



HOKKAIDO UNIVERSITY

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Development and Implementation of a Compound Screening Assay
to Identify Antivirals against Rabies Virus
(狂犬病ウイルスに対する抑制作用を有する化合物の
スクリーニングアッセイ法の開発と化合物の探索)

Rabies caused by rabies virus (RABV) remains a fatal neurological disease despite the availability of immunoprophylaxis treatment that serves both as preventive and therapeutic measures. There is no currently available medication that provides efficacy in treating RABV-infected patients during the symptomatic phase of the disease leading to death. Novel antiviral agents that can inhibit RABV replication are then still urgently needed and such agents can be discovered through examination of compound libraries.

To facilitate the examination of compound libraries for RABV inhibitors, recombinant RABVs (rRABVs) encoding NanoLuc luciferase (NanoLuc). The RABV were generated by inserting either non-secreted NanoLuc (Nluc) or secreted NanoLuc (secNluc) in the intergenic region. The infectivity of rRABVs was determined by measurement of viral titers and quantification of NanoLuc expression after inoculation of rRABVs into cells, demonstrating the replication competency of the rRABVs. The luciferase activities could be maintained over ten serial passages of the rRABV and correlated with the viral inputs. Moreover,

the rRABV could be used to demonstrate a dose-dependent antiviral activity of ribavirin against RABV, reflected by the decrease of NanoLuc signal.

The rRABV encoding Nluc was then employed to examine the antiviral activity of ribavirin-related compounds which have similar chemical structure to ribavirin and are considered as ribavirin analogs. It was found that three ribavirin analogs could decrease the Nluc expression in the rRABV-infected human neuroblastoma cells with more than 5 to 27- fold higher anti-RABV activity compared to ribavirin in a dose-dependent manner. Among them, two ribavirin analogs harboring EC₅₀ values of less than 5 μ M were chosen for further studies. The antiviral activity of these two ribavirin analogs against RABV replication was examined in a time-of-addition, RABV minigenome, and qRT-PCR assays. These assays revealed that the viral transcription and genome replication stages of the RABV life cycle was inhibited by the addition of the compounds.

The findings presented in this thesis provide insights into screening methodologies that could be employed to discover antivirals against RABV. It is also suggested that nucleoside analogs could be one class of chemical compounds which is feasible to inhibit RABV infection in order to decrease the mortality associated with rabies.