The Libyan International Medical University

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Clinical trials of Ebola virus treatment

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Abstract

Ebola virus disease (EVD) is a deadly incurable illness; however there are ways and stratgies by which the treatment could be reashed . some of which are, by inhibiting the action of viruse glycoproteins therby preventing viral entery into the host cell, by interrupt transcraption of the viruse and that happeing by preventing the dephosphoraltion of VP30 viral factor that is needed in transcription of the virus and it must be in its dephosphoraltion form, by iteraction between ebola viurs protein (VP30) and human protein (RBBP6) which has an ability to stop Ebola viruse life cycle.

Introduction

Ebola virus disease (EVD), one of the deadliest viral diseases, was discovered in 1976 when two consecutive outbreaks of fatal hemorrhagic fever occurred in different parts of central Africa. The first outbreak occurred in the Congo in a village near the Ebola river, which gave the virus its name, the second outbreak occurs what is now south Sudan. The largest outbreak to date took place in West Africa between march 2014 and June 2016; it resulted in 28,646 cases and 11,323 deaths and affected 10 countries in Africa, Europe, and North America.[(1)]

Ebola virus (EBOV), member of the Filoviruses which are family of viruses that are appearing as long filamentous threads or as odd-shaped. Moreover, Filoviruses are highly virulent and require maximum containment facilities (Biosafety level 4). There are two important filoviruses that cause human disease one of which is EBOV and the other is Marburg virus. Focusing on the EBOV structure, the large filovirus genome is single-stranded, nonsegmented, negative-sense RNA contains seven genes encased in the nucleocapsid enveloped in a lipid membrane. Structural proteins associated with the nucleocapsid are the nucleoprotein (NP), VP30, VP35, and the polymerase(L) protein, there is an RNA-dependent RNA polymerase in the virion. The viral envelope contains spikes consisting of the glycoprotein (GP) trimer which plays key role in cellular attachment, and entry of the virus into host cells, and is a key target for immune and therapeutic approaches. After the virion envelope glycoproteins bind to the surface of the human cell, the nucleocapsid enters the

cytoplasm where the virion RNA polymerase transcribes the seven genes. During the early stages of EBOV infection, the negative stranded RNA genome is transcribed into mRNA encoding viral proteins in a process termed primary transcription. At later stages of infection, when viral proteins have accumulated, the RNA genome is replicated and packaged into new viral particles. Both transcription and replication are executed by the viral polymerase complex, which is composed of the polymerase L, VP35, and nucleoprotein (NP), while transcription in addition requires the transcriptional activator VP30.[(2)]

Unfortunately There is no antiviral available for the deadly disease, there is no licensed Ebola virus vaccines or treatments, on the other hand the number of cases highlighted the need to accelerate EVD therapeutics development treatments, moreover an experimental therapy has shown promise in a clinical trial. The research aim is focusing on studies and experiences that are promising for further development as therapeutic molecules against Ebola virus species, each study focus on particular ways and technics.

Materials and Methods

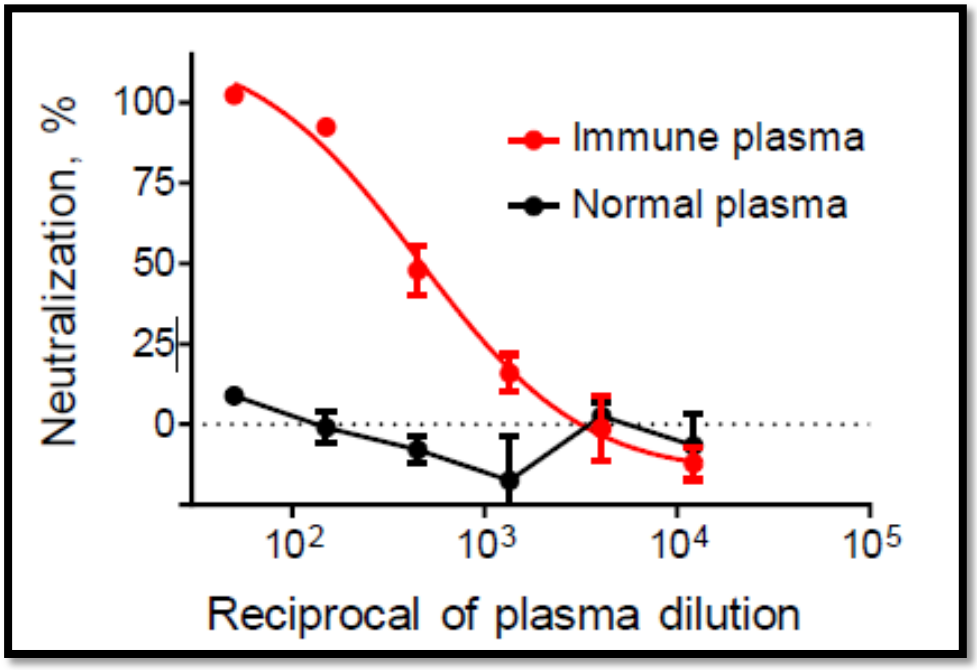
The research is determined by collect the data from three different articles each one focusing on specific ways and strategies that ends up to a treatment, each artical determining specific methods depending on the nature of the study.

