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Ebola Virus: To Ascertain Drug's Aim and Hitting the Target

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Abstract—*Ebola virus, one of the most deadly viruses known to human has remained a mystery to the world scientists with no specific diagnostic test in early infection stages and treatment. Resulting in ebola hemorrhagic fever, it can easily spread through direct contact. It was first recognized in 1976 and since then has caused thousands of death and many more are being suspected. In a recent breakthrough researchers were able to find certain drugs that can stop its infection by binding to Niemann-Pick C1 (NPC1) that is essential for the virus to enter cells. In this study, we attempt to ascertain how a drug, targeted for NPC1 to stop ebola, may cause disrupting effects on other biological processes of humans. Using databases, 11 such non-target proteins were found with similar structure- NPC1L1, PTCH1, PTCH2, PTCHD3, PTCHD4, SCAP, PTCHD1, DISP1, HMGCR, PTCHD2 and DISP1. All these belong to the patched family of proteins and have sterol-sensing domain in common. NPC1L1 protein takes up free cholesterol into cells through vesicular endocytosis and plays a critical role in the absorption of intestinal cholesterol. Polymorphic variations in NPC1L1 gene are associated with plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels and coronary heart disease (CHD) risk. PTCH1 and PTCH2 proteins function as tumor suppressor in hedgehog signaling pathway. Thus, a drug inhibiting NPC1 can affect other non-target proteins which will although prevent the deadly ebola infection but can also accidentally disrupt important biological processes and subsequently cause other diseases including cancer coronary heart disease and intellectual disabilities like autism.*

1. INTRODUCTION

Recently, researchers made a breakthrough in the understanding of the mechanism of infection of one of the world's most deadly human viruses, Ebola. Since its recognition in 1976, many outbreaks of ebola have occurred primarily in Africa. In August 2011, two different groups of researchers reported that in order to enter our cells and infect our bodies, the Ebola virus must bind to a protein called Niemann-Pick C1 ("NPC1"). To understand the role of NPC1 protein a large number of cells were collected and different genes were randomly disrupted in the different cells. These mutated cells were exposed to the Ebola virus and then checked for infection resistance. Analysis of resistant cells revealed that in these cells NPC1 gene was mutated confirming the key function of this gene in ebola infections [1]. NPC1 is found along the membrane of endosomes, which are small compartments in our cells that transport molecules from the outside of the cell to the inside. Inside the cell, molecules in endosomes are carried to lysosomes, which are compartments that break down molecules and cell debris.

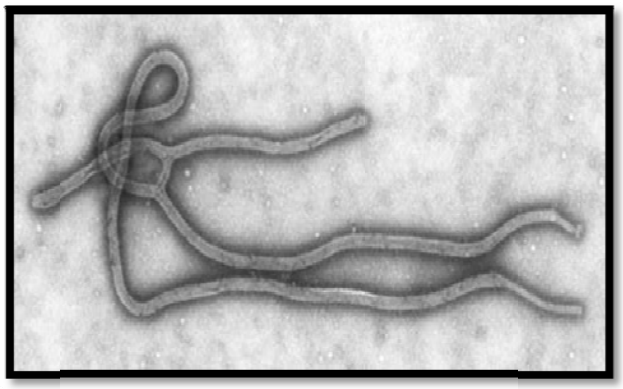

Fig. 1: A picture showing deadly Ebola virus.

Normally, NPC1 is important for transporting cholesterol in cells, but the Ebola virus uses NPC1 to gain entry into the endosomes and causes the endosomes to burst, releasing the virus into the cell [2]. One of the research groups already developed two anti-viral drugs that can block infection by binding the NPC1 protein. To find these successful drugs, the researchers first tested a large number of small molecules on cells exposed to the Ebola virus to see if any of the small molecules could prevent infection. One small molecule was able to stop the infection and was labeled 3.0. The researchers made 50 small molecules which were similar to the 3.0 molecule and found that one of these 50 small molecules that was labeled 3.47 better at preventing Ebola infection than the 3.0 molecule. (3.0 is technically a benzylpiperazine adamantine diamide molecule, and 3.47 is just like this molecule but has a methoxycarbonyl benzyl group added to it) [3].

