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ADAPTOR PROTEIN Ruk/CIN85 AFFECTS REDOX BALANCE IN BREAST CANCER CELLS

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Excessive reactive oxygen species (ROS) production may lead to damage of cellular proteins, lipids and DNA, and cause cell death. Our previous findings demonstrated that increased level of adaptor protein Ruk/CIN85 contributes to breast cancer cells malignancy. The aim of this study was to investigate the role of Ruk/CIN85 in the maintaining of the redox balance in cancer cells. Mouse breast adenocarcinoma 4T1 cells with different levels of Ruk/CIN85 expression were used as a model in this study. Activities of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), aldehyde dehydrogenase (ALDH) and formaldehyde dehydrogenase (FALDH), as well as H_2O_2 and aldehydes content were measured using fluorometric assays. Gene expression correlations between Ruk/CIN85 and antioxidant enzymes in breast cancer samples were analyzed using ist.medisapiens transcriptomic database. It was demonstrated that Ruk/CIN85-overexpressing 4T1 cells were characterized by increased production of H_2O_2 and reduced activities of CAT, GPx and SOD. Overexpression of Ruk/CIN85 resulted in decreased content of aldehydes together with increased activity of ALDH, while in Ruk/CIN85-knocked down 4T1 cells, activities of ALDH and FALDH were decreased. The data of transcriptomic analysis revealed the correlations between SH3KBP1 expression and CAT, GPX4, ALDH1A1, ALDH1L1, ALDH2, GSR, SOD1 in human breast carcinomas samples. The obtained results indicate that adaptor protein Ruk/CIN85 affects redox balance in mouse breast adenocarcinoma 4T1 cells.

K e y w o r d s: breast cancer, adaptor protein Ruk/CIN85, hydrogen peroxide, ROS, antioxidant enzymes.

Oxidative stress refers to the imbalance in the production and utilization of reactive oxygen species (ROS) and free radicals. ROS, namely hydrogen peroxide H_2O_2, superoxide anion $O_2^{-\bullet}$, hydroxyl radical $OH^{\bullet}$ etc, are generated in enzyme-dependent or independent ways from molecular oxygen, mainly during tissue respiration in mitochondria [1].

At low doses, ROS (especially H_2O_2) function as signaling molecule, that control multiple cellular processes, including survival, proliferation, differentiation, motility, inflammation, autophagy, adaptation to hypoxia, immunity and others [2]. On the contrary, excessive accumulation of ROS and free radicals may cause cell damage *via* oxidative modification of lipids, proteins and DNA. In order to control ROS content, cells developed several ROS scavenger systems, which transform or neutralize active molecules: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione, thioredoxin, antioxidant vitamins, such as E, C, A, and other small molecules [3, 4].

It has been demonstrated that oxidative stress is associated with development of numerous pathologies, including diabetes, pulmonary diseases, cardiovascular diseases, neurological diseases, inflammation, obesity, aging, and cancer [3]. Increased ROS accumulation is linked to cancer initiation and progression via DNA damage, mutagenesis, genome instability, induction of cells proliferation and angiogenesis [3, 5]. In cancer cells ROS molecules induce various signaling pathways,

such as MAPK, PI3K/Akt/mTOR, NF-κB, HIF, Wnt, integrins, p53, iNOS and others [3, 6]. Thus, moderate oxidative stress in cancer cells can be involved in the control of cell proliferation, survival, angiogenesis, chemo- and radioresistance, invasion [3], and cancer stem cells reprogramming [7].

Cellular signaling involves many different molecules, including receptors, kinases, phosphatases, transcription factors, and adaptor/scaffold proteins. The latter ones serve as dynamic binding platforms for other signaling molecules, thus facilitating, directing, and intensifying cellular signaling [8, 9]. Ruk/CIN85 is a member of CIN85/CMS family of adaptor proteins. It consists of three SH3 domains (A, B, and C) at the N-terminus of the molecule, proline-rich region, and coiled-coil motif at C-terminus [10]. In previous studies it has been demonstrated that high level of adaptor protein Ruk/CIN85 expression was associated with cancer metastasis and poor prognosis [11, 12]. Moreover, its overexpression in cancer cells led to increased motility, invasiveness [10], resistance to antitumor drugs and potentiation of cancer stem cells (CSCs) properties [13]. However, there is almost no data about ROS involvement in Ruk/CIN85-dependent cancer progression.

