INTERNATIONAL MASTER IN SUSTAINABLE DRUG DISCOVERY

Development of Zika Virus NS2B/NS3 Protease Reporter Systems for Analysis of PROTACs

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SUMMARY

Zika virus (ZIKV) is an arthropod-borne *Orthoflavivirus* belonging to Flaviviridae family, which has been reported to cause active transmission in 84 countries. And it is expected to see increased levels of infections due to expanding urbanization, excessive international travels and climate change. ZIKV outbreaks were associated with severe neurological complications including Congenital Zika Syndrome (CZS). The ability of ZIKV to infect placental cells can cause foetal human brain into developing microcephaly by a series of cell deaths in different regions of the brain. Currently, there are no vaccines approved or small molecule drugs advanced to clinical trials.

Zika Virus NS2B/NS3 protease is responsible for processing viral polyprotein into mature structural or non-structural proteins, therefore it is an appealing target for antiviral agent design. In this project, it is aimed to design a reporter system to analyse the expression of ZIKV NS2B/NS3 protease that can be utilized to screen PROTACs (Proteolysis Targeting Chimeras) targeting NS2B/NS3. To achieve this, pLenti vector system was used to clone ZIKV protease and HiBiT luminescent tag to quantitatively assess the levels of NS2B/NS3 expressed human cell lines and/or degraded upon PROTAC administration. Transfection of the designed construct in Lenti X cells proved successful transient expression by luminescence and western blot. Lentiviral transduction was performed for A549 cells and stable cells were selected with puromycin, however no stable expression of NS2B/NS3 could be observed. Consequently, PROTAC screening was performed with transiently expressed reporter system and MDZ4 showed significant ($p=0.0013$) reduction in luminescent signal.

The transgene expression in stable cell lines was non-detectable, which could be caused by gene silencing. For future improvements, different cell lines or viral systems can be used in transduction of stable cell lines. Considering the success of the reporter system to identify MDZ4 as an NS2B/NS3 degrader, it can be concluded that even when transiently expressed, the constructs have potential to be utilized in future applications for PROTAC screening.