The impact of glycosylation and phosphorylation on cellular localization of cystatin F active form

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M. Lačen[I], M. Perišić Nanut *[II], J. Sabotič *[II], J. Kos *[I]

[I]University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia, [II]Jozef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia

Cystatin F is a cysteine peptidase inhibitor. Among other differences that distinguish it from other members of type II family, cystatin F possesses three glycosylation sites and is localized in endo/lysosomes. Cleavage of 15 N-terminal amino acids enhances monomerization and turns cystatin F into an active form in which it becomes a potent inhibitor of cysteine cathepsines C, H and L, localized in the endo/lysosomes. It has been shown that degree of glycosylation influences secretion and uptake of cystatin F inactive form and that transition is facilitated by binding of phosphorylated glycans to M6P-receptor. The aim of this thesis was to determine whether the degree of glycosylation has an effect on secretion, reuptake and cellular localization of active form of cystatin F. For this purpose, we prepared single, double and triple non-glycosylated mutants of active form and examined their expression, secretion and reuptake on HeLa cells, that normally do not express cystatin F. We determined whether mutants are secreted and taken up by the cells by western blot analysis. Using fluorescent confocal microscopy, we analyzed the intracellular localization of our mutants. As a control, glycosylated active form was used, which is secreted from cells as well as localized in endo/lysosomes. Our results show that only active cystatin F,non-glycosylated on asparagine 115 can be taken up to the cell and is localized in endo/lysosomes.Other non-glycosylated mutants are not localized in the endo/lysosomes and cannot be taken up by the cells.Secretion of mutants that are not glycosylated on asparagines 61 and 62 shows us that glycosylation on asparagine 115 affects secretion of the protein. Glycosylation on asparagines 61 and 62 has a big impact on secretion, protein uptake and localization of active form of cystatin F. Results also indicate that the transfer of the active form across membranes is not affected by possible O-glycosylation or phosphorylation on glycosylation sites.

* The authors marked with an asterisk equally contributed to the work.