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Predicted side effects of NPC1 protein inhibitors

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ABSTRACT

Niemann-Pick C1 (NPC1) gene encodes a large protein that transports low-density lipoproteins to late endosomal / lysosomal compartments where they are hydrolyzed and released as free cholesterol. NPC1 facilitates Ebola and Marburg virus entry into the cytoplasm, serving as an intraluminal receptor after these viruses have been internalized. The recent Ebola virus outbreak clearly showed the lack of available vaccines to treat infected patients and to stop the spread necessitates the discovery of antivirals. Viral entry is the initial and an essential step in the viral replication cycle and blocking the entry into the target cell leads to suppression of viral infection and is therefore an attractive antiviral strategy. Several studies have found novel small molecule inhibitors to inhibit the interaction of Ebola virus glycoprotein (mediates viral entry into cells) with NPC1 and suggested NPC1 as antifiloviral therapeutic effect. NPC1 protein shares sequence homology with Protein patched homolog 1 and 2 which are tumor suppressor. NPC1 is being expressed in oocytes, testes and ovary at different amounts, but oocytes express far more strongly than other cell types. Therefore any therapeutic molecule developed to inhibit NPC1 may also affect the functions of other cell types where NPC1 expresses and interfere with the tumor suppressor function of PTCH 1 & 2.

Keywords: Niemann-Pick C1, Protein patched homologue 1, Ebola, lysosomal storage disease, cholesterol transport.

INTRODUCTION

Niemann-Pick C1 gene encodes a large protein involved in the transportation of cholesterol as cholesterol esters that are packaged into low-density lipoprotein (LDL) (Roth, 2006). LDL is endocytosed and transported to late endosomes and lysosomes where it is subjected to lipolysis and liberated as free cholesterols. These free cholesterols bound to an intra-lysosomal protein called NPC 2 (Naureckiene et al., 2000) to be transported to other cellular compartments. Mutation in NPC1 cause excessive accumulation of cholesterol in lysosomes within multiple tissues and induces a fatal lysosomal storage disease (Pentchev, 2004).

Besides this, recent researches have found that ebola virus glycoprotein interaction with NPC1 facilitates the Ebola virus entry into the cytoplasm. Ebola virus infection causes acute hemorrhagic fever in humans with mortality rate exceeding 50% (Leroy et al., 2009; Ray et al., 2004; Sullivan et al., 2000).

Currently, no FDA approved drug to treat ebola viral infection available in clinical setting and the treatment for Ebola infection primarily limited to palliative care and practicing methods to prevent transmission. Of the various approaches, inhibiting the entry of virus into cells is an attractive approach since entry of virus is an initial and essential step in the replication of virus. Investigations on the mode of entry of ebola virus revealed that, NPC1 is an absolute requirement for the entry into the cytoplasm (Herbert et al., 2015; Bruchez Anna 2012; Cote M et al., 2011). Several investigations explored small molecules as inhibitors of NPC1 (Cote M et al., 2011; Bruchez Anna, 2012; Basu A et al., 2015). The present study was aimed to predict the undesirable effects of inhibition of NPC1 by small molecule inhibitors through an *In silico* approach.

MATERIALS AND METHODS

Niemann Pick C1 amino acid sequence was retrieved from NCBI (GenBank: ANN44507.1). This sequence was subjected to blastp analysis leaving default parameter undisturbed. Blastp search returned a list of similar proteins including isoforms of NPC1. All the isoforms were ignored and two proteins, Protein patched homolog 1 (PTCH1) and Protein patched homolog 2 (PTCH2) were selected.

Kyoto Encyclopaedia of Genes and Genomes (KEGG) database was used to find a signalling pathway that is associated with NPC1 and PTCH1 and PTCH2. Amazonia! database was used to retrieve information pertaining to expression of NPC1 in different cell types.

RESULTS AND DISCUSSION

According to KEGG, NPC1 protein was associated with three pathways which include fat absorption and digestion, lysosome-phagocytosis and antidyslipidemic agents. In fat absorption and digestion pathway, NPC1 seemed to assist in transport of intestinal and hepatic cholesterol transport. If this protein is inhibited by small molecule inhibitors, could lead to poor cholesterol absorption in intestine and liver. However, recent research disproves this notion and suggests the existence of an alternate pathway for cholesterol uptake in humans (Dixit et al., 2007). Mutation in either NPC1 or NPC2 results in a disease called Niemann pick disease in which endocytosed cholesterol becomes sequestered in late endosomes / lysosomes and this consequently causes progressive neurodegenration in mice (Yu et al., 2005; Ong et al., 2001). The NPC1 is a polytopic protein of late lysosome / endosome (LE/L) limiting membrane whereas NPC2 is a soluble protein in the LE/L lumen.