The aim of the present study was to investigate the role of adaptor protein Ruk/CIN85 in maintaining the redox balance in breast cancer cells.

Materials and Methods

Reagents used in experiments were purchased from Sigma-Aldrich (benzamidine, PMSF, aprotinin, leupeptin, pepstatin, HEPES, folic acid, SOD Assay Kit-WST, NADH, H_2O_2), Gibco (RPMI-1640 medium, L-glutamine, penicillin, streptomycin), HyClone (fetal bovine serum). All other reagents were of purest available grade.

Cell culture. Mouse breast adenocarcinoma 4T1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C, 5% CO_2 in humidified atmosphere. Sublines with stable overexpression (RukUp-1) or downregulation (RukDown) of adaptor protein Ruk/CIN85, and corresponding control sublines (Mock and Scr) were generated as described previously [13].

Preparation of cytoplasmic extracts. Cultured cells were grown to 70-80% confluence. Proteins were extracted with hypotonic buffer containing 0.4% NP-40, 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM Na_3VO_4, 5 mM benzamidine, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 μg/ml pepstatin. Volumes of extraction buffer required were scaled up according to cell culture surface area as in the case of cell lysis. Cells were scraped, neatly pipetted, and transferred into 1.5 ml tubes. Samples were subjected to repeated freezing-thawing procedure in liquid nitrogen, pipetted, sonicated for 30 s, and centrifuged at 3,000 rpm for 15 min at 4 °C using centrifuge HERAEUS LABOFUGE 400 R (Thermo Scientific, USA). The supernatants were transferred to clean tubes and centrifuged again at 12,000 rpm for 30 min at 4 °C. The obtained supernatants were used for determination of protein concentration, enzymatic activities and metabolites concentration in cytoplasmic extracts.

Enzyme assays. For estimation of catalase activity (EC 1.11.1.6), fluorometric method, based on the measurement of residual amount of H_2O_2 after the catalase action during 3 min, was developed. The reaction mixture contained 150 μl of 10 mM H_2O_2 in 10 mM Na-K phosphate buffer, pH 7.5 and 5-20 μl of cells extract with protein concentration 1-2 mg/ml. The reaction was terminated by addition of cold 30% solution of $ZnSO_4$ followed by centrifugation at 5,000 rpm for 10 min. H_2O_2 detection was carried out by monitoring folic acid oxidative degradation using microplate fluorometer (FLx800, Biotek', USA) in black plates according to Hirakawa [14]. The mixture for H_2O_2 determination contained 140 μl of Na-phosphate buffer pH 7.4, 20 μl of 0.2 mM $CuCl_2$, 20 μl of control/experimental sample and 20 μl of 0.1 mM folic acid. After 45 min, the amount of H_2O_2 was measured at the excitation/emission wavelength of 360/460 nm. A calibration curve was used to calculate hydrogen peroxide. One unit of enzyme activity was defined as nmol of H_2O_2 decomposed per minute per mg protein.

GPx activity (EC 1.11.1.9) was determined with Elman's reagent (DTNB - 5,5′-dithiobis-2-nitrobenzoic acid) according to the protocol proposed in the paper [15]. The 500 μl of reaction mixture contained 2.5 mM H_2O_2, 2 mM reduced glutathione (GSH), 50 mM Tris-HCl buffer, pH 7.2, 5 mM EDTA, 10 mM NaN_3 and 150 μg of cell protein extract. The reaction was carried out at 37 °C for 10 min and terminated by 10% TCA addition followed by centrifugation at 3,000 rpm for 10 min. The amount of GSH in the supernatant was determined using Elman reagent. For this purpose a standard solution of glutathione (STD), testing (TS) or appropriate con-

trol samples (CS) (CS1 - which did not used for reaction, CS2 - which contained water instead of GSH), Elman's reagent were added to the microplate wells and the total volume was brought to 130 µl with 10 mM sodium-potassium phosphate buffer pH 8.0. The optical density was measured at 412 nm using µQuant reader (BioTech, USA). The amount of GSH in the reaction was determined by the equation: (TS-CS1-CS2). One unit of the activity corresponds to 1 µmole of GSH per 1 min of reaction per 1 mg of protein.

SOD activity (EC 1.15.1.1) was analyzed using the "SOD Assay Kit-WST" according to the manufacturer's protocol. The method is based on the inhibition of superoxide anion formation in the xanthine/xanthine oxidase system in the presence of superoxide dismutase. The generation of superoxide was detected by the formation of a chromophore, a water-soluble formazan, at 450 nm. The enzyme activity unit corresponds to the amount of protein that caused 50% inhibition of superoxide formation compared to control samples that did not contain cytoplasmic extract.

