DATE: Day____Month____Year 2023

SUMMARY of
2022 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Crystallographic fragment screening and structure determination for anticancer target proteins
Applicant	**Name**	soo Jae Lee
	Affiliation	College of Pharmacy, Chungbuk National University
	Present Title	Professor
Research Collaborator (Host PI)		Prof. Atsushi Nakagawa

Summary

This report describes a research project focused on the crystallization of the PAK4 protein complex with various inhibitors or pseudo-substrates, with the aim of designing new lead compounds for anticancer drug development. PAK4 is a kinase protein that has been found to have pro-oncogenic functions when overexpressed, and inhibiting its activity shows promise in combating cancer.

We conducted X-ray diffraction experiments to determine the crystal structures of three types of PAK4 complexes: PAK4-Methotrexate, PAK4-Pseudo-substrate3, and PAK4-Pseudo-substrate4. Methotrexate, an anticancer drug, was discovered to directly bind to PAK4 and activate its activity. The pseudo-substrates bind to the kinase domain of PAK4 but cannot accept a phosphate group from PAK4, offering insights for designing non-kinase domain inhibiting drugs.

The X-ray diffraction experiments were conducted at Beamline BL44XU, and the crystal structures were determined at resolutions of 1.2 Å for PAK4-Methotrexate and 1.5 Å for both PAK4-Pseudo-substrate3 and PAK4-Pseudo-substrate4. Additionally, native PAK4 crystals were soaked in a 5% DMSO solution for further experiments in Fragment-based Lead Discovery (FBLD), and the diffraction quality and resolution were satisfactory.

The structures of PAK4 complexes revealed that Pseudo-substrate3 and Pseudo-substrate4 bind to the substrate binding site in a similar manner, while Methotrexate binds at a different site, distant from the ATP binding site. The binding of Methotrexate may influence the conformational changes of the active loop of PAK4 near the ATP binding site.

In conclusion, PAK4 is an important target for cancer therapy due to its role in cancer progression. Designing PAK4 inhibitors based on the findings from the crystal structures could lead to the development of more effective anticancer drugs.

***Deadline: May 12, 2023**
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