MDMX interactions [96]. Scorpion poisons can

kill tumor cells. Adding a fluorescent molecule to chlorotoxin bioconjugates makes it possible to better see cancer cells via infrared imaging. Human clinical trials with chlorotoxin/Cy5.5 have been reported (Figure 3) [97].

Until now, molecular dynamic simulation has been used to study the mechanism of RIP-VGK channel interaction with RIPs. Such as the interaction of scorpion toxins (such as noxiustoxin, charybdotoxin, noxiustoxin, agitoxin 2, kalitoxin, Pandinus margaritoxin) with the human voltage-gated potassium channel was investigated using a Brownian dynamics (BD) simulation approach that included Molecular Dynamics (MD). The results demonstrated that scorpion poisons use electrostatic interactions to link to the channels in extracellular entry routes. In the channel, the binding process between Arg-25 or Arg-24 in toxin structures, Lys-27 or Lys-28, Tyr-400, His-404, Asp-402, Gly-380, and Asp-386, is dominated by electrostatic interactions (Figure 4) [97].

CONCLUSION

This study aimed to highlight RIPs' possibilities as chemotherapeutic drugs for various tumors and malignancies. Some biobarriers interfere with the efficient distribution of RIPs, making it difficult to function. Their clinical studies are hindered by suboptimal nonselective and underutilized uptake, limiting cancer treatment options. Most papers have focused on cell-penetrating peptide-based materials, making it feasible to introduce pharmaceuticals into cells successfully. Due to its facilitating capabilities, this peptide promotes transfection and offers a new method of administration. We described how RIPs might be administered using various ways, including antibody-targeted electrically modified prodrugs, pH-triggered, enzyme-triggered, nanomaterials-based, and light-induced approaches. Although numerous promising results for clinical application of these strategies have been generated, they still require additional investigation in selectivity, delivery efficiency, and cell-penetrating techniques that are stable in the circulatory system and cancerous cells.

AUTHOR CONTRIBUTION

Safir Ullah Khan devised and designed the study. Munir Ullah Khan performed surveys and analyzed data. The authors read and approved the manuscript.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by the author.

References

- Shapira A, Benhar I. Toxin-based therapeutic approaches. *Toxins*. 2010;2(11):2519-2583. doi: 10.3390/toxins2112519.
- Maraganore JM, Joseph M, Bailey MC. Purification and characterization of trichosanthin. Homology to the ricin A chain and implications as to mechanism of abortifacient activity. *J Biol Chem*. 1987 Aug 25;262(24):11628-33. PMID: 3624228.

