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Molecules of Life: Towards New Horizons



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Indexing

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P-06.3-14**Human and horse milk extracellular vesicles: proteins and peptides**S. Sedykh^{1,2}, A. Kuleshova¹, L. Purvinsh¹, G. Nevinsky¹¹Novosibirsk State University, Novosibirsk, Russia, ²SB RAS ICBFM, Novosibirsk, Russia

Extracellular vesicles are secreted in milk by breast cells. Exosomes are vesicles with a diameter of 40–100 nm and contain CD9, CD63, and CD81 tetraspanins on the surface. Horse milk is a unique source of exosomes since is less allergenic and not prion-prone comparing to bovine milk and can be obtained in larger amounts than human milk. Different nucleic acids (plasmid DNA, microRNA) and drugs can be delivered in cells with milk vesicles. According to the literature data, milk exosomes present hundreds and thousands of proteins, mRNA, and microRNA molecules. Our recent findings show that the results on the protein and nucleic content in milk exosomes are significantly overestimated. Isolation of exosomes with an additional step of gel-filtration allows us to decrease the number of proteins, that co-isolate with vesicles. Here we show the content of proteins and nucleic acids in human and horse milk exosomes before and after gel-filtration of sediment obtained after ultracentrifugation. According to these data, human and horse milk exosomes may contain only a few major proteins with well-known biological functions and just several dozens of different microRNA. Our data confirm that horse milk exosomes can be used for the delivery of biologically active molecules to cells in vitro since their protein and nucleic acid content is highly similar to exosomes obtained from human milk. The study was funded by the Russian Scientific Foundation (research project 18-74-10055 to S. Sedykh).

P-06.3-15**Angiogenic factors and miRNA as diagnostic markers**

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Endometriosis is an estrogen dependent gynaecology condition and almost 7–10% of women in reproductive age are affected by this pathological condition that can complicate pregnancy and cause the infertility. A novel research data considers a combination of pro/angiogenic factors that play an important role in angiogenesis during endometriosis and endometrial cancer development. The aim of presented work was detection of expression changes of selected miRNA molecules, of mRNA and proteins levels of specific pro/anti angiogenic factors of patients with different stages of endometriosis (n = 22) and endometrial cancer of corpus uteri (n = 13) compared with the control group (n = 63). Expression of individual genes was detected by qRT-PCR and the results were compared with the control group. The enzyme-linked immunosorbent assay were used for detection of proteins level. Expression levels of specific miRNA molecules were different, and depend on the differential disease of the patients. The mRNA level for sEng was about 273% higher in patients with frozen pelvis and about 126% higher in patients with peritoneal endometriosis than in controls. We analysed significantly the high level of Endoglin protein of patients with endometriosis. Similarly, ROC analysis showed that Endoglin protein level could

distinguish patients with endometriosis versus patients with endometrial carcinoma of the corpus uteri with a sensitivity of 92% and 67% of AUC specificities of 0.801 (0.625–0.977); $P = 0.01$. The Notch 3 protein could differentiate uterine endometrial cancer from a control group with 61% sensitivity and 75% AUC specificity of 0.792 (0.634–0.951) $P = 0.01$. Study of the specific markers expression is highly current topic, can help understand of the mechanisms and can lead to the development of new diagnostic and therapeutic applications in monitoring and treatment of patients with endometriosis. This work was supported by grant project VEGA 1/0620/19

P-06.3-16**Cooperative interaction of dimeric enzyme prostaglandin H synthase with substrate – arachidonic acid**A. Krivoshey^{1,2}, V. Barkhatov^{1,2}, A. Efremov², E. Matveishina^{2,3}, V. Panova², P. Vrzhesch^{1,2}¹International Biotechnological Center, Lomonosov Moscow State University, Moscow, Russia, ²Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia, ³Institute of Bioengineering, Research Center of Biotechnology, Russian Academy of Science, Moscow, Russia

Prostaglandins are regulators of inflammation, immune response and many other physiological processes. Synthesis of prostaglandins is a result of cyclooxygenase oxidation of arachidonic acid (AA), which is catalyzed by homodimeric enzyme prostaglandin H synthase (PGHS). PGHS is irreversibly inactivated during catalyzed reaction and inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). There was some evidence of cooperative interactions between enzyme subunits, but cooperativity of AA binding to the active sites was not investigated sufficiently. In this study, we used the PGHS-1 isoform purified from sheep vesicular glands. The cyclooxygenase reaction was detected amperometrically from the consumption of dissolved molecular oxygen. Obtained data were analyzed using Origin and MATLAB. It was shown that the dependence of cyclooxygenase oxidation rate in the wide range of AA concentrations is not described by Michaelis-Menten equation. However, it can be described by model taking into account the negative cooperativity of AA binding to PGHS subunits (the enzyme affinity to the first AA molecule is 14 μ M, and the affinity to the second AA molecule is 1 mM). The investigations of integral kinetics of cyclooxygenase reaction demonstrated that total enzyme turnover number (from the beginning of the reaction until complete inactivation) decreases with the increase of AA concentration. The experimental dependences were described by cooperative model, and the values of kinetic constants were found. Obtained results should be taken into account during the investigation of prostaglandin synthesis and pharmacological effects of NSAIDs *in vivo*. The reported study was funded by RFBR according to the research project № 19-04-01150a. It was carried out using the equipment purchased via the Moscow State University Development Program and the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.