Cell lines and virueses, enzyme-linked immunosorbent assay (ELISA) ,Co- immunoprecipitation (co-IP), spectrometry, Immunofluorescence Analysis.[(3)]

The methodology of the first analysis were calculated by taking human samples of 17 plasma from 16 human survivors of the 2014 EVD outbreak in the Democratic Republic of the Congo (DRC) and one survivor of the 2013–2016 West African EVD epidemic, for determining the neutralization of EBOV with antibodies.[(4)] Since the second study target and prevent the VP30 dephospharsation, Mutations in the coding region of the VP30 were introduced and that was by site directed mutagenesis of the intermediate plasmid.[(5)] Affinity tag-purification mass spectrometry (AP-MS) has been used in the thierd study to generate an EBOV-host protein-protein interaction (PPI) map.[(6)]

Results

A small subset of Monoclonal antibodies (mAbs) mediate broadly reactive responses in human survivors of EVD *Figure (1)*.[4]



Figuer 1 Neutralization activity of donor plasma was determined using EBOV

As regards the second study result, the non-phosphorylated VP30 facilitates the transcription of the EBOV in contrast to the phosphorylated form that inactivate it *Figure (2)*.[5]

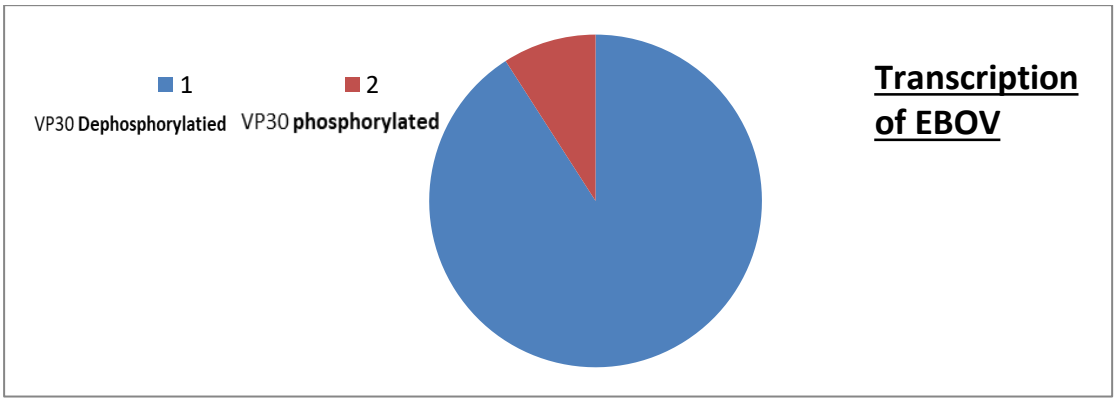


Figure 2 Expression of permanently phosphorylated and permanently nonphosphorylated VP30 in EBOV-infected cells.