- Shi WW, Wong KB, Shaw PC. Structural and Functional Investigation and Pharmacological Mechanism of Trichosanthin, a Type 1 Ribosome-Inactivating Protein. *Toxins (Basel)*. 2018 Aug 20;10(8):335. doi: 10.3390/toxins10080335. PMID: 30127254; PMCID: PMC6115768.
- Zhang C, Gong Y, Ma H, An C, Chen D, Chen ZL. Reactive oxygen species involved in trichosanthin-induced apoptosis of human choriocarcinoma cells. *Biochem J*. 2001 May 1;355(Pt 3):653-61. doi: 10.1042/bj3550653. Erratum in: *Biochem J* 2001 Sep 15;358(Pt 3):792. PMID: 11311127; PMCID: PMC1221780.
- Li M, Li X, Li JC. Possible mechanisms of trichosanthin-induced apoptosis of tumor cells. *Anat Rec (Hoboken)*. 2010 Jun;293(6):986-92. doi: 10.1002/ar.21142. PMID: 20225201.
- Sha O, Niu J, Ng TB, Cho EY, Fu X, Jiang W. Anti-tumor action of trichosanthin, a type 1 ribosome-inactivating protein, employed in traditional Chinese medicine: a mini review. *Cancer Chemother Pharmacol*. 2013 Jun;71(6):1387-93. doi: 10.1007/s00280-013-2096-y. Epub 2013 Feb 3. PMID: 23377374; PMCID: PMC3668121.
- Fang EF, Ng TB, Shaw PC, Wong RN. Recent progress in medicinal investigations on trichosanthin and other ribosome inactivating proteins from the plant genus *Trichosanthes*. *Curr Med Chem*. 2011;18(28):4410-7. doi: 10.2174/092986711797200499. PMID: 21861819.
- Li Z, Qu Y, Li H, Yuan J. Truncations of gelonin lead to a reduction in its cytotoxicity. *Toxicology*. 2007 Mar 7;231(2-3):129-36. doi: 10.1016/j.tox.2006.11.074. Epub 2006 Dec 8. PMID: 17222956.
- Wang P, Chen LL, Yan H, Li JC. Trichosanthin suppresses HeLa cell proliferation through inhibition of the PKC/MAPK signaling pathway. *Cell Biol Toxicol*. 2009 Oct;25(5):479-88. doi: 10.1007/s10565-008-9102-x. Epub 2008 Aug 28. PMID: 18751959.
- Jiang Q, Bai T, Shen S, Li L, Ding H, Wang P. Increase of cytosolic calcium induced by trichosanthin suppresses cAMP/PKC levels through the inhibition of adenyl cyclase activity in HeLa cells. *Mol Biol Rep*. 2011 Apr;38(4):2863-8. doi: 10.1007/s11033-010-0432-4. Epub 2010 Nov 19. PMID: 21088904.
- Kang M, Ou H, Wang R, Liu W, Mao Y, Tang A. Effect of trichosanthin on apoptosis and telomerase activity of nasopharyngeal carcinomas in nude mice. *J BUON*. 2013 Jul-Sep;18(3):675-82. PMID: 24065482.
- Huang Y, Song H, Hu H, Cui L, You C, Huang L. Trichosanthin inhibits DNA methyltransferase and restores methylation-silenced gene expression in human cervical cancer cells. *Mol Med Rep*. 2012 Oct;6(4):872-8. doi: 10.3892/mmr.2012.994. Epub 2012 Jul 18. PMID: 22825485.
- Cui L, Song J, Wu L, Huang L, Wang Y, Huang Y, Yu H, Huang Y, You CC, Ye J. Smac is another pathway in the anti-tumour activity of Trichosanthin and reverses Trichosanthin resistance in CaSki cervical cancer cells. *Biomed Pharmacother*. 2015 Feb;69:119-24. doi: 10.1016/j.biopha.2014.10.027. Epub 2014 Nov 11. PMID: 25661347.
- Chen GF, Huang WG, Chen FY, Shan JL. [Protective effects of trichosanthin in Herpes simplex virus-1 encephalitis in mice]. *Zhongguo Dang Dai Er Ke Za Zhi*. 2006 Jun;8(3):239-41. Chinese. PMID: 16787600.
- Fang EF, Zhang CZ, Zhang L, Wong JH, Chan YS, Pan WL, Dan XL, Yin CM, Cho CH, Ng TB. Trichosanthin inhibits breast cancer cell proliferation in both cell lines and nude mice by promotion of apoptosis. *PLoS One*. 2012;7(9):e41592. doi: 10.1371/journal.pone.0041592. Epub 2012 Sep 5. PMID: 22957017; PMCID: PMC3434199.
- Wang JH, Nie HL, Tam SC, Huang H, Zheng YT. Anti-HIV-1 property of trichosanthin correlates with its ribosome inactivating activity. *FEBS Lett*. 2002 Nov 6;531(2):295-8. doi: 10.1016/s0014-5793(02)03539-1. PMID: 12417329.
- Stirpe F, Olsnes S, Pihl A. Gelonin, a new inhibitor of protein synthesis, nontoxic to intact cells. Isolation, characterization, and preparation of cytotoxic complexes with concanavalin A. *J Biol Chem*. 1980 Jul 25;255(14):6947-53. PMID: 7391060.
- Pagliaro LC, Liu B, Munker R, Andreeff M, Freireich EJ, Scheinberg DA, Rosenblum MG. Humanized M195 monoclonal antibody conjugated to recombinant gelonin: an anti-CD33 immunotoxin with antileukemic activity. *Clin Cancer Res*. 1998 Aug;4(8):1971-6. PMID: 9717827.
- Varkouhi AK, Scholte M, Storm G, Haisma HJ. Endosomal escape pathways for delivery of biologicals. *J Control Release*. 2011 May 10;151(3):220-8. doi: 10.1016/j.jconrel.2010.11.004. Epub 2010 Nov 13. PMID: 21078351.
- Nicolas E, Beggs JM, Haltiwanger BM, Taraschi TF. Direct evidence for the deoxyribonuclease activity of the plant ribosome inactivating protein gelonin. *FEBS Lett*. 1997 Apr 7;406(1-2):162-4. doi: 10.1016/s0014-5793(97)00267-6. PMID: 9109409.
- Shin MC, Zhang J, David AE, Trommer WE, Kwon YM, Min KA, Kim JH, Yang VC. Chemically and biologically synthesized CPP-modified gelonin for enhanced anti-tumor activity. *J Control Release*. 2013 Nov 28;172(1):169-178. doi: 10.1016/j.jconrel.2013.08.016. Epub 2013 Aug 23. PMID: 23973813; PMCID: PMC3849409.

