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FIBRINOGEN-SPECIFIC PROTEASE IN THE *Vipera renardi* SNAKE VENOM

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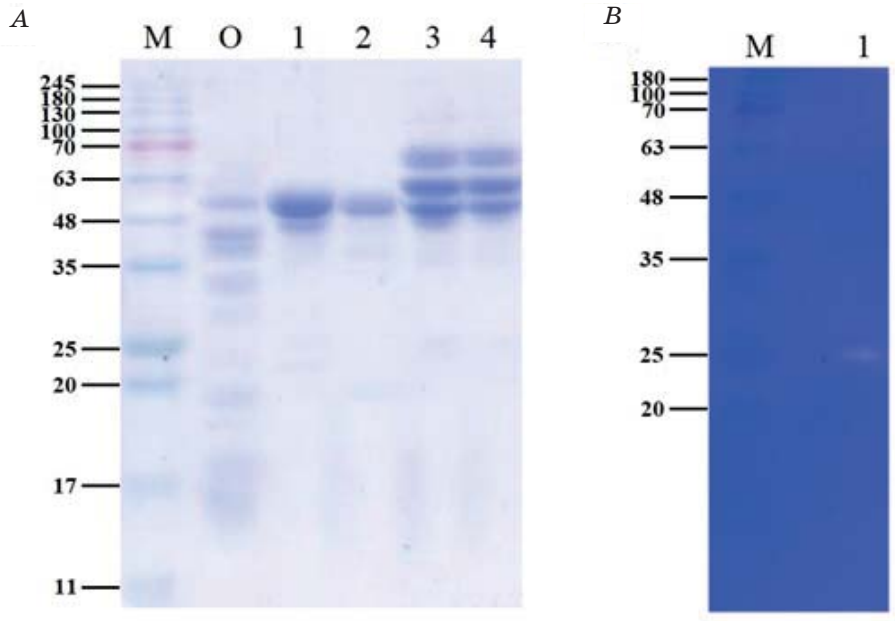
Venoms of snakes of Viperidae family contain the mixtures of physiologically active proteins. Evolutionary they were developed for the effective envenomation of the prey, however, their high specificity towards components of mammal blood makes them a prospective pharmacological agent [1]. Some of these enzymes are targeted preferentially to fibrinogen [1–3]. Fibrinogen-specific proteases of snake venoms are assumed as a possible agent for defibrin(ogen)ation and the achieving of antithrombotic effect. Also, their application is one of the possible approaches for studying structure and functions of fibrinogen molecule. That is why the aim of the present study was the search of fibrinogenolytic enzymes among protein components of *Vipera renardi* snake venom.

Methods. Venom of *V. renardi* as the lyophilized powder was supplied by Trypillia serpentarium. It was dissolved in 0.05 M Tris-HCl buffer pH 8.3 and fractionated on Superdex G-75 using FPLC system Acta Prime. Peaks were tested for their ability to directly cleave fibrinogen. For this, the fraction with the lowest light absorption at 280 nm was diluted 5 times and fibrinogen was added to its final concentration of 2 mg/ml. Other peaks were diluted an appropriate number of times so that all fractions were commensurate. Incubation continued for 2 hours at a temperature of 37 °C. Hydrolytic products were analyzed by SDS-PAGE. Enzyme-electrophoresis with fibrinogen co-polymerized in 12% polyacrylamide gel was used for the identification of protein that can cleave fibrinogen.

Results. Venom of *V. renardi* was fractionated on 4 fractions using size-exclusion chromatography. SDS-PAGE of fibrinogen hydrolysis products showed the presence of fibrinogen-specific protease in the 1st and 2nd fractions of venom. 2nd fraction was much more active and according to the data of enzyme electrophoresis contained protease with molecular mass 25 kDa.

