Tensin Phosphatase-Like System of Hantavirus Facilitates

Membrane Fusion to Disrupt Vascular permeability

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Abstract

Increased vascular permeability is a characteristic of Hantavirus illness, for which there is now no treatment. We employed the domain search method to investigate the Hantavirus protein in this present work. The results indicated that the membrane glycoprotein E protein (containing Gn-Gc) of Hantavirus had lipid phosphatase and C2-like domains. The E protein was a tensin phosphatase-like (PTEN) enzyme that could shuttle in the cytoplasm and cell membrane. In an acidic endosomal environment, Gn dissociates, exposing Gc's autophosphorylation region to complete autophosphorylation and activating the C2 domain. The C2 domain facilitates Gc's conformational transition, which is followed by Gc binding to the endosomal membrane. After being inserted into the endosomal membrane, the phosphatase domain of Gc phosphorylates PI(3,4,5)P3 on the endosomal membrane. Then converted PI(3,4,5)P3 to PI(4,5)P2 . PI(4,5)P2 bound to the N-terminal of Gc, completely anchoring the tetramer-shaped Gc to the endosomal membrane and forming a fusion hole. Then analogous to PTEN, phosphorylation of PI(3,4,5)P3 directly induced the disintegration of Gc tetramer. The enlargement of the fusion pore speeded up the fusion of the viral and endosomal membranes. Through the fusion hole, the virus's intracellular material was swiftly discharged into the cytoplasm. The C2 domain promoted the PKC signaling route during Hantavirus membrane fusion, whereas the phosphatase inhibited the PI3K signaling pathway. E protein's PTEN-like action impaired lipid metabolism and endothelial cell remodeling, increasing blood vessel permeability and resulting in renal and cardiac syndromes. Additionally, E protein inhibited the immune system and Akt-mediated eNOS activation, resulting in a cascade of consequences.

Keywords: lipid phosphatase；C2；PTEN；PIP3；PIP2；renal syndrome；pulmonary syndrome

1 Background

Hantavirus is a rodent-borne zoonotic pathogen. Humans can contract Hantavirus after coming into touch with rodent urine, saliva, or excrement. Hantavirus infection in humans is associated with two diseases: hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS) (alternatively called Hantavirus cardiopulmonary syndrome (HCPS))(1). Hantaviruses come from the Old and New Worlds(2). Hantaan illness has a mortality rate of up to 12% (HFRS) and 40% (HPS)(3). Fever, muscle aches, and gastrointestinal problems are all indications of human infections(4). Patients with HFRS have a greater degree of renal involvement, resulting in oliguria. Pulmonary symptoms are more severe in HPS patients, and pulmonary edema occurs in a significant number of individuals(4). Increased vascular

permeability is a common hallmark of these two illnesses. It is capable of resulting in hypotension, thrombocytopenia, and leukocytosis(5). There are currently insufficient interventions available. The primary cause for this is an insufficient understanding of Hantavirus pathophysiology and the virus's molecular mechanism of replication(6). As a result, understanding the molecular mechanism of vascular leakage in Hantaan disease is critical for vaccine development and disease prevention.

Hantavirus (HTNV) originated in bats, shrews, or moles and later established persistent infections in rodents(7). HTNV is a virus with an genome composed of three negative-sense RNA segments (L, M, S)(8). The segments L, S, and M of HTNV, respectively, encode RNA polymerase L, viral nucleocapsid protein N, and membrane glycoprotein M(8). CK II can phosphorylate HTNV N at threonine residues(8). N protects RNA from cellular nucleases and collaborates with L to guarantee that the viral genome replicates effectively(9). Segment M contains the glycoprotein precursor (GPC) sequence(10). Cell signal peptide complex cleaves GPC to form Gn and Gc at a conserved WAASA sequence(11). Peptides containing Gc residues interact with artificial membranes, preferably cholesterol and sphingomyelin-rich, and are typically found in lipid rafts (12). Gc residues are believed to be involved in the membrane contact(13). Once the proteolytic process is complete, Gn and Gc are individually anchored to the phospholipid membrane via their transmembrane domains, with their N-terminal domains pointed toward secretion the pathway's lumen(14).