22. Nolan PA, Garrison DA, Better M. Cloning and expression of a gene encoding gelonin, a ribosome-inactivating protein from *Gelonium multiflorum*. *Gene*. 1993 Dec 8;134(2):223-7. doi: 10.1016/0378-1119(93)90097-m. PMID: 7916721.
23. Park T, Min KA, Cheong H, Moon C, Shin MC. Genetic engineering and characterisation of chlorotoxin-fused gelonin for enhanced glioblastoma therapy. *J Drug Target*. 2019 Nov;27(9):950-958. doi: 10.1080/1061186X.2018.1516221. Epub 2018 Sep 11. PMID: 30156929.
24. Veenendaal LM, Jin H, Ran S, Cheung L, Navone N, Marks JW, Waltenberger J, Thorpe P, Rosenblum MG. In vitro and in vivo studies of a VEGF121/rGelonin chimeric fusion toxin targeting the neovasculature of solid tumors. *Proc Natl Acad Sci U S A*. 2002 Jun 11;99(12):7866-71. doi: 10.1073/pnas.122157899. Erratum in: *Proc Natl Acad Sci U S A* 2002 Aug 6;99(16):10941. PMID: 12060733; PMCID: PMC122986.
25. Lyu MA, Cheung LH, Hittelman WN, Marks JW, Aguiar RC, Rosenblum MG. The rGel/BLyS fusion toxin specifically targets malignant B cells expressing the BLyS receptors BAFF-R, TACI, and BCMA. *Mol Cancer Ther*. 2007 Feb;6(2):460-70. doi: 10.1158/1535-7163.MCT-06-0254. Epub 2007 Jan 31. PMID: 17267661.
26. Lyu MA, Rai D, Ahn KS, Sung B, Cheung LH, Marks JW, Aggarwal BB, Aguiar RC, Gandhi V, Rosenblum MG. The rGel/BLyS fusion toxin inhibits diffuse large B-cell lymphoma growth in vitro and in vivo. *Neoplasia*. 2010 May;12(5):366-75. doi: 10.1593/neo.91960. PMID: 20454508; PMCID: PMC2864474.
27. Nimmanapalli R, Lyu MA, Du M, Keating MJ, Rosenblum MG, Gandhi V. The growth factor fusion construct containing B-lymphocyte stimulator (BLyS) and the toxin rGel induces apoptosis specifically in BAFF-R-positive CLL cells. *Blood*. 2007 Mar 15;109(6):2557-64. doi: 10.1182/blood-2006-08-042424. Epub 2006 Nov 21. PMID: 17119117.
28. Lyu MA, Cao YJ, Mohamedali KA, Rosenblum MG. Cell-targeting fusion constructs containing recombinant gelonin. *Methods Enzymol*. 2012;502:167-214. doi: 10.1016/B978-0-12-416039-2.00008-2. PMID: 22208986.
29. Ham S, Min KA, Yang JW, Shin MC. Fusion of gelonin and anti-insulin-like growth factor-1 receptor (IGF-1R) antibody for enhanced brain cancer therapy. *Arch Pharm Res*. 2017 Sep;40(9):1094-1104. doi: 10.1007/s12272-017-0953-7. Epub 2017 Sep 12. PMID: 28900896.
30. Shin MC, Min KA, Cheong H, Moon C, Huang Y, He H, Yang VC. Preparation and Characterization of Gelonin-Melittin Fusion Biotxin for Synergistically Enhanced Anti-Tumor Activity. *Pharm Res*. 2016 Sep;33(9):2218-2228. doi: 10.1007/s11095-016-1959-4. Epub 2016 Jun 1. PMID: 27251414; PMCID: PMC4967393.
31. Ham SH, Min KA, Shin MC. Molecular tumor targeting of gelonin by fusion with F3 peptide. *Acta Pharmacol Sin*. 2017 Jun;38(6):897-906. doi: 10.1038/aps.2017.20. Epub 2017 Apr 17. PMID: 28414205; PMCID: PMC5520187.
32. Esser C, Rannug A. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacol Rev*. 2015;67(2):259-79. doi: 10.1124/pr.114.009001. PMID: 25657351.
33. Pohorille A, Deamer D. Self-assembly and function of primitive cell membranes. *Res Microbiol*. 2009 Sep;160(7):449-56. doi: 10.1016/j.resmic.2009.06.004. Epub 2009 Jul 4. PMID: 19580865.
34. Chenab KK, Eivazzadeh-Keihan R, Maleki A, Pashazadeh-Panahi P, Hamblin MR, Mokhtazadeh A. Biomedical applications of nanoflares: Targeted intracellular fluorescence probes. *Nanomedicine*. 2019 Apr;17:342-358. doi: 10.1016/j.nano.2019.02.006. Epub 2019 Feb 28. PMID: 30826476; PMCID: PMC6520197.
35. Su C, Liu Y, Li R, Wu W, Fawcett JP, Gu J. Absorption, distribution, metabolism and excretion of the biomaterials used in Nanocarrier drug delivery systems. *Adv Drug Deliv Rev*. 2019 Mar 15;143:97-114. doi: 10.1016/j.addr.2019.06.008. Epub 2019 Jun 28. PMID: 31255595.
36. Jones AR, Shusta EV. Blood-brain barrier transport of therapeutics via receptor-mediation. *Pharm Res*. 2007 Sep;24(9):1759-71. doi: 10.1007/s11095-007-9379-0. Epub 2007 Jul 10. PMID: 17619996; PMCID: PMC2685177.
37. Upadhyay RK. Drug delivery systems, CNS protection, and the blood brain barrier. *Biomed Res Int*. 2014;2014:869269. doi: 10.1155/2014/869269. Epub 2014 Jul 20. PMID: 25136634; PMCID: PMC4127280.
38. High KP. Overcoming barriers to adult immunization. *J Am Osteopath Assoc*. 2009 Jun;109(6 Suppl 2):S25-8. PMID: 19553634.
39. Xu W, Hou W, Yao G, Ji Y, Yeh M, Sun B. Inhibition of Th1- and enhancement of Th2-initiating cytokines and chemokines in trichosanthin- treated macrophages. *Biochem Biophys Res Commun*. 2001 Jun 1;284(1):168-72. doi: 10.1006/bbrc.2001.4940. PMID: 11374886.
40. Madan S, Ghosh PC. Interaction of gelonin with macrophages: effect of lysosomotropic amines. *Exp Cell Res*. 1992 Jan;198(1):52-8. doi: 10.1016/0014-4827(92)90148-2. PMID: 1727057.
