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Nanoparticle-Mediated Delivery of Viperin: Advancing Therapy Across Diverse Viral Infections

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ABSTRACT

Nanotechnology-based delivery systems hold immense potential to revolutionize antiviral therapy by providing efficient mechanisms of gene delivery systems. Nanoparticles, with their specific characteristics and various properties, offer intriguing potential for improving the efficacy and specificity of antiviral therapies. Viral infection in a host cell triggers a range of host immune responses to prevent viral invasion through the activity of different antiviral proteins. Among these proteins, viperin plays a crucial role in the host's innate immune response against a broad range of viral diseases. Despite its promise, challenges with the delivery and expression of viperin in host cells, restrict its therapeutic application. This review investigates nanoparticle-based gene delivery systems as a potential approach for enhancing viperin activity. Nanoparticles offer a diverse platform for the targeted delivery of therapeutic genes such as viperin, improving efficacy while reducing off-target consequences. Viperin's encapsulation within nanoparticles protects it from degradation, resulting in efficient delivery to target cells and stimulating its antiviral activity. This highlights the latest advances in applications of nanoparticle design and engineering strategies for viperin delivery, including as lipid-based nanoparticles, polymeric nanoparticles, chitosan and carbon-based nanoparticles. These nanocarriers offer precise control over viperin distribution, release dynamics, and cellular absorption, enhancing viperin's therapeutic potential against viral infections. So, the current approach can use a nanoparticle-based gene delivery system to improve antiviral therapies by employing viperin's diverse properties in dealing with a wide variety of viral diseases.

1. INTRODUCTION

Nanotechnology is the creation and deployment of techniques to build structures in the range of 1–100 nanometers (nm), study physical phenomena and also integration of these structures into practical implementations of efficient gene delivery system [1,2]. Nanotechnology has been applied to improve medicine, improving drug and gene delivery and combating various adverse diseases [3,4].

The use of nanotechnology in medicine connects two cross-disciplinary subjects and because of this combination great societal and economic benefits can arise by combining specific accomplishments in nanotechnology and medicine [5]. In medicine, nanotechnology has been applied broadly for the prevention of certain diseases, vaccination, diagnostics and treatment of various diseases and the list doesn't stop here [6,7]. A few examples of the

Table 1. A few applications of nanotechnology in vaccine research, diagnostic and therapeutic

Application of nanotechnology	Disease	Example
Vaccines research	Hepatitis B (HBV)	The ferritin nanoparticle vaccine targets preS1, triggering a robust anti-preS1 response in mice, enabling efficient HBV eradication [8].
	Malaria	Malaria diagnosis improved through piezo-actuated nanoresonators, which offer high sensitivity despite a small sample volume [9].
	Brucellosis	Novel nanovaccines designed for the prevention of brucellosis, were tested for their immunological effectiveness in mice [10].
Diagnostics and therapeutics	SARS-CoV-2	Antimicrobial and antiviral nanotechnology formulations can reduce the spread of SARS-CoV-2, while sensitive biosensors detect COVID-19 [11]. Engineered nanocarriers for improved COVID-19 therapy is prime of how nanointerventions offer viable solutions [12].

significant contributions of nanotechnology in these fields broadly are tabulated in Table 1.

1.1 Nanoparticles as Effective Gene Delivery Systems

Nanoparticles are emerging as effective gene delivery systems over conventional multi-dose therapy as they present several advantages such as high stability [13], capability for controlled release [14], high specificity, higher gene and drug carrying capacity [15], possibility to use in different routes of administration and the ability to deliver both hydrophilic and hydrophobic molecules [16]. A well-designed gene delivery system is important as it can help to overcome some of the challenges of conventional therapy and increase the therapeutic efficacy against particular infectious diseases [17].