Hantavirus primarily infects endothelial cells in the bloodstream. However, it also infects epithelial cells, mononuclear phagocytes (MNP), follicular dendritic cells (DC), and perhaps other types of cells(15).. Hantavirus causes endothelial cell infection and is responsible for two distinct illnesses with vascular permeability(16). Damage to the endothelium barrier induced by an overactive innate immune response is thought to be central to the pathophysiology(17). Hantavirus directs endothelial cell permeability by sensitizing them to the vascular permeability factor VEGF. Angiogenesis 1 and sphingosine 1-phosphate prevent Hantavirus-induced permeability(18). VEGF, also known as vascular permeability factor, is a critical regulator of endothelial permeability(19). By amplifying the response of the VEGFR2-VE-cadherin pathway, pathogenic Hantavirus degrades the fluid barrier capabilities of endothelial cell adhesion and junctions, hence increasing the cell's permeability(16). Hantavirus is a pathogenic response to Endothelial growth factor (VEGF) of blood vessels to increase endothelial cell permeability(16). Pathogenic Hantavirus, Andes virus, and New York 1 Hantavirus dramatically boost endothelial cell permeability to VEGF. In contrast, non-pathogenic Hantaviruses Prospect Hill and Tula have no effect(18). The N protein of the Andes virus (ANDV) increases the permeability of microvascular endothelial cells (20). But during hantavirus infection, the vascular endothelium remains intact, and there is no clear cytopathic impact to account for leakage and edema(21).

The vascular endothelium that lines the vascular intima is involved in various processes, including smooth muscle tension, host defense response, angiogenesis, and tissue fluid hemostasis (22). Phosphatase and tensin homolog(PTEN) is required for adequate cardiovascular homeostasis. It may be involved in angiogenesis and vascular integrity regulation(23). PTEN is a tumor suppressor gene demonstrated to be critical for the proliferation of vascular smooth muscle cells (SMC). It is a possible therapeutic target for vascular remodeling(24). The Notch-PTEN signaling axis is identified as the coordinator of blood vessel density, implying that the PTEN-APC/C-Fzr1/Cdh1 hub participates in angiogenesis(25). PTEN mutation carriers exhibit

vascular problems. In patients with PTEN mutations, vascular abnormalities typically manifest as a multifocal intramuscular mix of rapid flow channels and ectopic fat(26). Increased PTEN expression limits the growth of vascular smooth muscle cells and arterial restenosis(24). PTEN also plays a critical role in differentiating vascular smooth muscle cells (SMCs)(27). PTEN is an anti-inflammatory and anti-fibrotic protein that is required for SMC differentiation to occur(27). SMC deficiency in PTEN results in pathological artery remodeling(27). Cardiovascular development during embryonic development and postnatal neovascularization are intricate processes that share signaling molecules(28). PTEN reduces vascular germination and endothelial tube formation generated by VEGF in vitro; a dominant-negative mutation of PTEN abolishes these effects(29). PTEN has a role in cardiovascular development and angiogenesis by regulating the production of vascular signaling molecules such as VGF(30).Mutations in these molecules result in abnormalities in cardiovascular development(30). Beside, PTEN promotes late endosomal maturation via its protein phosphatase activity. It inhibits epidermal growth factor receptor (EGFR) signaling(31). PTEN deficiency affects ligand-bound EGFR's passage from early to late endosomes(31).

EGFR and PTEN are abundant in clathrin-coated pits (CCP) when coupled to EGF(32). PTEN regulates clathrin-coated pits (CCP) dynamically(32). The formation of cytoplasmic-coated vesicles by clathrin-coated pits (CCPs) in the plasma membrane may take 20 seconds to 1 minute (32). Viruses exploit both clathrin-mediated endocytosis and macropinocytosis as cellular routes to induce productive infection(33). Hantavirus enters the host cell via clathrin-dependent endocytosis after it comes into contact with the cell surface(34). Hantavirus particle is internalized when it attaches to cell receptors by clathrin-mediated, dynein-independent, or macropinocytosis-like pathways(35). After that, the particle is sent to the endosomal compartment(35). There is a possibility that the virus and endosomal membrane fuse there(35). Hantaviruses from the old world (HTNV, SEOV) are absorbed by clathrin-mediated endocytosis(36). Dynein, clathrin heavy chain, and adaptor protein AP2 (clathrin's three principal components) are the primary target of ANDV internalization and the dependent endocytic pathway(37). Multiple genes involved in cholesterol sensing, regulation, and biosynthesis are required for Hantaviruses from the old and new worlds(38). ANDV has been demonstrated to be dependent on host components unrelated to dyneins, such as cholesterol and the Rho GTPase Rac11(39). Following membrane fusion, which requires cholesterol and an acidic pH, the viral nucleocapsid escapes into the cytoplasm and commences genome replication(6). It implies that the hantavirus membrane glycoprotein protein possesses PTEN activity, participating in endocytosis mediated by clathrin.

