Poster

ULK1 inhibition for autophagy deregulation recovery

Marta S. Semrau[1*], Theo Battista[1*^], Viktorja Sidarovich[2], Rachele Ortolan[1], Andrea Dalle Vedove[3], Ivana Manini[4], Daniela Cesselli[4,5], Valentina Adami[2], Graziano Lolli[6], Paola Storici[1]

[1]Protein Targets for Drug Discovery Lab, Structural Biology, Elettra, Sincrotrone Trieste S.C.p.A, SS 14 Km 163.5 in AREA Science Park, Basovizza, 34149 - Trieste, Italy, [2]HTS and Validation Core Facility, Department of Cellular, Computational and Integrative Biology, University of Trento, via Sommarive 9, 38123 - Povo, Trento, Italy, [3]Area Science Park, Padriciano, 99, 34149 - Trieste, Italy, [4]Department of Medicine, University of Udine, via Palladio 8, 3310 - Udine, Italy, [5]Institute of Pathology, Academic Hospital Santa Maria della Misericordia, Udine, via Pozzuolo 330, 3310 Udine, Italy, [6]Department of Cellular, Computational and Integrative Biology, University of Trento, via Sommarive 9, 38123 – Povo, Trento, Italy

paola.storici@elettra.eu

Autophagy is a multistep lysosomal pathway which regulates cellular homeostasis by nutrient recycling and degradation of damaged cellular components, including misfolded proteins and organelles. Autophagy dysfunction is implicated in a plethora of human illnesses including cancer as well as cardiovascular, neurodegenerative, metabolic, pulmonary, renal, infectious, musculoskeletal, and ocular disorders [1]. In human cells, ULK1 complex initiates autophagy in response to nutrient deprivation or energy stress. The ULK1 complex consists of a large cluster of proteins which includes the serine/threonine protein kinase ULK1 (unc-51-like kinase 1), FIP200, ATG13, and ATG101 [2]. Activation of ULK1, which is essential for autophagosome assembly, is tightly regulated by phosphorylation/dephosphorylation events that control the complex formation. ULK1 gets further activated by the autophosphorylation of Thr180, the mutation of which results in a significant reduction in its activity.

ULK1 has garnered considerable attention as a potential target for modulating autophagy, particularly in the context of cancer progression and drug resistance [3]. To this end, we performed a Medium-Throughput Screening campaign at the HTS and Validation Core Facility of CIBIO (Trento, IT). Starting from an autophosphorylation-based screening of 4027 small-molecules we identified 22 active compounds with varying chemotypes, endowed with micromolar to nanomolar potencies. Subsequent structure-based drug discovery studies and *in-cellulo* validation experiments were initiated. A novel R245A/E246A mutant of the ULK1 kinase domain was engineered to improve crystal packing, enabling the determination of 6 co-crystal X-ray structures with 5 selected compounds (resolution range 1.8-2.2 Å). We could reveal the molecular details of the binding modes, and laying the basis for future structure- activity relationship (SAR) studies. Preliminary investigations using a commercial autophagy detection assay on the human glioblastoma U87 cell line, have shown that two out of three of the tested compounds effectively inhibit autophagy in cells.

In conclusion, these findings offer new tools to further investigate the role of ULK1 as a druggable target for modulating autophagy for cancer treatment laying the foundation for a drug discovery initiative.

[1] Klionsky, D. J.; Petroni, G.; Amaravadi, R. K.; Baehrecke, E. H.; Ballabio, A.; Boya, P.; Bravo-San Pedro, J. M.; Cadwell, K.; Cecconi, F.; et al., Zhong, Q.; Galluzzi, L.; Pietrocola, F. Autophagy in Major Human Diseases. (2021) *The EMBO Journal*, **40**, pp. 1-63.

[2] Zou, L.; Liao, M.; Zhen, Y.; Zhu, S.; Chen, X.; Zhang, J.; Hao, Y.; Liu, B. Autophagy and beyond: Unraveling the Complexity of UNC-51-like Kinase 1 (ULK1) from Biological Functions to Therapeutic Implications. (2022). *Acta Pharm Sin B* **12**, 3743–3782.

[3] Bhattacharya, S.; Piya, S.; Ma, H.; Sharma, P.; Zhang, Q.; Baran, N.; Ruvolo, V. R.; McQueen, T.; Davis, R. E.; Pourebrahim, R.; Konopleva, M.; Kantarjian, H.; Cosford, N. D. P.; Andreeff, M.; Borthakur, G. Targeting Unc51-like Autophagy Activating Kinase 1 (ULK1) Overcomes Adaptive Drug Resistance in Acute Myelogenous Leukemia. (2023). *Mol Cancer Res* **21**, 548-563.

* These authors equally contributed to the work.

^ Present address: Laboratory of Structural Dynamics of Receptors, Max Planck Society - Dioscuri Centre for Scientific Excellence, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

Acknowledgements: Partially Funded by the Regional Law No. 13, 6 Aug 2021, Art. 8, par. 28 30.