1.2 Nanoparticles-Based Antiviral Therapies

Nanoparticles have recently been used in many researches as efficient vehicles for various antiviral therapies by improving the delivery of various antiviral agents [18,19]. The

nanoformulations used for the said purpose can be of multiple types: lipid-based, polymer-based, carbon-based, inorganic-based nanoformulations, or even hybrid nanoformulations [20,21]. Examples of each of these are to combat and improve antiviral therapy as mentioned in table 2.

Traditional medicines and vaccines are reported to combat harmful and deadly viruses, but they don't prove effective as most viruses have reduced incubation time, high mutation rates, and high reproduction rates of various viral strains [27][28][29].

Conventional antiviral therapies present many challenges which include poor solubility or permeability [30], short half-life [31], multi-drug resistance [32], failure in targeting [33], and inefficiency in viral reservoir sites because of the conventional methods and routes used for their delivery [34]. Nanotherapeutics and medicines based on it, intend to overcome these hurdles to achieve better targeting, efficacy, and safety [35]. The use of nanotechnology in antiviral therapies has enormous promise, particularly in improving the potency of different antiviral proteins such

Table 2. Examples of various types of nanocarriers used for delivering agents in antiviral therapies

Nanocarrier type	Disease	Antiviral drug	Type of nano delivery system	Benefits
Nanostructured lipid carriers	HIV	Etravirine	Liposomes	Better targeting to a reservoir site [22]
Polymer-based hydrogel	SARS-CoV-2	viral antigens or mRNA-based molecules	Alginate, chitosan, and hyaluronic acid	Hydrogels encapsulate drugs, control release, target cells display viral antigens, and aid tissue regeneration [23]
Carbon Dots (C-dots)	Coronavirus	-	Curcumin and citric acid	Inhibition of the entry of the virus, ROS formation and generation of an RNA-negative strand by CCM-C-dots to prevent the virus replication [24]
Inorganic	Dengue	—	<i>Malvastrum coromandelianum</i> encapsulated with AgNPs	Increased the mosquitocidal effects and potential for dengue control [25]
Hybrid	SARS-CoV-2	Fluoxetine hydrochloride (FH)	LPH (Lipid polymer hybrid) nanoparticles	Increase the effectiveness of FH in the treatment of the SARS-CoV-2 infection [26]

as viperin. Researchers intend to enhance the targeted delivery of viperin by using the specific features of nanomaterials, and enhancing its antiviral effectiveness while limiting off-target effects.

2. VIPERIN

Viperin is an acronym of virus inhibitory protein, is an endoplasmic reticulum-associated, interferon-inducible effector. Viperin was first reported about 20 years ago, the product of an IFN- γ -inducible as a response of human macrophages [36]. It is a member of the radical SAM (S-adenosylmethionine) superfamily, inhibits the replication of RNA and DNA viruses. It has recently gained special importance in triggering innate immune signaling events, combating bacterial

infections, and various endotoxins, acting as an antiviral agent [37]. This protein has been reported to be directly and indirectly stimulated by either type I, II, and III interferons or through some virus particles respectively [38] [39].

2.1 Characterization of Viperin Structural and Functional Domains

The viperin sequence mainly comprised of three structural domains, known as N-terminal extension, central SAM terminal domain and C-terminal extension as shown in Figure 1 [40]. The N-terminal extension contains 50 residues which indicate variation in both length and sequence between species but most of such sequence properties are absent in microorganisms. The sequence at this site may