Numerous viruses' infection and replication processes are influenced by PTEN-related lipid metabolism. Hepatitis C virus (HCV) impairs the host's lipid metabolism and frequently results in liver steatosis(40). PTEN protein phosphatase activity affects hepatitis C virus secretion via regulating cholesterol metabolism, and the formation of large lipid droplets enhances viral particle outflow(41). A translational variation of PTEN is PTEN with a long N-terminal extension (PTEN-L). PTEN-L increases type I interferon response and innate antiviral immunity during viral infection in a phosphatase activity-dependent way(42). Through the PTEN and AMP-activated protein kinase (AMPK) and dsRNA-dependent protein kinase (PKR)/eIF2 signaling pathways, the p17 non-structural protein of sick avian reovirus induces autophagy to promote virus replication (43). PTEN activity as a lipid phosphatase promotes dengue fever virus (DENV) generation by the Akt/FoxO1/Mafl signaling pathway(44). DENV hijacks the host's lipid metabolism for virus

replication, with lipid droplets (LD) playing a critical part in the virus's life cycle(44). The cholesterol content of the target membrane has a significant effect on the efficacy of membrane fusion mediated by Hantavirus Gn/Gc(45). Although Hantavirus infection is primarily directed at capillary endothelial cells, virus replication does not appear to cause direct harm to these cells or blood vessels endothelial cells(46). As a result, vascular damage is likely to occur at the site of Hantavirus infection.

PTEN can behave as a phosphatase in most situations, controlling the phosphorylation status of lipids and proteins and hence their activities. The structure of PTEN's N-terminal domain determines its dephosphorylation activity. However, it is regulated by its C2 and C-tail domains, which are boosted when homodimers develop. Although it is not directly connected with the PTEN dimer interface, the C2 domain is required to produce PTEN dimers(47). PTEN may flip between open and closed conformations in solution, and phosphorylation of the C tail promotes the closed conformation(48). PTEN's C2 domain is structurally identical to the C2 domains of phospholipase C1, phospholipase A2, and protein kinase C (PKC). It is capable of binding to phospholipid vesicles and regulating PTEN's subcellular distribution(47). The C2 domain of PTEN interacts with the phosphatase domain and modulates the activity of the phosphatase domain(47). PTEN has a broader spectrum of actions due to its interaction with proteins via the C-2 and PDZ-BD domains(49). The eight residues "HTQITKVT" in the C-terminal tail of PTEN is required for specific recognition of the PDZ domain. Phosphorylation of the proximal C-terminal tail residues of PTEN (Ser380/Thr382/Thr383) can decrease binding to the PDZ domain. It preserves the closed conformation of PTEN's C-terminus(50). PTEN-L is a secreted translational variation(51), a PTEN phosphatase that enters cells to change signaling and survival. It contains an additional 173 amino acids at the PTEN N-terminus. PTEN's membrane recruitment requires and is facilitated by the phosphatase and C2 domains. Phosphorylation of the C-terminal tail decreases its membrane affinity(52). PTEN must shuttle dynamically between the cell membrane and the cytoplasm to exhibit PI(3,4,5)P3 phosphatase activity(53). The positively charged C2 domain has been shown to limit excessive PTEN jumping and stabilize the membrane binding of PTEN(53). Phosphatidylinositol 3-phosphate (PI(3)P), an endosomal marker lipid, is associated with vesicles(54). PTEN is a protein that specifically targets the endosomal membrane. PTEN's non-catalytic C2 domain binds to PI(3)P(54), mainly via the CBR3 loop. The phosphorylation/dephosphorylation of the C-terminal region acts as an electrostatic switch that regulates protein-membrane translocation(52). Phosphorylation affects PTEN's plasma membrane targeting by inhibiting the PDZ domain's binding site and interfering with PTEN's electrostatic membrane binding.