Discussion. Fibrinogen is a large blood plasma glycoprotein. When blood vessels are damaged, a cascade of reactions is triggered, the final result of which is the transformation of fibrinogen into fibrin, which polymerizes to form the core of a blood clot that terminates bleeding [4]. However, certain pathological conditions increase the risk of intravascular blood clotting which affect the normal blood supply of tissues and can be a deadly threat. Therefore, the search for proteases targeted fibrinogen and fibrin can be assumed as agents for direct defibrination [5]. The protease found in the present study effectively cleaves fibrinogen and can be tested for its ability to act on blood clots in the bloodstream.

Proteases that exhibit fibrinogenolytic activity can also be tools for studying of the structure and function of fibrin(ogen) molecule. Under the action of fibrinogenolytic enzymes we can obtain fragments of the molecule which study can indicate the role of different sites in the functioning of fibrinogen [6]. Therefore, the next stage of the study of the obtained protease will be the detection of the hydrolysis point within the fibrinogen molecule, which will allow obtaining unique proteolytic fragments.



SDS-PAGE of fibrinogen hydrolysis products by fractions obtained using Superdex G-75 (*A*):
M —markers, kDa; O — venom of V. renardi; 1–4 —studied samples.
Enzyme-electrophoresis with fibrinogen co-polymerized in 12% polyacrylamide gel of fraction with fibrinogenolytic activity of Vipera renardi snake venom (*B*):
M — markers, kDa; 1 — studied sample

Conclusions. Fractionation of *V. renardi* snake venom allowed to detect a protease with apparent molecular mass 25 kDa that can cleave fibrinogen molecule.

Key-words: proteases; fibrinogen; snake venom; vipera.

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Authors' contribution. KB and OP fractionated venom of *V. renardi*. KB and YS studied the ability of obtained fractions to cleave fibrinogen.

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OVEREXPRESSION/KNOCKDOWN OF ADAPTOR PROTEIN RUK/CIN85 IN HUMAN LUNG ADENOCARCINOMA A549 CELLS RESULTS IN OPPOSITE CHANGES BETWEEN MMP-2/MMP-9 EXPRESSION LEVELS/ACTIVITIES AND CELL`S INVASION

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To acquire the ability to metastasize, cancer cells undergo molecular reprogramming in the course of epithelial-mesenchymal transition resulting in an increased motility and invasiveness [1]. Cells with elongated spindle-like mesenchymal phenotype require ECM-degrading enzymes, mainly MMP-2 and MMP-9, to generate the path for migration. In our previous works we demonstrated that overexpression of adaptor protein Ruk/CIN85 in breast cancer cells was associated with their aggressive metastatic behavior [2, 3]. In this study we aimed to investigate the changes in MMPs expression and activity as well as invasiveness of human lung adenocarcinoma A549 cells with up-/down-regulation of Ruk/CIN85.

Methods. We used A549 cells with stable overexpression (subline RukUp) and knockdown of Ruk/CIN85 (subline RukDown), as well as corresponding vector control sublines Mock and Scr.

