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CXCR4 リガンド候補化合物の探索を指向した高感度スクリーニング手法の開発

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Background and Objective of Research: There is a continuous search and development of potent CXCR4 ligands to modulate effect of the CXCR4/SDF-1(CXCL12) signaling axis. However, existing screening methods can be improved by applying advanced screening system. NanoBRET was applied to develop a sensitive screening method for potent CXCR4 ligands.

Methods: TAMRA-AcTZ14011 was synthesized as a fluorescent probe and an acceptor of bioluminescent energy transfer from NanoLuc tagged to the N-terminal of CXCR4 expressed in CHO stable cells. Evaluation of NanoBRET was conducted in 96 well plate where the ratio of intensity at 460nm and 620 nm indicates interaction between ligands and CXCR4.

Results and Discussion: In our present study, it was shown that the NanoBRET assay system is feasible for the evaluation of CXCR4 ligands using TAMRA-AcTZ14011 as an acceptor, and NanoLuc tagged to CXCR4 as bioluminescent donor expressed in living cells. IC_{50}s of known CXCR4 antagonists were determined and found to correspond to values obtained by other sensitive but disadvantaged methods. Initial screening was conducted to evaluate inhibition rates at pre-determined concentration of 23 possible CXCR4 antagonists, MKN series. Inhibition potency of the MKN series was proved, and values were even higher with zinc ions.

Conclusion: A NanoBRET assay system useful for real time-high throughput screening of CXCR4 potent ligands was successfully developed. The method will be utilized for multi-well plate based screening.