The Design, Synthesis and Structural Analysis of Small-Molecule Probes for the Targeting of USP2 Deubiquitinase.

The tumorigenesis of several types of cancer is linked with cyclin D1 overexpression. Cyclin D1 is responsible for regulation of cell-cycle progression during G1/S transition state.The USP2 hydrolase deubiquitinates polyubiquitinated target proteins such as cyclin D1, MDM2 and MDMX. USP2 deubiquitinates MDM2 without reversing MDM2-mediated p53 ubiquitination, therefore indirectly promotes p53 degradation, decreasing its antioncogenic activity. Deubiquitination is the counterpart of the ubiquitination that may be perceived as a kind of signal for a proteasome to obliterate the targeted protein. Deubiquitinase USP2 also prevents MDM2 mediated degradation of MDMX and by modulating its level affects the cell growth. The promotion of cyclin D1 stabilization and antagonizing ubiquitin-dependent degradation makes USP2 to play an important role in the G1/S cell- cycle progression in normal and cancer cells. Therefore, targeting of USP2 might be an effective approach for induction of growth suppression in the tumor cells addicted to cyclin D1 expression.

The design of small molecular chemical probes based on the information available for the complex of ubiquitin and USP2, supported by STD NMR screening for active fragments will provide the structures that will be capable to interact with USP2. The synthesis of these compounds comprises two approaches the linear synthesis and the multicomponent chemistry. The activity of candidates for potential chemical probes against USP2 can be initially deterred in assay experiments by measuring the level of free AMC fluorescence increase. The marker is released from the complex of Ub-AMC as a product of an enzymatic reaction catalyzed by USP2. The more decreased fluorescent the stronger chemical probe is as its affinity towards USP2 leads to its deactivation and inability to perform Ub-AMC hydrolysis. Once the activity of the compound is established the compound will be tested by NMR methods. The most potent probes will also be tested with application of NMR spectroscopy to be finally used as the ligands for the crystallization with the USP2 and examined on living cell lines according to their eventual toxicity and *in vivo* activity.

Targeting USP2 is very likely to become a promising strategy for cancer therapies nevertheless; finding promising candidates for potent chemical probes of USP2 are still in its initial stadium. The early stage of the research provides the vast perspectives for innovational research of the USP2 structural and metabolic aspects.