Form 2

Dissertation Abstract

Report no.	(Course-based) ☑ No. **1216**	Name	**Raef Soliman Ahmed Shams**
Dissertation title	Identification of mTORC1 allosteric inhibitors by in-silico and in-cell hybrid selection (In-silico および in-cell 複合選択法による、mTORC1アロステリック阻害剤の同定)		

Abstract

The mammalian target of rapamycin (mTOR) is the critical regulator of cell growth and proliferation. It works as a sensor for nutrients, energy, and growth factors levels to control the metabolic processes through the connection between the input state (feeding or fasting) and the output state (growth). Therefore, diseases-associated metabolic alterations result in defective mTOR regulatory function specially in cancer. Therefore, mTOR has been assigned as a therapeutic target for anti-cancer drugs through multiple pathways of targets. However, until now these inhibitors showed to have some challenges which limit their therapeutic efficacy like immune suppression, incomplete inhibition, or non-specificity. Thus, we aimed in this study to find new and selective inhibitors for mTORC1 for efficient tumor suppression. In addition, we aimed to evaluate the binding kinetics of the mTORC1 activator, RHEB, to further validate RHEB for targeting by small molecule or peptide inhibitors.

In **chapter 2**, I assigned FKBP-rapamycin binding (FRB) domain of mTORC1 for specific allosteric inhibition by non-rapalog ligand for the first time. The FRB domain is the main substrate recruiter for the ribosomal S6 kinase-1 (S6K1) but not for the Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). FRB domain is being blocked by the FK506-binding protein 12 (FKBP12)-rapamycin complex to inhibit mTORC1 activity allosterically. However, the rapamycin was reported to induce cancer cell invasion due to its immunosuppressive properties. Therefore, replacing rapamycin became inevitable. To select new ligands, I used a hybrid strategy of in-silico method of virtual ligand screening based-docking, and in-cell method of split-luciferase reporters for protein-protein interaction. As a result, the best ligand, WRX606, showed to have stronger inhibition effect on mTORC1 kinase activity by inhibiting the phosphorylation of both substrates, S6K1 and 4E-BP1, while rapamycin only inhibited S6K1 phosphorylation. In turn, WRX606 strongly suppressed tumor growth in the experimental mice without promotion of metastasis. Overall, I could identify new mTORC1 allosteric nan-rapalog inhibitor by in-silico and in-cell method for the first time. The challenging her is to target two proteins at the same time to form ternary complex.

In addition, RHEB-GTPase is the last activation signal for mTORC1 which responsible for the lysosomal translocation of mTORC1 by binding to the mTOR portion resulting in conformational changes that makes the active site accessible. Thus, RHEB targeting may give a promise for prospective mTORC1 inhibition. At first, binding kinetics of RHEB with mTOR should be determined

at the molecular level. In **chapter** 3, I conducted the protein: protein interactions of RHEB with mTOR by in vitro methods (AlphaLISA and BLItz) and in-cell method (NanoBiT). After proteins preparation, I could measure the binding kinetics of RHEB with the whole mTOR for the first time using the established AlphaLISA-based assay. RHEB showed to bind mTOR either in the presence or absence of GTP which may contribute to further understanding of this activation mechanism. Also, by preparing different mTOR fragments including the structurally identified binding site (N-heat, M-heat, and FAT domains) and the biochemically identified binding site (kinase domain), I observed the binding of RHEB to these mTOR fragments by in-cell and in vitro BLItz methods. Surprisingly, RHEB bound the kinase domain at higher affinity than the reconstituted allosteric site composed of N-heat, M-heat, and FAT domains. These differential binding may incorporate RHEB in a dual role for mTORC1 activation process. Overall, I could determine the binding kinetics of RHEB with mTOR for the first time at the molecular level by different methods. These results, in turn, may be involved in better understanding the RHEB-dependent activation mechanism of mTORC1.

In conclusion, I discovered a new small-molecule allosteric inhibitor, WRX606, for mTORC1. This compound inhibited mTORC1 activity with a consequent cancer cytotoxicity in vivo without promotion of cancer cell metastasis. Furthermore, I evaluated the binding kinetics of RHEB-mTORC1 interaction by in vitro methods for better understanding the binding machinery. This will benefit to develop new small-molecule or peptide inhibitors to interfere RHEB-mTORC1 binding which in turn promote mTORC1 inactivation.