As can be seen from the structural properties of PTEN, if the hantavirus protein possesses PTEN function, it should contain both C2 and lipid phosphatase domains. Simultaneously, it can form dimers and bind to phospholipid membranes. Hantavirus enters cells through clathrin-mediated methods. Because membrane glycoprotein E can form multimers and interact with phospholipid membranes, it may possess PTEN action.Gn and Gc are included in E. The Gn and Gc glycoproteins of Hantavirus are referred to as glycoprotein precursors (GPC) (55). According to sequence-based predictions, there are four transmembrane domains, two of which are thought to be signal peptides for translocation into the endoplasmic reticulum lumen. The remaining two are thought to be transmembrane domains that attach each protein to the phospholipid membrane. The glycoprotein precursor may be cleaved in the conserved WAASA

motif by the cell signal peptidase complex(56), yielding Gn and Gc proteins derived from the N- and C-termini of GPC, respectively. Hantavirus enters cells via contact between the viral glycoprotein Gn-Gc and integrins(57). Gn-Gc is made up of tetramer spikes that interact laterally(58). In contrast to other class II fusion proteins, Gn H release at acidic pH causes additional conformational changes at the tip of Gc domain II, exposing non-polar side chains for endosomal membrane insertion(58). Gn-Gc is a member of the Envelope protein family of Hantavirus. So the Envelope protein had a phosphatase and a C2 domain, showing PTEN activity. On Gn or Gc sub proteins all had phosphatase and C2 domains. It may be concluded that E's PTEN activity is required for the entire process of Hantavirus and endosomal membrane fusion.

The C2-like domain contributed to the multimeric conformation of the E protein. The domain facilitated E's translocation to the phospholipid membrane on the cell surface. However, the C2-like domain of the E protein activated the PKC pathway as well. Protein kinase C (PKC) isoforms are expressed in varying quantities in various vascular beds' smooth muscle (VSM)(59). The primary regulator of vascular smooth muscle (VSM) function is protein kinase C (PKC)(60). VSM is critical for maintaining vascular tone(60). Protein kinase C is first implicated in smooth muscle contraction control. Phorbol ester has been shown to generate a slow-developing persistent contraction. PKC that has been inactivated is primarily cytoplasmic. After activation, it undergoes phosphorylation, maturation, and translocation to the surface membrane, nucleus, endoplasmic reticulum, and other organelles(60). Protein kinase C's long-term association with the membrane may render it proteolytic. The produce is protein kinase M, released into the cytoplasm, phosphorylating and relaxing myosin(61). PKC inhibitors effectively decrease vasoconstriction generated by agonists. PKC initiates a cascade of phosphorylation processes(62). It phosphorylates calmodulin directly and activates mitogen-activated protein kinase and filament-related proteins. The phosphorylation will reduce these filament proteins' inhibitory effect on the rate of cross-bridge circulation. Due to the sluggish creation of force, it results in a slower cross-bridge circulation(62). Thus, phosphorylation of MLCK by PKC may result in vascular relaxation(63). It may the a significant contributor to vascular leakage.

The lipid phosphatase domain aided the E protein in binding to the phospholipid membrane on the cell surface by phosphorylating PtdIns(3,4,5)P3. PTEN's phosphorylation status also influences its binding to the plasma membrane. PTEN is found in the cytoplasm and nucleus of the cell and can be secreted. PTEN is exportable by exosomes(64). PTEN can be transported to the cytoplasmic side of the plasma membrane under normal conditions. It can dephosphorylate PtdIns(3,4,5)P3 to PtdIns(4,5)P2, thereby blocking the PI3K signaling pathway in the cell. Not only is PtdIns(4,5)P2 the result of PtdIns(3,4,5)P3 dephosphorylation by PTEN, but it is also a prerequisite for PTEN membrane binding(48). PTEN comprises three domains: an N-terminal phosphatase domain, a C2 domain, and a C-terminal tail region containing a PSD-95/Dlg/ZO-1 homology (PDZ) domain binding sequence and several phosphorylation sites(65). PTEN is activated to target the plasma membrane when its C-terminal tail residues are dephosphorylated. Then it exposes its surface cationic residues and fast electrostatic membrane interaction(65). Once attached to the plasma membrane, PTEN will bind to and dephosphorylate 3-phosphoinositides, the most important of which is PtdIns(3,4,5)P3, resulting in PTEN breakdown(65). The C-terminal region's phosphorylation/dephosphorylation works as an electrostatic switch regulating protein-membrane translocation(65). PTEN interacts with the membrane via many locations. However, only when PI(4,5)P2 binds to the N-terminal domain, a conformational shift and rise in

-helicity occurs(66).

