HAMLET CYTOTOXICITY IN COLORECTAL CANCER CELL MODELS WITH DIFFERENT MUTATION STATUS IN VITRO

Miglė Pivoriūnaitė[1,3], Darius Stukas[1,3], Justas Žilinskas[2], Giedrė Šilkūnienė[1], Mantas Šilkūnas[1], Žilvinas Dambrauskas[1,2], Antanas Gulbinas[1], Algimantas Tamelis[2]

[1]Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences, Lithuania
[2]Clinic of Surgery, Lithuanian University of Health Sciences, Lithuania
[3]Faculty of Natural Sciences, Vytautas Magnus University, Lithuania
m.pivoriunaite@gmail.com

HAMLET (Human Alpha-lactalbumin Made LEthal to Tumor cells) is a proteolipid complex of partially unfolded α-lactalbumin and several oleate residues. Its efficacy as a selective killer of tumor cells has been documented *in vitro* and *in vivo* in several animal models [1]. HAMLET interacts with multiple tumor cell compartments, affecting cell morphology, metabolism, proteasome function, chromatin structure and cell viability [2]. Colorectal cancer is one of the most frequent malignancies worldwide, being second in males and third in females for its frequency and ranking fourth and third for cancer-related deaths among males and females, respectively [3]. KRAS and BRAF are major oncogenic drivers of colorectal cancer (CRC) [4].

The aim of this study was to evaluate antitumoral activity of the HAMLET complex on three different CRC cell lines (LoVo, WiDr, Caco-2) with different mutation status (KRAS/BRAF, wild type).

HAMLET complex was prepared using controlled temperature (partial protein unfold) combined with mixing/shaking with olein acid additive (acid incorporation in protein structure) [5]. Cytotoxicity of complex (metabolic activity and viability of the cells) was evaluated using 6 h exposition and different concentration in compliance with MTT and clonogenic assay protocols.

The results suggest that HAMLET affects cell metabolism, this effect is severe and at the same time irreparable for cells, leading to cell death. The complex exhibits cytotoxicity in dose-response manner against all cell lines. However, BRAF mutant cells seems to be more resistant to HAMLET in comparison to KRAS mutants and wild type cells. HAMLET has anticancer potency for CRC in *in vitro* model.

Acknowledgments: This project has received funding from European Social Fund (project No 09.3.3-LMT-K-712-15-0288) under grant agreement with the Research Council of Lithuania (LMTLT).

[1] Ho, J., Nadeem, A., Rydström, A. *et al.* Targeting of nucleotide-binding proteins by HAMLET—a conserved tumor cell death mechanism. *Oncogene* 35, 897–907 (2016) doi:10.1038/onc.2015.144

[2] Ho, J., Sielaff, H., Nadeem, A., Svanborg, C., & Grueber, G. (2015). The molecular motor F-ATP synthase is targeted by the tumoricidal protein HAMLET. *Journal of molecular biology*, *427 10*, 1866-74

[3]Testa, U., Pelosi, E., & Castelli, G. (2018). Colorectal cancer: genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. *Medical sciences (Basel, Switzerland)*, *6*(2), 31. doi:10.3390/medsci6020031

[4] Morkel, M., Riemer, P., Bläker, H., & Sers, C. (2015). Similar but different: distinct roles for KRAS and BRAF oncogenes in colorectal cancer development and therapy resistance. *Oncotarget*, *6*(25), 20785–20800. doi:10.18632/oncotarget.4750

[5] Kamijima T1, Ohmura A, Sato T, Akimoto K, Itabashi M, Mizuguchi M, Kamiya M, Kikukawa T, Aizawa T, Takahashi M, Kawano K, Demura M. Heat-treatment method for producing fatty acid-bound alpha-lactalbumin that induces tumor cell death. Biochem Biophys Res Commun. 2008 Nov 7;376(1):211-4.