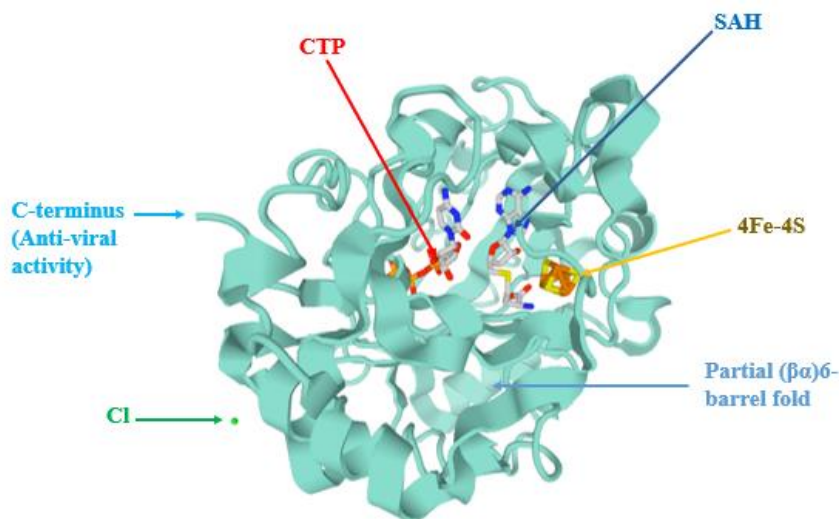


Figure 1. Cartoon representation of crystal structure viperin-like enzyme, having 5 ligands, reported in mouse, adapted and modified from the Protein Data Bank database (ID: 6Q2P). The radical SAM domain, in blue, adopts the similarity with radical SAM enzymes i.e., "partial β -barrel" fold ($\beta\alpha$)₆, dark blue and grey sticks indicate SAH (S-adenosylhomocysteine), yellow and brown sticks show [4Fe-4S] cluster and red sticks represent cytidine triphosphate residues of the [4Fe-4S] cluster-binding motif and two green balls indicate negatively charged chloride ions

have an amphipathic α -helix conformation which localizes the viperin to the ER (endoplasmic reticulum) cytosolic face and assembled viruses in the lipid droplets, such as flaviviruses to restrict their replication. The other two domains contain conserved sequences i.e., C-terminal extension and central SAM (S-adenosylmethionine)-binding domain [41]. The residues of C-terminal extension are involved in binding CTP (cytidine triphosphate) to stimulate the CIAO1 (Fe-S cluster-installing protein) for insertion of [Fe-S] cluster. The functions of the C-terminal domain remain unknown but studies suggested its role in the identification of substrate and protein interactions to target NS5A (non-structural 5A) protein which in turn stimulates antiviral properties against HCV [42]. The central domain shows partial beta-barrel fold which is the common characteristic of radical SAM enzymes. 71-182 residues of this domain share significant structural similarity with the MoA/PQQIII motif associated with the

superfamily of enzymes that require cofactor SAM for its activity. The sequence of this domain comprises four motifs which are linked with the radical SAM superfamily, together with the consensus CXXXCXXC (tricysteine) motif, which are involved in the binding of catalytic [4Fe-S] clusters [36]. So, viperin is named RSAD2 (radical SAM domain-containing 2) in which radical SAM enzymes act on associated [Fe-S] clusters and mediate a variety of reactions to generate highly oxidizing deoxyadenosyl radical species [43,44].

3. POTENTIAL ROLE OF VIPERIN AGAINST VIRAL INFECTION

3.1 Antiviral Activity

Viperin was earlier reported to have antiviral properties against CMV (Cytomegalovirus) in humans and has since been identified to suppress different viral infections [44]. The

antiviral activity of viperin involves the release of a cascade of interferons in response to viral infection which in turn stimulate various signaling molecules including JAK/STAT pathway [45]. Studies showed that viperin has a great potential to interact with viral proteins and inhibits a broad spectrum of RNA and DNA viruses replications belonging to flaviviruses [46], herpesviruses [47], retroviruses [38], paramyxoviruses [48,49], orthomyxoviruses [49] and alphaviruses [50]. Recently, a study revealed that viperin poisons numerous viral replications including Newcastle disease virus, Human immunodeficiency virus, Zika virus, and West Nile virus [38,46,51]. Another *in vitro* study depicted that although viperin is anti-viral but is dispensable for preventing the replication of dengue virus [52]. The antiviral role of viperin has been elucidated in different *in vitro* experiments in infected cell lines where the overexpression or pre-expression of viperin inhibits the expression of viral proteins to restrict virus invasion or observing the reversal of viperin action through the RNAi-mediated knockdown of viperin [53]. Moreover, the antiviral activities of viperin are also reported to stop some enveloped viruses from budding. This study established a link between the expression of viperin and the reduction of cholesterol biosynthesis observed in transiently responsive genes of human cell line i.e., HEK293T (human embryonic kidney 293T) that exerts its antiviral effects against enveloped viruses. The results indicated that cholesterol levels downregulated by 20% to 30% through the expression of viperin in HEK293T cells [54]. Other studies reported that viperin restricts the replication of the Zika virus by restricting both viral and cellular translation in a manner based on its radical SAM activity. The collision of ribosome sensor ZAK triggered the viperin-induced translation inhibition through the activity of eIF2a kinase GCN2 that subsequently stimulates the downstream ISR [38].