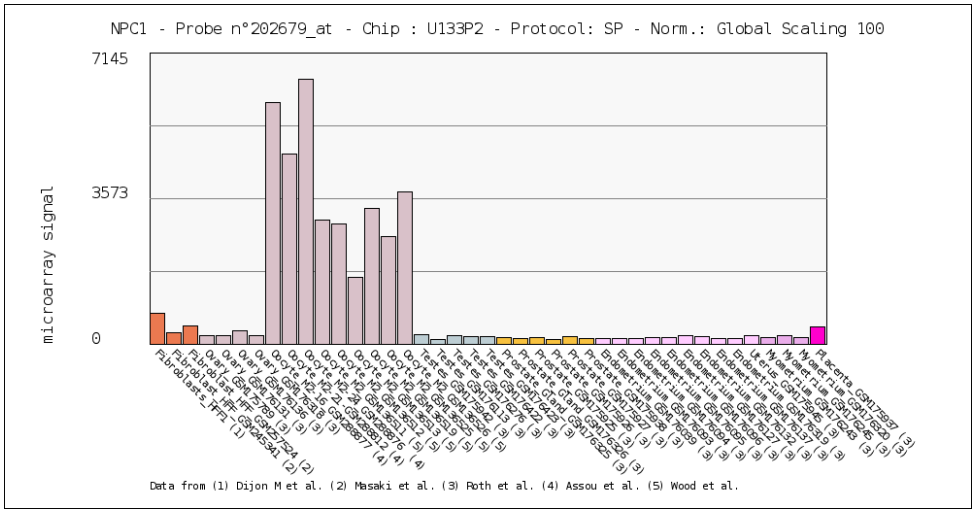

Figure 1: NPC1 expression in oocytes as shown by Amazonia!

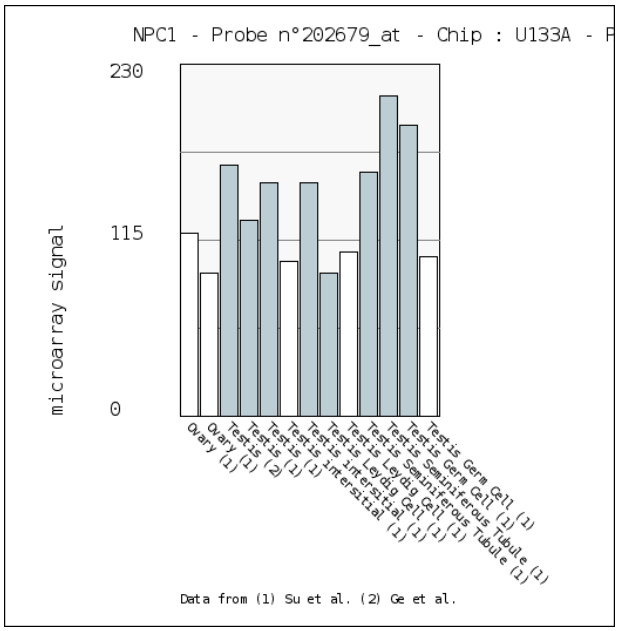

Figure 2: NPC1 expression testis as shown by Amazonia!

Both NPC1 and NPC2 act in tandem and help the transport of cholesterol from LE/Ls. Hence a defect in either NPC1 or NPC2 results in accumulation of cholesterol in LE/Ls, therefore there exists a risk that inhibition of NPC1 by small molecule inhibitors could result in Niemann Pick C disease. In the antidyslipidemic pathways, NPC1 protein acts as triglyceride and LDL cholesterol reducing agent. Inhibition of NPC1 protein may result in accumulation of LDL cholesterol which may increase the risk of getting cardio vascular diseases. Protein patched homologue 1 and 2 are tumor suppressor proteins and according to blastn search, PTCH 1 and 2 shared sequence homology with NPC1. Greer et al. (1999) reported a significant sequence homology throughout NPC1 transmembrane spans with transmembrane domain of plasma membrane PTCH1. Therefore, small molecule inhibitors inhibiting NPC1 may also inhibit PTCH1 which is a tumor suppressor gene.

Amazonia! search with NPC1 revealed that, NPC1 gene expresses strongly in oocytes (Fig. 1) (Masaki et al., 2007; Assou et al., 2009; Wood et al., 2007). Inhibitors which inhibit NPC1 may also affect the maturation of oocytes thereby affecting reproductive health. In addition to this, testis also expresses NPC1 (Fig. 2) (Ge et al., 2005; Su et al., 2004). The NPC1 knock out studies in mice revealed that, NPC1 $1^{-/-}$ male mice infertile because of partial arrest of spermatogenesis and spermatozoa also showed morphological head abnormalities and are unable to fertilize an oocytes *in vitro* (Fan et al., 2006)..

CONCLUSION

Our study concludes that, while exploring inhibitors to inhibit NPC1, it is recommended to check for embryonic and oocytes toxicity using embryonic and oocytes cell lines and animal models. We feel that, it is also necessary to carry out extensive studies on animal models to elucidate the effect of NPC1 inhibitors have on cancer. Further, all volunteers for clinical trials must be tested for Niemann' pick type C disease.

Conflicts of interest: The authors stated that no conflicts of interest.

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