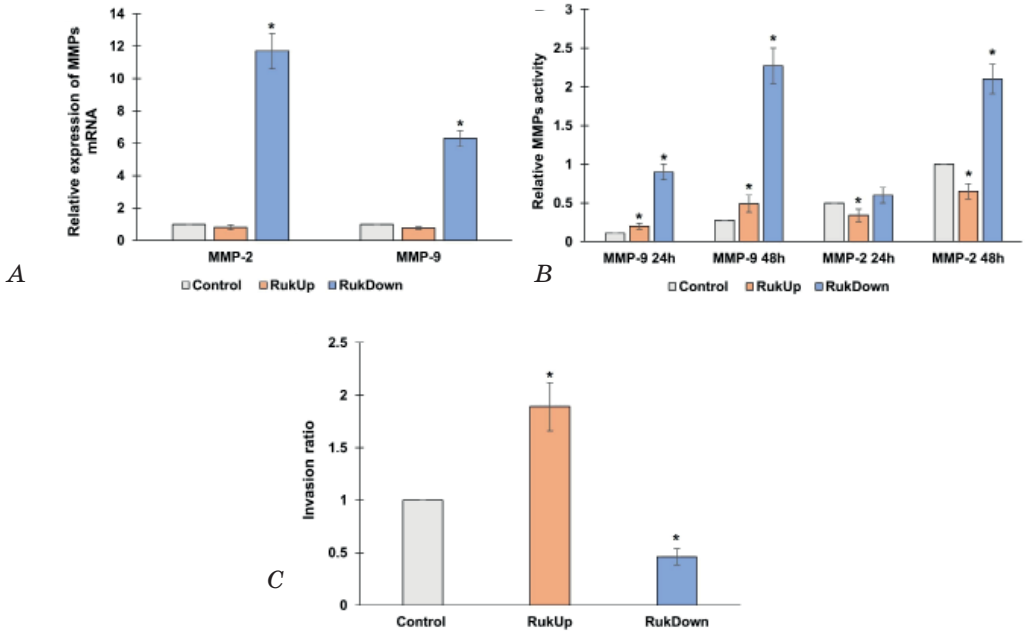

Fig. **Up-/down-regulation of Ruk/CIN85 in human lung adenocarcinoma A549 cells lead to opposite changes between MMP2/MMP9 genes expression (*A*); their enzymatic activities (*B*) and invasion ratio of cell sublines (*C*):**
$M \pm m$, $n = 3$; * — $P < 0.05$ compared to the corresponding control

Cells were cultured in the complete DMEM medium under standard conditions. mRNA expression levels were estimated by RT^2-PCR, enzymatic activity was assessed using gelatin zymography. Invasiveness of cancer cells was studied using Boyden chambers coated with Matrigel.

Results. Analysis of mRNA expression of MMPs in RukUp and RukDown cells revealed that MMP-2 and MMP-9 were preferentially overexpressed in RukDown cells, while RukUp subline did not exhibit significant difference compared with corresponding control (Fig. *A*). These findings were confirmed and complemented by study of enzyme activities. As can be seen from Fig. ***B***, the gelatinolytic activities of both MMP-2 and MMP-9 were dramatically increased in RukDown subline, compared to respective control (Fig. *B*). Surprisingly, we revealed that MMPs regulation was inversely correlated with invasion potential of Ruk/CIN85 up/down A549 cells. In particular, it was established that invasiveness of RukUp cells was 2 times higher in comparison with respective control subline. Alternatively, invasion ratio was significantly decreased in RukDown cells (0.5 times) in comparison with control (Fig. *C*).

Discussion. In this study we found that expression level of Ruk/CIN85 in A549 cells is strongly associated with opposite changes between their invasiveness and MMPs expression/activities. It is known that the role of MMPs in carcinogenesis remains ambiguous. MMPs are involved in tumor progression. In particular, MMP`s associated degradation of ECM components modulates cancer cells motility as well as leads to activation of proangiogenic factors in various cancerous tissues. On the other hand, MMP-2/MMP-9 take part in digestion of plasminogen resulting in generation of angiostatins (kringle–containing fragments of plasminogen) that could function as inhibitors of angiogenesis and tumor growth *in vitro* and *in vivo* [4].

Conclusions. According to the data received, it is possible to suggest that up-regulation of adaptor protein Ruk/CIN85 in A549 cells can lead to the very aggressive MMP-independent mode of migration that rely on cycles of expansion and contraction of the cell body mediated by the cortically localized actin and myosin [5].

Key words: Lung Adenocarcinoma; Motility; Invasion; Epithelial-Mesenchymal Transition; MMPs; Adaptor Protein Ruk/CIN85.

Author`s contribution. Bekala M. I. has performed estimation of mRNA expression levels by RT^2-PCR as well as was involved in zymography assay, data analysis and thesis writing. Geraschenko D. S. has received sublines of A549 cells with stable expression and knockdown of Ruk/CIN85. Khudiakova O. V. worked on the RNA extraction. Skaterna T. D. has performed zymography and Boyden chamber assays, as well as has curated research planning, data analysis and thesis writing.

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