Another study determined the viperin mechanism in a type of coronavirus PEDV (Porcine epidemic diarrhea virus)-infected IPEJ-C2 cells and found an upregulation of viperin expression in PEDV-infected IPEC-J2 cells which has been related to the binding of SAM domain with the PEDV N-protein. It resulted in the inhibition of virus replication which suggested the viperin role in antiviral response [55]. Recently it was suggested that antiviral effects of viperin have been associated with the formation of ddhCTP (3'-deoxy-3',4'-didehydro-CTP), an antiviral nucleotide, and interactions of viperin with numerous viral proteins. It has been found that the interactions between viperin, HCV virus NS5A (nonstructural 5A) protein and vesicle-associated membrane protein A trigger antiviral activity of viperin resulting in NS5A degradation [36].

3.2 Innate Immune Response

Viperin has an influential role in the stimulation of immune response to activate the cells against foreign invaders and pathogens. PPR (Pattern recognition receptors) such as MDA-5, TLRs, and RIG-I can recognize foreign invaders and activate different interferon-releasing factors including the IRAK1-TRAF6-TAK1 axis to impair the innate immune response after Mtb infection [45,56,57]. Thus, stimulates the expression of viperin in an IFN-independent manner to combat foreign attack as illustrated in Figure 2. The same study suggested that viperin is associated with lipid droplets and causes mediation of numerous signaling pathways including TLR4 (Toll-like receptor 4) signal transduction pathway and MAVS (mitochondrial antiviral-signaling protein)-dependent pathway [57]. The comparative study of viperin-positive and negative pDCs (plasmacytoid dendritic cells) showed that viperin together with this cell type could mediate the induced production of TLR7 and