While the researchers found that their drugs bind NPC1, and that this can block Ebola infection, extensive pharmaceutical testing still needs to be done before doctors can use these

drugs to fight Ebola infection in people. For example, it needs to be determined whether the drugs bind other proteins that are similar to NPC1. Additionally, because researchers had the goal to study how the drugs prevent infection, using only cells grown in a lab, they did not find out how the drugs affect the overall health and function of the cells. For example, the drugs might interfere with important signaling pathways (biochemical pathways) or maybe the drugs affect the body of an organism as a whole. In summary, currently researchers do not know whether these will be good clinical drugs. In this study, using bioinformatics tools we explore how Ebola virus drugs could bind non-target proteins, and disrupt the normal function of NPC1 and these non-target proteins, interfering with the normal cellular and bodily functions.

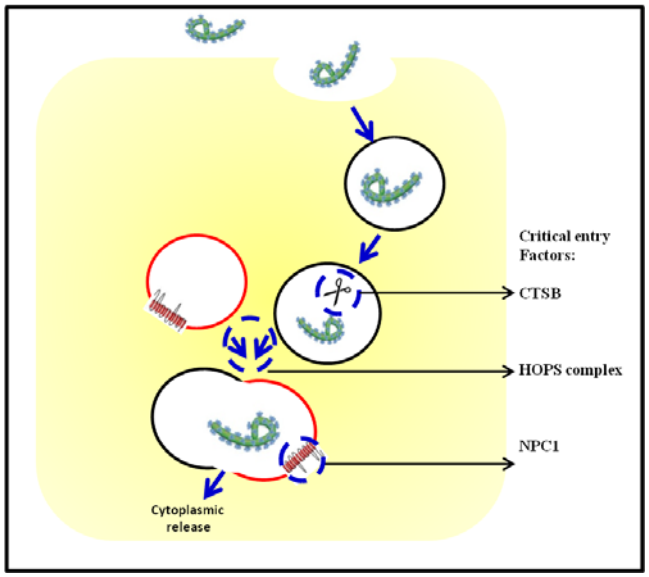

Fig. 2: Proposed Model for Entry of Ebola Virus into the host cells

2. METHODOLOGY

NCBI Gene Database was used to learn about NPC1 gene including its symbol, gene type, lineage and summary. Further, the amino acid sequence of the NPC1 protein was retrieved. Next, other human proteins with similar amino acid sequence were identified. "Distance tree of results" was selected to obtain a visual representation of how different proteins are related based upon the sequences that they share. The two most closely related non-target proteins were identified by analyzing the Distance tree of results. Other genes related to NPC1 were found using UCSC Gene Sorter. For better understanding of the functions of NPC1 gene in humans, Kyoto Encyclopaedia of Genes and Genomes (KEGG) Pathway Database was used from which various signaling pathways were obtained in which NPC1 and related proteins are involved and studied. Moreover, Amazonia database was used to identify the tissues and organs that will be most disrupted by the drug targeting NPC1. The expression levels of the non-target proteins that were found similar to NPC1 in different human tissues and organs were also observed using the Amazonia database.

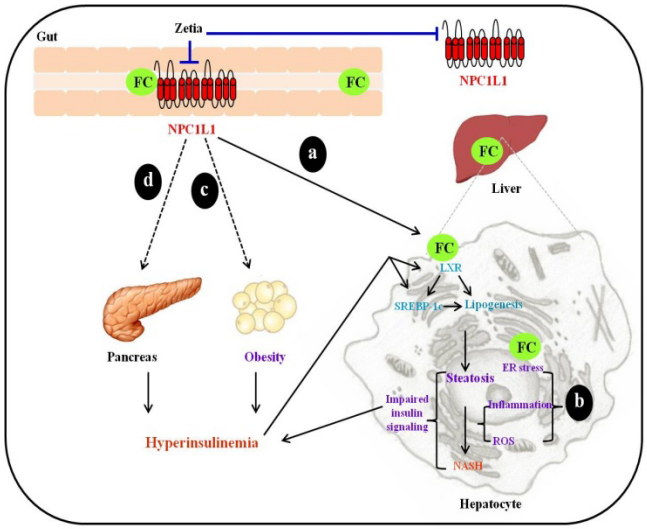

Fig. 3: NCP1L1 in intestinal and hepatic cholesterol transport.

