ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Pharmacognosy

Candidate: Ivana Šachová
Supervisors: PharmDr. Jan Martin, PhD., Dipl. Ing. Mgr. Ferdinand Molnár, PhD.
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The aim of this study was to express wild type and mutated synthetic human SRC1 central domains in *E. coli* BL21 DE3 cells. Expressed recombinant proteins were purified afterwards and used for investigation of SRC1-CAR protein interaction using pull down experiments**.**

The constitutive androstane receptor (CAR), a nuclear receptor (NR), is a transcriptional regulator, which influences the expression of various proteins, such as enzymes of biotransformation and transporters important for metabolism of both endogenous and exogenous compounds. CAR can be fully active only in presence of coactivators, such as steroid receptor coactivator-1 (SRC1). The most important fragment of SRC1 for binding to NRs is the central domain, made of three α-helical Leu-X-X-Leu-Leu (LXXLL) L1, L2, L3 motifs, in where L is a leucine and X represents any amino acid. hSRC1, as well as hCAR, can be successfully produced by expression of cDNA of interest in *E. coli* strains. In case of SRC1, codon optimised wild type and mutated synthetic human SRC1 central domains, where some of the motifs have been removed, were expressed in *E. coli* BL21 DE3 cells. Both expressed proteins, hSRC1 and hCAR, bear 6xHis-tag to facilitate the purification through the use of immobilized metal ion affinity chromatography (iMAC) such as TALON resin. All expressed proteins, except for FΔL2, which contains L1 and L3 motif but not L2, were able to bind CAR in pull down experiments. Higher affinity to CAR was observed with L2 motif compared to L1 and L3. Interestingly, the affinity to CAR was observed also with the mutant ΔAll, that lacks all the motifs. Surprisingly the ligand effects of CITCO, SO7662 and Clotrimazole on the interaction between hCAR and hSRC1 have not been observed in pull down experiments.