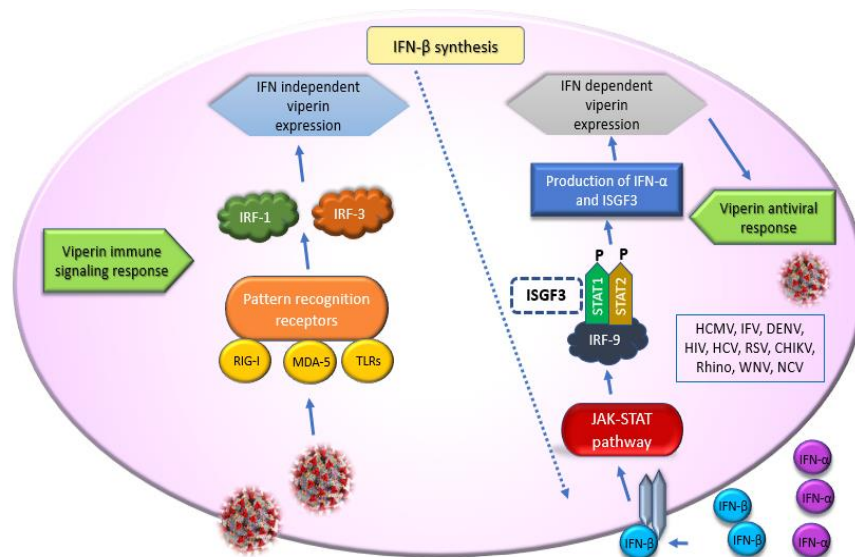


Figure 2. Schematic diagram depicts the synthesis of IFN- β (interferon-beta) and its significance in the immune response, with an emphasis on viperin. On the left, PRRs (pattern recognition receptors) such as RIG-I, MDA5, and TLRs stimulate viperin expression in response to viral infection, which is subsequently regulated by IRF-1 or IRF-3. IFN-dependent viperin expression on the right demands IFN- α/β production and ISGF3 induction, which activates the JAK-STAT pathway via ISGF3/IRF9. This pathway protects against a wide variety of viruses, including HCMV, HPV, and DENV. Overall, this approach improves antiviral defense by modulating viperin production in response to viral infections

TLR9 of IFN-1 in HIV viral proliferations [58]. Studies suggested the apparent role of viperin in pDCs to mediate the direct recruitment of signaling molecules TRAF6 and IRAK1 to lipid droplets. This results in IRAK1 ubiquitination which induces the entry of IRF7 in the nucleus and triggers the expression of type I IFN. At this stage, a direct link between the two conditions remains unclear, viperin knockout mice showed that the impaired Th2 cell development reflected the basic signaling function of viperin [57,59]. Another study suggests that viperin significantly involved in the regulation of induced ISG pathways in a variety of cells including macrophages and plasmacytoid dendritic cells, to activate type I interferons (IFNs), IFN- α and β , resulting in providing different innate immune responses against viral infection as shown in figure 2 [45,60,61]. Study showed that viperin

colocalized with many organelles driving the innate sensing response. The stimulation of MAVS (mitochondrial antiviral signaling protein) within the RLR (RIG-I like receptor) pathway in peroxisome (Pex19) suggested that viperin requires to modulate the antiviral cellular immune response for the optimal stimulation of IFN- β [62]. Similarly, viperin has been found to synergistically enhanced the expression of key molecules STING and TBK1 involved in innate immune dsDNA signaling pathway to restrict viral replication [63]. Viperin has formidable cellular immune response against viral hemorrhagic septicemia virus (VHSV). Its induction is regulated by both IFN-independent and IFN-dependent mechanisms, especially C-terminal domain playing a critical role in anti-VHSV activity. Viperin's immune response is produced by binding to VHSV nucleoprotein and

phosphoprotein and triggering their destruction via the autophagy route. VHSV, on the other hand, uses a counteractive strategy by targeting and degrading critical transcription factors that control viperin expression, indicating prospective therapeutic targets for VHSV prevention [64].

4. CHALLENGES IN VIPERIN-BASED THERAPIES

The major challenge, in viperin-based therapies, is an inefficient drug delivery system that weakly stimulates the viperin expression in host cells. Thus, the overexpression of viperin in host cells mainly relies on an efficient gene delivery system [65,66].

4.1 Plasmid-mediated vs Nano-particles-mediated Transfection of Viperin

Many investigators showed the increased antiviral activity of viperin while performing experiments in many different virus-infected cell lines. These include PK-15 (porcine kidney-15) cell lines infected with CSFV (classical swine fever virus) [46], HEK293, A549 and MARC-145 cells infected with Shaan virus [67], HEK293 cell lines infected with rotavirus [68], HK-2 cells infected with Zika virus [69] and L929 cells infected with NDV (Newcastle Disease Virus) [70]. For the induction of viperin expression, the plasmids were constructed using the sequence of viperin and then transfected in virus-infected cells to analyze the antiviral activity of viperin. The expression of viperin was found to be upregulated in the transfected cells with the reduction of virus replication [40,71]. Plasmid-based transfection introduces recombinant circular, double-stranded DNA (expressing viperin gene) into target cells, with better efficiencies in activating innate antiviral immune response [62]. This method allows stable gene expression but sometimes may induce cellular stress [38]. Nanoparticle-based transfection, applying nanometer-sized