3. RESULTS AND DISCUSSION

Eleven proteins having similar structure as NPC1 were found - NPC1L1, PTCH1, PTCH2, PTCHD3, PTCHD4, SCAP, PTCHD1, DISP1, HMGCR, PTCHD2 and DISP1. All these belong to the patched family of proteins and have sterol-sensing domain in common.

NPC1L1

This gene encodes a multi-pass membrane protein. It contains a conserved N-terminal Niemann-Pick C1 (NPC1) domain and a putative sterol- sensing domain (SSD). The SSD domain of this gene includes a YQRL motif that functions as a plasma membrane to trans-Golgi network transport signal in other proteins. The function of this protein is to take up free cholesterol into cells through vesicular endocytosis and thus, plays a critical role in the absorption of intestinal cholesterol. It also has the ability to transport vitamin E. This protein is targeted by the drug ezetimibe, which inhibits the absorption of intestinal cholesterol and alpha-tocopherol or Vitamin E. This protein may also play a critical role in regulating the lipid metabolism. Variations in this gene have been associated with plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels and coronary heart disease (CHD) risk [6, 7].

PTCH1

The protein encoded by this gene is a sonic hedgehog receptor (sonic hedgehog is a secreted molecule implicated in the

formation of embryonic structures and in tumorigenesis, as well as the desert hedgehog and Indian hedgehog proteins) [8, 9, 10]. Its main function is as a tumor suppressor [11, 12]. Mutations of this gene have been associated with basal cell nevus syndrome, trichoepitheliomas, and transitional cell carcinomas of the bladder, esophageal squamous cell carcinoma as well as holoprosencephaly 13, 14].

PTCH2

It encodes a transmembrane receptor of the patched gene family [15, 16]. The protein encoded functions as a tumor suppressor in the hedgehog signaling pathway. Polymorphic variations in this gene have been associated with basal cell carcinoma, nevoid basal cell carcinoma syndrome, medulloblastoma, and susceptibility to congenital macrostomia [17, 18].

PTCHD3 and PTCHD4

It may play a role in sperm development or sperm function. It is expressed in germ cells of the testis [19].

PTCHD1

It encodes a membrane protein with a patched domain. The encoded protein is similar to *Drosophila* proteins that act as receptors for the morphogen sonic hedgehog. Deletions in this gene (located on the X chromosome) are associated with intellectual disability and autism [20, 21, 22].

SCAP

This encodes a protein having a single sterol sensing domain (SSD) and seven WD domains. In the presence of cholesterol, this protein binds to sterol regulatory element binding proteins (SREBPs) and mediates their transport to the Golgi from the ER [23]. The SREBPs are proteolytically cleaved and then they regulate the sterol biosynthesis. Escort protein is required for cholesterol and lipid homeostasis. This gene regulates export of the SCAP/SREBF complex from the ER when the cholesterol level is low [24]. At high sterol concentrations there is the formation of a ternary complex with INSIG that leads to an ER-export signal masking in SCAP and retention of the complex in the ER. Low sterol concentrations result in a conformational change in the SSC domain of SCAP by triggering release of INSIG which unmasks the ER export signal, recruitment into COPII-coated vesicles occurs, followed by transport to the Golgi complex, proteolytic cleavage of SREBF in the Golgi, releasing of the transcription factor fragment of SREBF from the membrane, factor import into the nucleus and up-regulation of LDLR, INSIG1 and the mevalonate pathway (By similarity).

PTCHD2

This gene encodes a protein with hedgehog receptor activity [25].

DISP1 and DISP2

The pattern of cellular proliferation and differentiation of embryonic structures depends on the localized production of secreted protein signals. Cells that surround the source of a particular signal respond in a graded manner according to the sufficient concentration of the signal, and this response produces the pattern of cell types that constitute the mature structure. A gene known as dispatched with a novel segment-polarity has been identified in *Drosophila* and it has been found that its protein product is required for normal Hedgehog (Hh) signaling [26]. This gene is one of two human homologs of *Drosophila* dispatched. The encoded protein may play an essential role in Hh patterning activities in the early embryo based on sequence identity to its mouse counterpart [27].