41. Zervantonakis IK, Hughes-Alford SK, Charest JL, Condeelis JS, Gertler FB, Kamm RD. Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proc Natl Acad Sci U S A*. 2012 Aug 21;109(34):13515-20. doi: 10.1073/pnas.1210182109. Epub 2012 Aug 6. PMID: 22869695; PMCID: PMC3427099.
42. Azzi S, Hebda JK, Gavard J. Vascular permeability and drug delivery in cancers. *Front Oncol*. 2013 Aug 15;3:211. doi: 10.3389/fonc.2013.00211. PMID: 23967403; PMCID: PMC3744053.
43. Simonsen TG, Gaustad JV, Leinaas MN, Rofstad EK. High interstitial fluid pressure is associated with tumor-line specific vascular abnormalities in human melanoma xenografts. *PLoS One*. 2012;7(6):e40006. doi: 10.1371/journal.pone.0040006. Epub 2012 Jun 29. PMID: 22768196; PMCID: PMC3386940.
44. Walker C, Mojares E, Del Río Hernández A. Role of Extracellular Matrix in Development and Cancer Progression. *Int J Mol Sci*. 2018 Oct 4;19(10):3028. doi: 10.3390/ijms19103028. PMID: 30287763; PMCID: PMC6213383.
45. Eble JA, Niland S. The extracellular matrix in tumor progression and metastasis. *Clin Exp Metastasis*. 2019 Jun;36(3):171-198. doi: 10.1007/s10585-019-09966-1. Epub 2019 Apr 11. PMID: 30972526.
46. Au JL, Yeung BZ, Wientjes MG, Lu Z, Wientjes MG. Delivery of cancer therapeutics to extracellular and intracellular targets: Determinants, barriers, challenges and opportunities. *Adv Drug Deliv Rev*. 2016 Feb 1;97:280-301. doi: 10.1016/j.addr.2015.12.002. Epub 2015 Dec 11. PMID: 26686425; PMCID: PMC4829347.
47. Chen B, Le W, Wang Y, Li Z, Wang D, Ren L, Lin L, Cui S, Hu JJ, Hu Y, Yang P, Ewing RC, Shi D, Cui Z. Targeting Negative Surface Charges of Cancer Cells by Multifunctional Nanopores. *Theranostics*. 2016 Aug 7;6(11):1887-98. doi: 10.7150/thno.16358. PMID: 27570558; PMCID: PMC4997244.
48. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell Biol*. 2009 Sep;10(9):597-608. doi: 10.1038/nrm2755. PMID: 19696797; PMCID: PMC3038567.
49. Shin MC, Zhang J, Min KA, Lee K, Byun Y, David AE, He H, Yang VC. Cell-penetrating peptides: achievements and challenges in application for cancer treatment. *J Biomed Mater Res A*. 2014 Feb;102(2):575-87. doi: 10.1002/jbm.a.34859. Epub 2013 Jul 30. PMID: 23852939; PMCID: PMC3929953.
50. Chen Y, Zhang M, Min KA, Wang H, Shin MC, Li F, Yang VC, Huang Y. Improved Protein Toxin Delivery Based on ATTEMPTS Systems. *Curr Drug Targets*. 2018 Feb 19;19(4):380-392. doi: 10.2174/1389450118666170302094758. PMID: 28260497; PMCID: PMC5581292.
51. Powell AK, Yates EA, Fernig DG, Turnbull JE. Interactions of heparin/heparan sulfate with proteins: appraisal of structural factors and experimental approaches. *Glycobiology*. 2004 Apr;14(4):17R-30R. doi: 10.1093/glycob/cwh051. Epub 2004 Jan 12. PMID: 14718374.
52. Wang H, Moon C, Shin MC, Wang Y, He H, Yang VC, Huang Y. Heparin-Regulated Prodrug-Type Macromolecular Theranostic Systems for Cancer Therapy. *Nanotheranostics*. 2017 Mar 3;1(1):114-130. doi: 10.7150/ntno.18292. PMID: 29071181; PMCID: PMC5646728.
53. Shin MC, Zhang J, Min KA, He H, David AE, Huang Y, Yang VC. PTD-Modified ATTEMPTS for Enhanced Toxin-based Cancer Therapy: An In Vivo Proof-of-Concept Study. *Pharm Res*. 2015 Aug;32(8):2690-703. doi: 10.1007/s11095-015-1653-y. Epub 2015 Feb 21. PMID: 25701313; PMCID: PMC4490053.
54. Shin MC, Zhao J, Zhang J, Huang Y, He H, Wang M, Min KA, Yang VC. Recombinant TAT-gelonin fusion toxin: synthesis and characterization of heparin/protamine-regulated cell transduction. *J Biomed Mater Res A*. 2015 Jan;103(1):409-419. doi: 10.1002/jbm.a.35188. Epub 2014 Apr 23. PMID: 24733757; PMCID: PMC4198515.
55. Shin MC, Zhao J, Zhang J, Huang Y, He H, Wang M, Min KA, Yang VC. Recombinant TAT-gelonin fusion toxin: synthesis and characterization of heparin/protamine-regulated cell transduction. *J Biomed Mater Res A*. 2015 Jan;103(1):409-419. doi: 10.1002/jbm.a.35188. Epub 2014 Apr 23. PMID: 24733757; PMCID: PMC4198515.
56. Chen Y, Zhang M, Jin H, Li D, Xu F, Wu A, Wang J, Huang Y. Glioma Dual-Targeting Nanohybrid Protein Toxin Constructed by Intein-Mediated Site-Specific Ligation for Multistage Booster Delivery. *Theranostics*. 2017 Aug 15;7(14):3489-3503. doi: 10.7150/thno.20578. PMID: 28912890; PMCID: PMC5596438.
57. Chen Y, Zhang M, Jin H, Tang Y, Wang H, Xu Q, Li Y, Li F, Huang Y. Intein-mediated site-specific synthesis of tumor-targeting protein delivery system: Turning PEG dilemma into prodrug-like feature. *Biomaterials*. 2017 Feb;116:57-68. doi: 10.1016/j.biomaterials.2016.11.033. Epub 2016 Nov 27. PMID: 27914267; PMCID: PMC5527826.
58. Chen Y, Zhang M, Jin H, Tang Y, Wu A, Xu Q, Huang Y. Prodrug-Like, PEGylated Protein Toxin Trichosanthin for Reversal of Chemoresistance. *Mol Pharm*. 2017 May 1;14(5):1429-1438. doi: 10.1021/acs.molpharmaceut.6b00987. Epub 2017 Feb 14. PMID: 28195491.
59. Kato Y, Ozawa S, Miyamoto C, Maehata Y, Suzuki A, Maeda T, Baba Y. Acidic