The PTEN/PI3K pathway is involved in forming normal blood vessels and tumor angiogenesis(30). The PI3K-PKB/Akt-Pten pathway in endothelial cells is required for cardiovascular development, and loss of Pten-mediated regulation of this system can promote tumor angiogenesis(30). The PtdIns(3,4,5)P3 in cytoplasm is the optimal substrate for PTEN (67). PTEN dephosphorylates PIP3 via its lipid phosphatase activity. PTEN acts as a negative regulator of the phosphoinositide 3-kinase (PI3K)-PKB/Akt pathway, exerting a tumor suppressor effect(68). By decreasing PTEN activity in glomerular vascular endothelial cells (EC), TIMAP promotes PI3K-dependent Akt phosphorylation(25). Inhibition of PKC inhibits ANDV-directed vascular endothelial cell (EC) permeability and alleviates edema in HPS patients(69). PtdIns(3,4,5)P3 is a phospholipid found on the plasma membrane that rapidly increases the concentration in response to the physiological stimulus. Increased PI(3,4,5)P3 results in an increased fraction of short-lived clathrin-coated tiny pits (CCPs), a phenomenon that PTEN deficiency(32). So phosphorylation of PI(3,4,5)P3 by the E inhibited the PI3K signaling pathway

In summary, the hantavirus proteins exhibited PTEN activity, which was required for the virus entry pathway. PtdIns(3,4,5)P3 dephosphorylation by hantavirus protein facilitated the virus protein's binding to the cell membrane independent of Ca^{2+}. However, it would impair lipid metabolism and endothelial cell remodeling in the vascular endothelium cells. It increased vascular permeability and resulted in kidney and cardiac syndrome. We employed the domain search method to investigate the PTEN active domain of the Hantavirus protein in this present study. The findings revealed that Hantavirus membrane glycoprotein E contained intact lipid phosphatase domains and a C2-like domains. Hantaan and Andes had slightly different lipid phosphatase domain characteristic motifs HCXXGXXR. It may be a significant factor in the development of kidney and cardiopulmonary syndromes.

2 Method

2.1 Data set

1. The sequences of hantavirus proteins. Protein sequences of Andes and Hantaan downloaded from UniProt data set. There are RNA polymerase L, viral nucleocapsid protein N, and membrane glycoprotein envelope E.

2. Tensin Related sequences. The Tensin related sequence was downloaded from UniProt data set. Keywords is "Tensin".

2.2 The localized MEME tool of scanning for conserved domains

The analysis steps are listed as follows:

1. Downloaded MEME from the official website and subsequently install it in the virtual machine ubuntu operating system. The virtual machine was VM 15.2.

2. Downloaded the hantavirus E protein sequence from NCBI official website.

3. Downloaded the fasta format sequence such as Tensin-related ones from Uniprot official website, respectively. The search keyword was "Tensin".

4. For each sequence in all Tensin-related protein, paired with each hantavirus E protein sequence to generate fasta format files for MEME analysis.

5. For the files generated in Step 4, a batch of 50000 was used to create several batches, and

it was considered as the limited space of the virtual ubuntu system.

6. In ubuntu, searched the conserved domains (E-value<=0.05) of hantavirus E protein and Tensin-related with MEME tools in batches.

7. Collected the result files of conserved domains. Then, found the domain name corresponding to the motif from the uniprot database.

8. The domains' activity of each hantavirus E protein was analyzed according to the characteristics of the Tensin-related protein domains.

2.3 Multiple sequence alignment

The local version of Clustal X 2.1(70) was used to perform multiple sequence alignments on the E proteins of Andes and Hantaan, when the parameters were the default values.

3 Results

3.1 Membrane glycoprotein E had lipid phosphatase and C2 domains.

We discovered domain annotations for PTEN-related by scanning the Interpro database. Tensin phosphatase contains two adjacent domains: one encoding a lipid phosphatase (PS51181) and another encoding a C2-like domain (Interpro ID: IPR014020). The structure of the type C2 domain in Tensin is comparable to that of the classic C2 domain. The tensin type C2 domain is similar to the PKC type C2 domain in that it lacks two of the three conserved loops that bind Ca2+. Tensin C2 type domain can attach to the phospholipid membrane in a Ca2+-independent manner. The lipid phosphatase domain dephosphorylates the D3 location of the inositol ring of the lipid's second messenger, phosphodiinositol-3-4-5-triphosphate (PIP3). In the active protein tyrosine phosphatase (PTP) and dual specific phosphatase, the lipid phosphatase domain comprises the distinctive pattern HCXXGXXR (DSP). Only two consistent lysines were discovered in the Tensin-type phosphatase motif (HCKXGKXR) Acid. These are suspected of interacting with the phosphate groups at the D1 and D5 locations of the inositol ring. Tensin, Kinase (linked to cytochrome G) and auxiliary protein 2, chromosome 10 protein (PTEN) are proteins that contain phosphatase and C2 tensin-type domains.