The result of the third study show a strong evidence of interaction between Ebola virus protein VP30 and human protein RBBP6. Fluorescence polarization assay

showing RBBP6 peptide (green) displaces NP peptide (red) from the VP30-NP peptide complex *Figure (3)*.[6]

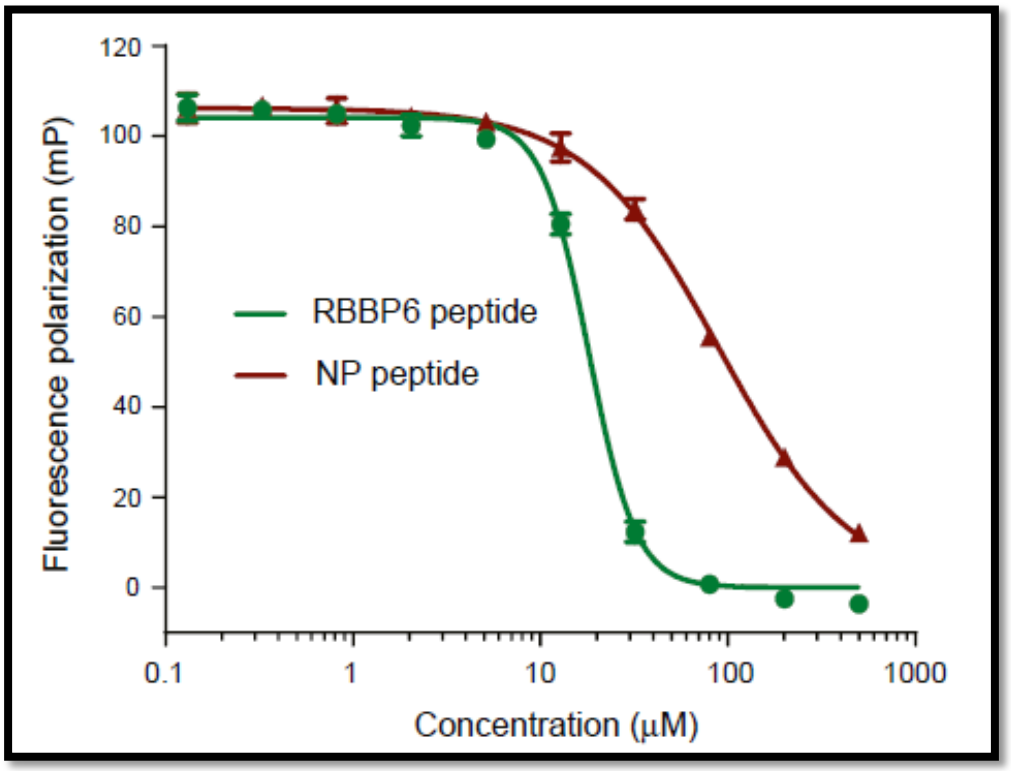


Figure 3 VP30 Interacts with 549–571 Amino Acid Region of RBBP6.

Discussion

Beginning with the first study, a research team from Vanderbilt University Medical Center and University of Texas Medical Branch, 2018 analyzed blood plasma from 17 people who had recovered from EVD, the plasma of survivors were used to find any cross-reactivity against EBOV and to identify survivors that most likely have circulating memory B cells encoding broadly neutralizing antibodies (bNABs). Plasma from two survivors have showed *(Figure 1)* the highest activity of antiboidies that bound to an essential viral protein known as glycoprotein (GP) and prevented the virus of entering the host cell. The team delineated interactions between various forms of viral GP and three of the newly isolated bNAbs, the results provided evidence of multiple mechanisms by which the antibodies inhibit actions of all forms of GP, therefor preventing infection by halting viral entry into the host cell. Moreover, one of the bNAbs, EBOV-520, was found binding to virus GP in such a way that prevents it from interacting with a cell surface protein known as Niemann–Pick C1 *protein (*NPC1).[4]