HMGCR

HMG-Coenzyme A reductase is the rate-limiting enzyme for cholesterol synthesis. It is regulated by a negative feedback mechanism which is mediated by sterols and non-sterol metabolites that are derived from mevalonate, the product of the reaction catalyzed by reductase. Normally in mammalian cells HMG-Coenzyme A reductase is suppressed by cholesterol, which is derived from the internalization and degradation of low density lipoprotein (LDL) by the LDL receptor. Competitive inhibitors of the reductase induce the expression of LDL receptors in the liver, which increases plasma LDL catabolism and lowers the plasma cholesterol concentration, and are an important determinant of atherosclerosis [28].

4. CONCLUSION

The work focuses on the potential non-target protein that a drug targeted for ebola infection can bind and then disrupt their normal functions. Pathway analysis of NPC1 in humans shows that blocking NPC1 may affect glycerolipid metabolism in small intestine epithelial cells. NPC1 binds to cholesterol in the 3ß-hydroxyl buried and isooctyl side chain exposed [29]. It was found that 11 proteins have similar domain to that of NPC1 which are NPC1L1, PTCH1, PTCH2, PTCHD3, PTCHD4, SCAP, PTCHD1, DISP1, HMGCR, PTCHD2 and DISP1. All these proteins belong to the patched family and have one sterol-sensing domain (SSD). Out of these 11 proteins, two of them, i.e. NPC1L1 and PTCH2 were found to be most similar to NPC1. NPC1L1 is the molecular target of ezetimibe, a potent cholesterol absorption inhibitor that is widely used in treating hypercholesterolemia. NPC1L1 deficiency or ezetimibe treatment also prevents diet-induced hepatic steatosis and obesity in addition to reducing blood cholesterol. Inhibition blocks the intestinal absorption and promotes biliary excretion of free cholesterol (FC) and may also dramatically decrease blood insulin concentrations. PTCH2 is an encoded protein that may function as a tumor suppressor in the hedgehog signaling pathway. Frameshift

mutation in the PTCH2 gene can cause nevoid basal cell carcinoma syndrome [30]. The major functions of these involves: sonic hedgehog activity, cholesterol absorption, lipid metabolism etc. Inhibition of their expression could result in disruption of the normal biological processes adversely affecting the body, for example, coronary heart disease in case of NPC1L1, carcinoma in case of PTCH1 and atherosclerosis in case of HMGCR etc.

Table 1: Non-target proteins with similar structure to NPC1

Protein	Blastp E-Value	Genome Position	Description	Highest gene expression level
NPC1L1	0	chr7 44,526,925	Homo sapiens NPC1-like 1 (NPC1L1), transcript variant 1, mRNA.	Liver and small intestine
PTCH1	1e-29	chr9 95,475,765	Homo sapiens patched 1 (PTCH1), transcript variant 1b, mRNA.	Endometrium followed by Colon
PTCH2	1e-26	chr1 44,832,605	Homo sapiens patched 2 (PTCH2), transcript variant 1, mRNA.	Ovary and Salivary Gland followed by Testes and Dorsal root ganglia
PTCHD3	1e-22	chr10 27,406,278	Homo sapiens patched domain containing 3 (PTCHD3), mRNA.	
PTCHD4	3e-21	chr6 47,973,358	Homo sapiens patched domain containing 4 (PTCHD4), transcript variant 1, mRNA.	
PTCHD1	3e-17	chrX 23,365,834	Homo sapiens patched domain containing 1 (PTCHD1), mRNA.	Cerebellum and fundus of stomach
SCAP	0.00000000000001	chr3 47,444,824	Homo sapiens SREBF chaperone (SCAP), mRNA.	
PTCHD2	0.000000002	chr1 11,508,410	Homo sapiens patched domain containing 2 (PTCHD2), mRNA.	Oocytes, Monocytes, B cells and T cells. It is expressed mostly in all cells.
DISP1	0.0000003	chr1 222,910,542	Homo sapiens dispatched homolog 1 (Drosophila) (DISP1), mRNA.	Highly expressed in adrenal gland.
HMGCR	0.00006	chr5 75,349,634	Homo sapiens 3-hydroxy-methylglutaryl-CoA reductase (HMGCR), transcript variant 1, mRNA	Oocytes

Thus, a drug inhibiting NPC1 can affect other non-target proteins which will although prevent the deadly ebola infection but can also accidentally disrupt important biological processes and subsequently cause other diseases including cancer coronary heart disease and intellectual disabilities like autism.

5. ACKNOWLEDGEMENT

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