Furthermore, PID, SH2, and DAG are also connected domains. Most Shc-like PID domain (PFAM: PF00640) ligands are RTKs or cytokines. Phosphotyrosine-independent Dab-like PID domains appear to mediate alternative signaling pathways such as endocytosis/processing or exocytosis. The PTB domains are classified as phosphotyrosine-dependent IRS-like, phosphotyrosine-dependent Shc-like, and phosphotyrosine-independent Dab-like. Phosphotyrosine interaction domains are the names given to the final two PTBs (PID or PI domains).

Src homology 2 (PFAM:PF00017) , SH2 domain, was initially found as a conserved sequence area shared by Src and Fps oncoproteins. The SH2 domain is the regulatory module of the intracellular signal cascade. It is sequence-specific and interacts with the target peptide containing phosphotyrosine to recognize the 3-6 residues at the C-terminal phosphorylated tyrosine. The SH2 domain is distinct from the others in that it is phosphorylation-dependent. Tyrosine kinases are associated with the catalytic domain of phospholipase Cy (PLCy) and non-receptor proteins. They often bind monophosphorylated and diphosphorylated substrates in structural proteins such as fibrin and tensin. Phorbol ester (PE) is a DAG analog that acts as a

potent tumor promoter, causing a variety of physiological alterations in cells and tissues when injected.

Table 1. Tensin related domains and motifs of E protein

Virus	Domain	Motif	Start	End	HCXXGXXR
Hantaan	C2 tensin-type	HACNMMK	149	155	-
		IDLHIEIEEQTIGVDVHALGHWFDGRLNLKTSFHCYGACTKYEYPWHTAKCHYERDYQYE	701	760	HCYGACTK
		FQSFNTSTMHFTDERIEWKDPDGMLRDHINILVTKDID	924	961	-
	Phosphatase tensin-type	RKSITCYDLSCNNTYCKPTLYMIVPIHACNMMKSCLIALGPYRVQVVYER	123	172	-
		QNRFRLTEQQVN	410	421	-
		YKELKAHGVSCPQSQCPYCFTHCEPTEAAFQAHYKVCQVTHRFRDDLKKTVTPQNFTPGCYRTLNLFRYKSRCYIFTMWIF	555	635	-
		HWFDGR	721	726	-
		CTKYEYPWHTAKCHYERDYQYETSW	739	763	CTK
		MHFTDERIEWKDPDGMLR	932	949	-
	Protein kinase	WHTAKCHYERD	746	756	-
	Phorbol-ester/DAG-type	MVCDICKYECETYKELKAHGVSCPQ	543	567	-
Andes	C2 tensin-type	YRRKLTNPANKEESIPFHFQMEKQVIHAEIQPLGHWMDATFNIKTAFHCYGACQKYSYPWQ	690	750	HCYGACQK
		PVCEYQGNTISGYKRMMATKDSFQSFNLTEPHITTNK	904	940	-
		EWIDPDGNTRDH	942	953	-
		NRDVSFQDLSDNPCK	959	973	-
	Phosphatase tensin-type	NQTHCQPTVYLIAPVLTCMSIRSCMASVF	138	166	-
		DHDAIQNSQSHLRIV	284	298	-
		QRFRGSEQKINFICQRVDQDVVVYCN	414	439	-
		IEYQKTMGSMVCDVCHHECETAKELESHRQSCINGQ	537	572	-
		YSYRRKLTNPANKEESIPFHFQMEKQVIHAEIQPLGHWMDATFNIKTAFHCYGACQKYSYPWQTS	688	752	HCYGACQK
		MRCPEH	885	890	-
		MMATKDSFQSFNLTEPHITTNKLEWIDPDGNTRDH	919	953	-
	Tyrosine-protein phosphatase	MATKDSFQSFNLTEPHITTNKLEWIDPDGNTRDHVNLVLNR	920	960	-
	PID	WMDATFNIKTAFHCYGACQKYSYPWQTSKCFFEKDYQYETGWGCN	725	769	HCYGACQK
	SH2	WMDATFNIKTAFHCY	725	739	HCY

DAG stimulates the activity of serine/threonine protein kinases collectively referred to as protein kinase C. (PKC). Phorbol ester can trigger PKC directly. The C1 domain of PKC's N-terminal region has been found to bind PE and DAG in a phospholipid and zinc-dependent manner.