particles, with comparatively high efficiencies with minimal cellular stress [66,72,73]. This makes the nanoparticle-based method outperforms plasmid-based transfection in achieving higher efficiency of viperin's antiviral response against a wide range of viral infections [65,74]. Recent studies reported the plasmid-mediated transfection of the viperin gene in virus-infected cells and showed that viperin reduces the cellular proliferation and cytotoxicity of infectious Foot and Mouth disease Asia I virus [71]. So, the need is to improve the success of transfection efficiency of viperin using modified and advanced technology. In this respect, the trend of nanotechnology will gain significant importance in the future. The plasmid construct coated with nanoparticles as compared with non-nanoparticles appeared to enhance the transfection efficiency [74,75]. Nanotechnology has improved the delivery and advantages of various antiviral agents as summarized in Table 2. Viperin has shown extraordinary antiviral properties to treat various viral infections and its delivery and advantages can further be enhanced similarly using nanotechnology [76].

5. POTENTIAL THERAPEUTIC APPLICATIONS OF NANOPARTICLE-MEDIATED VIPERIN DELIVERY

Nanodisc-based carriers maintained the viperin's membrane-bound state, which is essential for antiviral activity. The full-length viperin purification from HEK293T cells inserted with viperin gene reveals reduced catalytic activity, suggesting interactions with ER-membrane components, are crucial for its function in the therapies of different viral diseases [66]. Another study reported that CS-gp90@M-M NPs (carboxymethyl chitosan encapsulated gp90 gene, encapsulated with mannose-modified fowl erythrocyte membranes nanoparticles) greatly enhance the expression of viperin, IFIH1 and TLR3, which

are important elements of the cellular immune response, in both the bursa and spleen. This modification shows that nanotechnology-mediated viperin delivery systems can successfully stimulate antiviral immunity against reticuloendothelial tissue hyperplasia virus, particularly via viperin-mediated pathways [72]. In accordance with the findings of another study on the transcript level of immune genes, including viperin, using carboxymethyl chitosan (CMCS) as a delivery polymer material for producing nanoparticle CMCS-20H (C-I20) could increase viperin's antiviral activity. The nanoparticle CMCS-20H (C-I20), as a nanocarrier system, could facilitate the administration and uptake of viperin or other antiviral medicines, therefore contributing to the apparent increased in viperin mRNA expression as well as strengthening its antiviral effectiveness against ISKNV (infectious spleen and kidney necrosis virus) [77].

6. LIMITATIONS OF NANOPARTICLE-MEDIATED DELIVERY OF VIPERIN

The selection of nanocarriers is a major challenge for the viperin delivery to the target site. The study demonstrated that nanoparticles have various impacts on the regulation of expression of the RSAD2 (viperin) gene against viral infection. The findings illustrated how nanotechnology-based delivery of RSAD2 (viperin), specifically graphene oxide, changes antiviral lipid metabolism and signaling pathways in VSMCs (vascular smooth muscle cells). Graphene oxide interaction with VSMCs results in significant morphological changes and cytotoxicity, along with decreased expression of antiviral genes such as IFIT1 (interferon-induced protein with tetratricopeptide repeats 1) and RSAD2 (radical S-adenosyl methionine domain containing 2), which are associated with lipid dysfunction. Furthermore, the study reported that administering a TLR3 (Toll-like receptor

3) agonist partially reverses the downregulation of TLR3 and downstream genes in graphene oxide-exposed VSMCs, indicating a potential route for reducing graphene oxide's side effects on antiviral responses. These findings emphasize the necessity of knowing the impact of nanomaterials on cellular functioning and provide potential consequences for antiviral activity through the manipulation of RSAD2 (viperin) [78].

