Abstract and keywords

The ubiquitin-proteasome system (UPS) is a tightly and specifically regulated system of protein degradation in eukaryotic cells. Inhibition of an UPS component might represent a strategy to control human diseases, including cancer. Modulation of the UPS can also be employed in basic research strategies. This thesis deals with two independent yet methodologically connected research aims – first, to search for the target of the newly identified UPS inhibitor CBU79, and second, to develop a fluorescent cell-based reporter exploiting proteasomal degradation. In the first part of my work, previous findings regarding the molecular mechanisms of CBU79 inhibiton on the UPS were confirmed. In the next step, I characterized how the UPS inhibitor CBU79 affects protein synthesis using the metabolic labelling of proteins based on click chemistry. I also examined the cytotoxic effect of CBU79 treatment on different cell lines. Finally, I performed a CRISPR/Cas9 whole-genome enrichment screen with the aim to find a potential target of the inhibitor. I found out that CBU79 probably decreases levels of protein synthesis by triggering cellular signalling *via* the unfolded protein response (UPR). Using the screen, I found 22 potential targets of the CBU79 inhibitor that will be further validated. In the second part of the work I utilized previous knowledge of the UPS to establish a non-infectious cell-based assay to monitor viral protease activity. This system exploits a short-lived yellow fluorescent protein (YFP) that is targeted for proteasomal degradation through an N-terminal ubiquitin-fusion degradation (UFD) signal. The reporter contains a cleavage site for Zika protease between the YFP and the degradation signal. Hence, removal of the UFD signal upon cleavage of the reporter by Zika protease generates a stable YFP product that accumulates in cells. I have developed, tested and optimized 21 reporter variants and found a possible setup for further screens. In summary, both the novel UPS inhibitor and the reporter assay for Zika protease inhibitor screening might pave the way for promising new strategies for the treatment of human diseases.

Keywords: Ubiquitin-proteasome system, flaviviral proteases, Zika virus, inhibition, fluorescent proteins