We obtained the Andes and Hantaan protein sequences from the Uniprot database for this work. This collection contains complete sequences for the membrane glycoprotein Envelope E, RNA polymerase L, and nucleocapsid protein N. As a result, I investigated whether these three proteins possess PTEN action. Tensin-related sequences were acquired from the Uniprot database. Then, using the local MEME tool, the Hantavirus proteins were compared to the Tensin-related proteins to identify conserved domains. Due to many motif fragments, we had combined motif fragments according to protein and domain. This approach was used to analyze both the Andes and Hantaan viruses. According to the search findings, E, L, and N contain C2 and lipid phosphatase motif fragments. However, only the E motif fragment contains Tensin-related sequences that match the "HCXXGXXR" motif. The PTEN structure of E is active based on the facts.

Table 1 shows the PTEN domains and motifs of E of Andes and Hantaan. Both have C2 Tensin-type and Phosphatase tensin-type domains, as shown in Table 1. These two domains contain sites that overlap. Both domains include the pattern "HCXXGXXR" and are at C-terminal. The "HCXXGXXR" motif of Hantaan is "HCYGACQK". The "HCXXGXXR" motif of Andes is "HCYGACTK". The penultimate amino acid residue distinguishes the two motifs. Andes' E is denoted by the letter T, while the letter Q denotes Hantaan's E, at penultimate amino acid residue. This mutation results in the Andes' E has PID and SH2 domains with the "HCXXGXXR" motif. The G and R in the "HCXXGXXR" motif were changed to A and K in the two viral sequences, respectively. The two mutations G->A and R->K may confer a particular membrane-binding function on Hantavirus. Hantaan E has a shorter protein kinase domain. Hantaan E's phorbol-ester/DAG structure is capable of activating serine/threonine-protein kinase. It suggests that Hantaan E may function as a serine/threonine-protein kinase. Andes E contains a "Tyrosine-protein phosphatase" domain, implying that it contains a tyrosine-protein kinase domain. These mutations and changes in Andes E may enhance Andes Hantavirus endocytosis.

The result of multiple sequence alignment of E between Andes and Hantaan viruses using the tool Clustal X 2.1 is depicted in Figure 1. As illustrated in Figure 1, Andes' "HCYGACTK" and Hantaan's "HCYGACQK" are located in the same C-terminal region, a highly conserved area. The sequences of the two viruses are somewhat different in the region immediately upstream and downstream of the "HCXXGXXR" motif. These significant structural changes may explain why Andes' E "near HCXXGXXR" exhibits PID and SH2 functions.
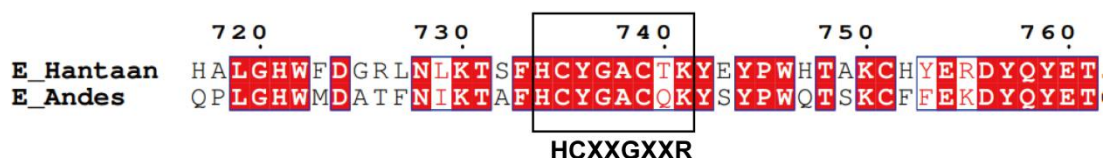

Figure 1. "HCXXGXXR" motif of E between Andes and Hantaan virus

3.2 The Hantavirus membrane fusion process

When vesicles containing Hantavirus matured into acidic endosomes, Gn dissociated under

an acidic environment, exposing Gc's autophosphorylation domains. Gc's autophosphorylation domain initiated and completed autophosphorylation. Gc autophosphorylation activated the C2 domain. The C2 domain facilitated the conformational shift of Gc necessary for activation of the phosphatase domain. Then Gc had the activity of PTEN-like. Under conditions of PTEN-like activity, Gc was bound to the endosomal membrane. Gc's phosphatase domain phosphorylated PI(3,4,5)P3 on the endosomal membrane following its insertion into the membrane. Then conversion of PI(3,4,5)P3 to PI(4,5)P2. PI(4,5)P2 bound to the N-terminus of Gc, allowing the tetramer-shaped Gc to adhere to the endosomal membrane fully. In the endosomal membrane, Hantavirus generated fusion holes. A slight coating of hydrated calcium ions may exist at the point of contact between the Gc tetramer and the endosomal membrane. The Gc tetramer was a phospholipid flippase. It drained water from the hydrated calcium ion layer, quickly fliped phospholipid and spliced membranes to form the fusion hole. Because phosphorylation of PI(3,4,5)P3 directly triggered the breakdown of the Gc tetramer on the inner membrane, similar to PTEN breakdown. The expansion of the fusion hole speeded the fusion of the viral membrane and the endosomal membrane. The virus's intracellular material was swiftly ejected into the cytoplasm.