- extracellular microenvironment and cancer. *Cancer Cell Int.* 2013 Sep 3;13(1):89. doi: 10.1186/1475-2867-13-89. PMID: 24004445; PMCID: PMC3849184.
60. Reshetnyak YK. Imaging Tumor Acidity: pH-Low Insertion Peptide Probe for Optoacoustic Tomography. *Clin Cancer Res.* 2015 Oct 15;21(20):4502-4. doi: 10.1158/1078-0432.CCR-15-1502. Epub 2015 Jul 29. PMID: 26224874; PMCID: PMC4609264.
61. Khanmohammadi Chenab K, Sohrabi B, Esrafil M. pHsensitive organic diimide materials-based superhydrophobic surface for oil-water separation applications. *Mater Res Express.* 2020; 6(12):125112.
62. Vila-Caballer M, Codolo G, Munari F, Malfanti A, Fasso M, Rugge M, Balasso A, de Bernard M, Salmaso S. A pH-sensitive stearyl-PEG-poly(methacryloyl sulfadimethoxine)-decorated liposome system for protein delivery: An application for bladder cancer treatment. *J Control Release.* 2016 Sep 28;238:31-42. doi: 10.1016/j.jconrel.2016.07.024. Epub 2016 Jul 18. PMID: 27444816.
63. Kisovec M, Rezelj S, Knap P, Cajko MM, Caserman S, Flašker A, Žnidaršič N, Repič M, Mavri J, Ruan Y, Scheuring S, Podobnik M, Anderluh G. Engineering a pH responsive pore forming protein. *Sci Rep.* 2017 Feb 8;7:42231. doi: 10.1038/srep42231. PMID: 28176876; PMCID: PMC5296754.
64. Khanmohammadi Chenab K, Sohrabi B, Jafari A, Ramakrishna S. Water treatment: functional nanomaterials and applications from adsorption to photodegradation. *Mater Today Chem* 2020;16:100262. doi: 10.1016/j.mtchem. 2020.100262.
65. Khanmohammadi Chenab K, Sohrabi B, Jafari A, Ramakrishna S. Effect of electron-donating and -withdrawing substitutions in naphthoquinone sensitizers: the structure engineering of dyes for DSSCs. *J Mol Struct.* 2018;1167:274–279. doi: 10.1016/j.molstruc.2018.05.014.
66. Khanmohammadi Chenab K, Sohrabi B, Zamani Meymian MR, Mousavi SV. Naphthoquinone derivative-based dye for dye-sensitized solar cells: experimental and computational aspects. *Mater Res Express* 2019;6(8):085537.
67. Khanmohammadi Chenab K, Sohrabi B, Zamani Meymian MR. Cobalt complex dye as a novel sensitizer in dye sensitized solar cells. *Mater Res Express.* 2020;6(12):125536.
68. Khanmohammadi Chenab K. Anti-Icing Properties of vertically aligned TiO₂ nanopyllars. *Langmuir.* 2020; 36(21):6041–6050. doi: /10.1021/acs.langmuir.0c00093.
69. Eivazzadeh-Keihan R, Maleki A, de la Guardia M, Bani MS, Chenab KK, Pashazadeh-Panahi P, Baradaran B, Mokhtarzadeh A, Hamblin MR. Carbon based nanomaterials for tissue engineering of bone: Building new bone on small black scaffolds: A review. *J Adv Res.* 2019 Mar 28;18:185-201. doi: 10.1016/j.jare.2019.03.011. PMID: 31032119; PMCID: PMC6479020.
70. Eivazzadeh-Keihan R, Chenab KK, Taheri-Ledari R, Mosafer J, Hashemi SM, Mokhtarzadeh A, Maleki A, Hamblin MR. Recent advances in the application of mesoporous silica-based nanomaterials for bone tissue engineering. *Mater Sci Eng C Mater Biol Appl.* 2020 Feb;107:110267. doi: 10.1016/j.msec.2019.110267. Epub 2019 Oct 15. PMID: 31761248; PMCID: PMC6907012.
71. Eivazzadeh-Keihan R, Bahojb Noruzi E, Khanmohammadi Chenab K, Jafari A, Radinekiyan F, Hashemi SM, Ahmadpour F, Behboudi A, Mosafer J, Mokhtarzadeh A, Maleki A, Hamblin MR. Metal-based nanoparticles for bone tissue engineering. *J Tissue Eng Regen Med.* 2020 Dec;14(12):1687-1714. doi: 10.1002/term.3131. Epub 2020 Sep 30. PMID: 32914573.