7. FUTURE DIRECTIONS

A recent review has explored the advancements of nanotechnology for the optimization of the design and delivery of nanovaccine for the targeted induction of other ISGs. Another study demonstrated a novel method for designing a dendritic mesoporous silica-based nanocarrier encapsulated with a metal-phenolic network (MPN) layer comprising divalent manganese ions and tannic acid (MSN@MT) for use as a nanovaccine. This vaccine is intended to trigger the cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) pathway in DCs (dendritic cells), predicted to activate the expression of ISGs, which are critical for raising immune responses against viral infections. This significant advancement illustrated the potential of nanotechnology-based vaccination techniques in triggering the innate immune pathways and immune responses against viral infections [79]. Overall, the study will underscore the applications of nanotechnology-based gene delivery systems in enhancing the therapeutic efficiency of other ISGs including viperin against viral infections as illustrated in Figure 3.

8. CONCLUSION

The nanoparticles have been used in many recent studies to design various antiviral therapies by improving the delivery of various antiviral agents. Viperin is one of the key

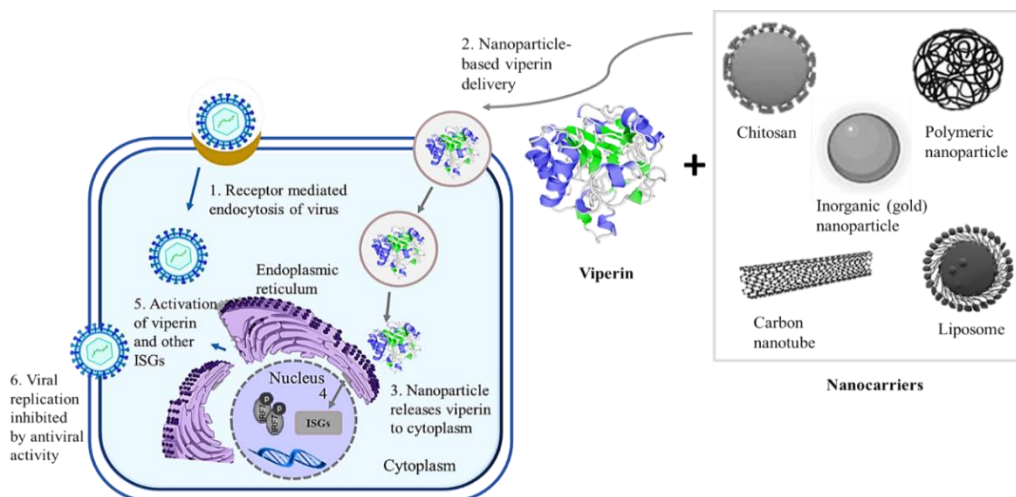


Figure 3. The figure illustrates nanoparticle-based delivery of viperin, crucial for cellular defense against viruses. On the left, virus entry via receptor-mediated endocytosis is shown, highlighting potential inhibition of viral replication by antiviral activity. In the middle, nanoparticle-mediated viperin delivery is depicted, enhancing cellular uptake and activation of immune response. Various nanocarriers, including chitosan, polymeric, inorganic (gold), carbon nanotube, and liposome, are depicted on the right, showcasing diverse administration options for nanoparticle-based viperin delivery to the target cell

interferon stimulating genes that has gained a significant importance in antiviral therapies and host immunity. The structure of this protein displays the characteristic features of its activity [80]. Viperin shows interaction with multiple proteins and is induced either directly or indirectly for antiviral activity and cellular immune response against different viral diseases and foreign invaders respectively. However, the main challenge is to increase the efficiency of viperin-based therapies against foreign invaders. So, this review is focused on the use of a nanoparticles-mediated viperin delivery system, which improved the antiviral therapies and stimulated the accessibility of viperin to the target virus-infected cells. Research studies conducted on nanoparticle-mediated delivery of viperin reveal intriguing strategies for improving delivery efficiency, including targeted delivery, formulation optimization, and safety testing. Future research should focus on in vivo efficacy, combination therapies, and clinical translation

to advance this strategy as a novel antiviral therapy with broad application.

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