The C2 domain activated the PKC pathway when the fusion of the Hantavirus membrane with the endosomal membrane. Dephosphorylation of PtdIns(3,4,5)P3 by the phosphatase domain inhibited the PI3K signaling pathway. Hantavirus's membrane fusion process had been shown to disrupt lipid metabolism and endothelial cell remodeling in vascular endothelial cells. It increased the permeability of the blood vessels and resulted in renal and cardiopulmonary syndromes.

4 Discussion

4.1 E protein impairs the PI3K signaling pathway for immunosuppression.

It is well established that PTEN plays a critical function in regulating immune cells' signal transduction(71). Abnormal PTEN signaling changes the immune system's interaction with the tumor, resulting in immune suppression and tumor escape. PTEN regulates many proteins involved in immune signaling pathways and immune cell growth(71). PTEN is required for the formation and control of adaptive immune cells. The majority of PTEN's functions are involved in the control of the PI3K signaling pathway. IFN- activates macrophages by downregulating miR-3473b and upregulating phosphatase and tensin homologs(72). miR-3473b targets PTEN to decrease macrophage activation and inflammation, boosting Akt/glycogen synthase kinase 3 signaling and IL-10 production(72). This study discovered that the E protein contains a mechanism similar to that of PTEN. This system's involvement with the PI3K signaling pathway also affects the signaling of immune cells, impairing macrophage activation and the inflammatory response.

4.2 E protein inhibits Akt-mediated eNOS activation resulting in endothelial dysfunction in the vascular system.

Overexpression of PTEN impairs the activation of endothelial nitric oxide synthase (eNOS) and the release of vascular endothelial growth factor (VEGF)(73). By upregulating PTEN, human CMV suppresses Akt-mediated eNOS activation(74). Endothelial nitric oxide synthase (eNOS) expression and activity are both early events in atherosclerosis. Inhibiting eNOS, which results in

endothelial dysfunction, may be the mechanism through which HCMV causes atherosclerosis(74). Notably, HCMV's inhibitory effect on eNOS activation is mediated via up-regulation of PTEN and activation of the stress signal p38 MAPK(74). SARS-COV-2 can interfere with NO metabolism and signaling pathways to break the Blood-Brain/Testi barrier(75). Hantavirus E protein's PTEN system is equivalent to upregulating PTEN. It suppressed the activation of eNOS via the Akt signaling pathway, resulting in inadequate nitric oxide generation. It caused endothelial dysfunction in the vascular system.

5 Conclusion

Hantavirus is a rodent-borne disease. Hantavirus infection is related to hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS) . In both of these disorders, increased vascular permeability is a common symptom. Hypotension, thrombocytopenia, and leukocytosis can occur. It affairs is a dearth of knowledge about Hantavirus pathophysiology and the molecular mechanism of virus replication.

We employed the domain search method to investigate the active domain of the Hantavirus protein in this work. The findings showed that Hantavirus membrane glycoprotein E comprised lipid phosphatase and C2-like domains. E was attached to the cell membrane independent of Ca^{2+} via tensin phosphatase (PTEN). Dephosphorylation of PtdIns(3,4,5)P3 by E resulted in PI(4,5)P2. The substance stimulated E's membrane binding and shedding. E's tensin phosphatase-like system affected lipid metabolism and endothelial cell remodeling in the vascular endothelium. The PTEN activity of E increased the permeability of the blood vessels and results in renal and cardiopulmonary syndromes. Hantaan and Andes had slightly different lipid phosphatase domain characteristic motifs HCXXGXXR. It may be a significant factor in the development of kidney and cardiopulmonary syndromes.

Declarations

Ethics approval and consent to participation

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets and results supporting the conclusions of this article are available at: https://pan.baidu.com/s/1LStMQ7YtvYiKf9EJtlawdg ; code：fsky
Or: https://mega.nz/folder/13QXiIxR#4WiXodfoygBpF_zdRbEFHw

Competing interests

The authors declare that they have no competing interests.

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