72. Khanmohammadi Chenab K, Sohrabi B, Rahmanzadeh A. Superhydrophobicity: advanced biological and biomedical applications. *Biomater Sci.* 2019 Aug 1;7(8):3110-3137. doi: 10.1039/c9bm00558g. Epub 2019 Jun 19. PMID: 31215913.
73. Zhang M, Liu E, Cui Y, Huang Y. Nanotechnology-based combination therapy for overcoming multidrug-resistant cancer. *Cancer Biol Med.* 2017 Aug;14(3):212-227. doi: 10.20892/j.issn.2095-3941.2017.0054. PMID: 28884039; PMCID: PMC5570599.
74. Pizzo E, Di Maro A. A new age for biomedical applications of Ribosome Inactivating Proteins (RIPs): from bioconjugate to nanoconstructs. *J Biomed Sci.* 2016 Jul 20;23(1):54. doi: 10.1186/s12929-016-0272-1. PMID: 27439918; PMCID: PMC4955249.
75. Provoda CJ, Stier EM, Lee KD. Tumor cell killing enabled by listeriolysin O-liposome-mediated delivery of the protein toxin gelonin. *J Biol Chem.* 2003 Sep 12;278(37):35102-8. doi: 10.1074/jbc.M305411200. Epub 2003 Jun 27. PMID: 12832408.
76. Tang Y, Liang J, Wu A, Chen Y, Zhao P, Lin T, Zhang M, Xu Q, Wang J, Huang Y. Co-Delivery of Trichosanthin and Albendazole by Nano-Self-Assembly for Overcoming Tumor Multidrug-Resistance and Metastasis. *ACS Appl Mater Interfaces.* 2017 Aug 16;9(32):26648-26664. doi: 10.1021/acsami.7b05292. Epub 2017 Aug 3. Erratum in: *ACS Appl Mater Interfaces.* 2020 Jan 15;12(2):3275. PMID: 28741923.
77. Martinez de Pinillos Bayona A, Moore CM, Loizidou M, MacRobert AJ, Woodhams JH. Enhancing the efficacy of cytotoxic agents for cancer therapy using photochemical internalisation. *Int J Cancer.* 2016 Mar 1;138(5):1049-57. doi: 10.1002/ijc.29510. Epub 2015 Mar 23. PMID: 25758607; PMCID: PMC4973841.
78. Bull-Hansen B, Berstad MB, Berg K, Cao Y, Skarpen E, Fremstedal AS, Rosenblum MG, Peng Q, Weyergang A. Photochemical activation of MH3-B1/rGel: a HER2-targeted treatment approach for ovarian cancer. *Oncotarget.* 2015 May 20;6(14):12436-51. doi: 10.18632/oncotarget.3814. PMID: 26002552; PMCID: PMC4494949.
79. Berstad MB, Cheung LH, Berg K, Peng Q, Fremstedal AS, Patzke S, Rosenblum MG, Weyergang A. Design of an EGFR-targeting toxin for photochemical delivery: in vitro and in vivo selectivity and efficacy. *Oncogene.* 2015 Oct 29;34(44):5582-92. doi: 10.1038/ncr.2015.15. Epub 2015 Feb 16. PMID: 25684137.
80. Dietze A, Peng Q, Selbo PK, Kaalhus O, Müller C, Bown S, Berg K. Enhanced photodynamic destruction of a transplantable fibrosarcoma using photochemical internalisation of gelonin. *Br J Cancer.* 2005 Jun 6;92(11):2004-9. doi: 10.1038/sj.bjc.6602600. PMID: 15886704; PMCID: PMC2361782.
81. Berg K, Selbo PK, Prasmickaite L, Tjelle TE, Sandvik K, Moan J, Gaudernack G, Fodstad O, Kjølsvrud S, Anholt H, Rodal GH, Rodal SK, Høgset A. Photochemical internalization: a novel technology for delivery of macromolecules into cytosol. *Cancer Res.* 1999 Mar 15;59(6):1180-3. PMID: 10096543.
82. Weyergang A, Fremstedal AS, Skarpen E, Peng Q, Mohamedali KA, Eng MS, Cheung LH, Rosenblum MG, Waltenberger J, Berg K. Light-enhanced VEGF₁₂₁/rGel: A tumor targeted modality with vascular and immune-mediated efficacy. *J Control Release.* 2018 Oct 28;288:161-172. doi: 10.1016/j.jconrel.2018.09.005. Epub 2018 Sep 11. PMID: 30217739.
83. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev.* 2005 Dec;57(4):397-409. doi: 10.1124/pr.57.4.4. PMID: 16382098.
84. Catterall WA. Structure and function of voltage-gated ion channels. *Annu Rev Biochem.* 1995;64:493-531. doi: 10.1146/annurev.bi.64.070195.002425. PMID: 7574491.
85. Payandeh J, Scheuer T, Zheng N, Catterall WA. The crystal structure of a voltage-gated sodium channel. *Nature.* 2011 Jul 10;475(7356):353-8. doi: 10.1038/nature10238. PMID: 21743477; PMCID: PMC3266868.
86. Zhang F, Xu X, Li T, Liu Z. Shellfish toxins targeting voltage-gated sodium channels. *Mar Drugs.* 2013 Nov 28;11(12):4698-723. doi: 10.3390/md11124698. PMID: 24287955; PMCID: PMC3877881.
87. Cestèle S, Catterall WA. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie.* 2000 Sep-Oct;82(9-10):883-92. doi: 10.1016/s0300-9084(00)01174-3. PMID: 11086218.
88. Lewis RJ, Garcia ML. Therapeutic potential of venom peptides. *Nat Rev Drug Discov.* 2003 Oct;2(10):790-802. doi: 10.1038/nrd1197. PMID: 14526382.
89. Morales-Lázaro SL, Hernández-García E, Serrano-Flores B, Rosenbaum T. Organic toxins as tools to understand ion channel mechanisms and structure. *Curr Top Med Chem.* 2015;15(7):581-603. doi: 10.2174/1568026615666150217110710. PMID: 25686735.
90. Girault A, Haelters JP, Potier-Cartreau M, Chantôme A, Jaffrès PA, Bougnoux P, Joulin V, Vandier C. Targeting SKCa channels in cancer: potential new therapeutic approaches. *Curr Med Chem.* 2012;19(5):697-713. doi: 10.2174/092986712798992039. PMID: 22204342.
91. Potier M, Joulin V, Roger S, Besson P, Jourdan ML, Leguennec JY, Bougnoux P, Vandier C. Identification of SK3 channel as a new mediator of breast cancer cell migration. *Mol Cancer Ther.* 2006 Nov;5(11):2946-53. doi: 10.1158/1535-7163.MCT-06-0194. PMID: 17121942.
92. Brown BM, Shim H, Christophersen P, Wulff H. Pharmacology of Small- and Intermediate-Conductance Calcium-Activated Potassium Channels. *Annu Rev Pharmacol Toxicol.* 2020 Jan 6;60:219-240. doi: 10.1146/annurev-pharmtox-010919-023420. Epub 2019 Jul 23. PMID: 31337271.
93. Jaffrès PA, Gajate C, Bouchet AM, Couthon-Gourvès H, Chantôme A, Potier-Cartreau M, Besson P, Bougnoux P, Mollinedo F, Vandier C. Alkyl ether lipids, ion channels and lipid raft reorganization in cancer therapy. *Pharmacol Ther.* 2016 Sep;165:114-31. doi: 10.1016/j.pharmthera.2016.06.003. Epub 2016 Jun 8. PMID: 27288726.
94. Pedarzani P, D'hoedt D, Doorty KB, Wadsworth JD, Joseph JS, Jeyaseelan K, Kini RM, Gadre SV, Sapatnekar SM, Stocker M, Strong PN. Tamapin, a venom peptide from the Indian red scorpion (*Mesobuthus tamulus*) that targets small conductance Ca²⁺-activated K⁺ channels and afterhyperpolarization currents in central neurons. *J Biol Chem.* 2002 Nov 29;277(48):46101-9. doi: 10.1074/jbc.M206465200. Epub 2002 Sep 17. PMID: 12239213.
95. Mayorga-Flores M, Chantôme A, Melchor-Meneses CM, Domingo I, Titax-Delgado GA, Galindo-Murillo R, Vandier C, Del Río-Portilla F. Novel Blocker of Onco SK3 Channels Derived from Scorpion Toxin Tamapin and Active against Migration of Cancer Cells. *ACS Med Chem Lett.* 2020 Jul 10;11(8):1627-1633. doi: 10.1021/acsmchemlett.0c00300. PMID: 32832033; PMCID: PMC7429966.
96. Li C, Liu M, Monbo J, Zou G, Li C, Yuan W, Zella D, Lu WY, Lu W. Turning a scorpion