On other view, another study focused on the fact that EBOV transcription is depends on viral factor known as viral protein 30 (VP30) in its unphosphorylated form. A team from Human Virology Department, Universite´ de Lyon, Claude Bernard University Lyon-1in franc, 2011, have investigated the role of VP30 phosphorylation in EBOV, they found that Non- or weakly phosphorylated forms of VP30 support transcription of the viral gene. In contrast, transcription interrupted when VP30 in phosphorylated form *(Figure 2).* The EBOV nucleoprotein recruit the host PP2A-B56 phosphatase through a B56-binding LxxlxE motif, leading to VB30 dephosphorylation. In other words, it have been showed that the EBOV nucleocapsid protein (NP) contains a functional PP2A-B56-binding LxxIxE motif in close proximity to VP30-binding PPxPxY motif which leading to VB30 dephosphorylation and viral transcription *(figure 4).*[5,3] Specific inhibitor of PP2A-B56 was generated and show that it suppresses EBOV transcription and infection, the B56-NP interaction is a possible target of therapeutic intervention, therefor it is to be expected that an exogenous peptide containing a high-affinity LxxIxE motif would displace NP from the B56-binding pocket by competitive inhibition and prevent VP30 dephosphorylation and activation.[5]

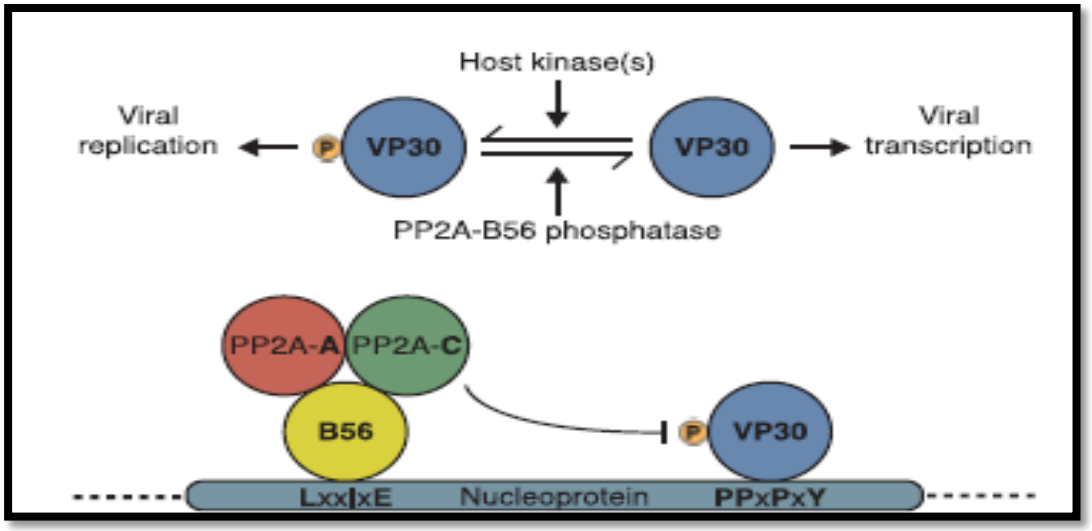


Figure 4 VB30 dephosphorylation

Another study give hopping to an effective therapy against the deadly disease. A human protein have been discovered which plays a role in fight the Ebola virus according to a new Northwestern Medicine study, 2018, the research was a close collaboration between Hultquist's lab at Feinberg and labs at Georgia State University

and the University of California, San Francisco. The newly discovered ability of the human protein (RBBP6) to interfere with Ebola virus replication suggests new ways to fight the infection. As viruses develop and evolve proteins to avoid the body's immune defenses, human cells on the other hand develop defense mechanisms against those viruses. Like other viruses, the ebola virus invades host cells and uses them to replicate, usurping cellular processes to build viral proteins, which eventually become new copies of the virus. In the current study, mass spectrometry had used to search for interactions between human proteins and Ebola virus proteins. Strong evidence for an interaction between the Ebola virus protein VP30 and the human protein RBBP6 had been found. Further structural and computational analysis narrowed the interaction down to a small, 23-amino acid-long peptide chain *(figure 3)*. This small group of amino acids alone is sufficient to disrupt the Ebola virus life cycle. They found out that putting the peptide into human cells leads to block Ebola virus infection, in contrast removing the RBBP6 protein from human cells result in Ebola virus replicates much faster.[7]

Conclusion

In conclusion, The studies concentrate on inhibiting replication with different steps and differet ways . Even though each study seems to have been successful, there is still no cure or specific treatment for the disease so far , and properly that the natural of the viruses could play a role to make them hard to study. But resershes and stdies always give us hope and assurance that the deadly virus will be cured.

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