toxin into an antitumor miniprotein. J Am Chem Soc. 2008 Oct 15;130(41):13546-8. doi: 10.1021/ja8042036. Epub 2008 Sep 18. PMID: 18798622; PMCID: PMC3810402.

97. Akcan M, Stroud MR, Hansen SJ, Clark RJ, Daly NL, Craik DJ, Olson JM. Chemical re-engineering of chlorotoxin improves bioconjugation properties for tumor imaging and targeted therapy. J Med Chem. 2011 Feb 10;54(3):782-7. doi: 10.1021/jm101018r.

Epub 2011 Jan 6. Erratum in: J Med Chem. 2013 Dec 12;56(23):9807. PMID: 21210710; PMCID: PMC3086956.

98. Yu K, Fu W, Liu H, Luo X, Chen KX, Ding J, Shen J, Jiang H. Computational simulations of interactions of scorpion toxins with the voltage-gated potassium ion channel. Biophys J. 2004 Jun;86(6):3542-55. doi: 10.1529/biophysj.103.039461. PMID: 15189853; PMCID: PMC1304258.

How to cite this article: Khan SU, Khan MU. Therapeutic Application of Genetically Engineered Ribosome-Inactivating Toxin Proteins for Cancer. J Biomed Res Environ Sci. 2021 Dec 22; 2(12): 1216-1228. doi: 10.37871/jbres1375, Article ID: JBRES1375, Available at: <https://www.jelsciences.com/articles/jbres1375.pdf>