Dehydrogenases activities in cytoplasmic extracts were determined using the fluorescence method based on the measurement of NADH formed. Alamar Blue (resazurin) and phenazine methosulfate were added to the reaction mixture to enhance and extend the range of NADH fluorescence to beyond 600 nm where biological samples have no fluorescence emission. The step was performed to distinguish biological samples from many other naturally occurring environmental fluorophores. Fluorescence was monitored at 528-545 nm excitation wavelength and 600 nm emission wavelength. The amount of NADH formed was determined from the calibration curve.

The reaction mixture (200 µl) for ALDH (EC 1.2.1.3) activity determination included: 100 mM Na-pyrophosphate, pH 9.0, 10 mM glyoxal, 0.5 mM NAD^+, 1 mM EDTA, 0.1 mM resazurin, 0.1 mM phenazine methosulfate. The reaction was started by the addition of cytoplasmic extract (20 µl) with the total protein concentration ~ 2 mg/ml [16].

The reaction mixture (200 µl) for FADH (EC 1.2.1.46) activity determination included: 50 mM K-phosphate buffer, pH 8.0, 2 µM formaldehyde, 0.2 mM NAD^+, 1.5 µM reduced glutathione (GSH), 0.1 mM resazurin and 0.1 mM phenazine methosulfate. The reaction was started by the addition of cytoplasmic extract (20 µl) with the total protein concentration ~ 2 mg/ml [17].

Metabolites measurement. Hydrogen peroxide and aldehydes concentrations were determined in deproteinized samples of cytoplasmic extracts. For this purpose cold 30% $ZnSO_4$ was added to the samples (10% of total volume) followed by centrifugation at 5,000 rpm for 10 min. The supernatant was used for further determinations. Fluorometric method was used for the detection of hydrogen peroxide [14]. The aldehydes content was determined by the Hantzsch reaction with the Nash Reagent (acetylacetone - 11 µl, 0.05 M acetic acid - 16.5 µl, ammonium acetate - 825 mg, H_2O - 5.5 ml). Supernatant was mixed with an equal volume of Nash Reagent (50 µl sample + 50 µl Nash Reagent) and incubated for 40 min at 37 °C. Absorption was measured at $\lambda = 412$ nm. The control sample contained distilled water instead of the supernatant. Formaldehyde calibration curve (5-75 µM) was used for calculations [18].

Protein concentration estimation. The protein concentration was determined by Bradford method [19].

Genes expression correlation analysis in breast cancer samples was performed using www.ist.medissapiens.com transcriptomic database. Correlation was considered as strong if $|r| > 0.5$; moderate – if $|r| > 0.3$; weak – if $|r| < 0.3$.

Statistical analysis was carried out using Origin 8.5 software. Pairwise comparisons (RukUp-1 group was compared to Mock control, and RukDown group – to Scr control) were performed by Student's *t*-test for independent samples with unequal variance. Significant difference between groups was declared at $P < 0.05$.

Results and Discussion

The involvement of adaptor protein Ruk/CIN85 in the maintaining of redox status in cancer cells was studied using mouse breast adenocarcinoma 4T1 cells model with stable overexpression (subline RukUp-1) and downregulation (subline RukDown) of Ruk/CIN85. As corresponding controls, we used sublines Mock and Scr [13].

It was found that H_2O_2 production was increased by 70% in Ruk/CIN85-overexpressing 4T1 cells in comparison with Mock control (Fig. 1, *A*). Analysis of antioxidant enzymes demonstrated that activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were de-

creased significantly, by 31, 23 and 39%, respectively (Fig. 1, *B, C, D*). We did not found any significant difference in H_2O_2 production or antioxidant enzymes activities in Ruk/CIN85-downregulated cells. The obtained results suggest that overexpression of adaptor protein Ruk/CIN85 in 4T1 breast adenocarcinoma cells led to increased ROS production associated with decreased activities of antioxidant enzymes.

Aldehydes may also lead to cell damage by spontaneous carbonyl modification of DNA and proteins, causing proteins cross-linking, generation of another free radicals, and inhibiting DNA reparation [20, 21]. It was demonstrated, that 4T1 RukUp-1 cells

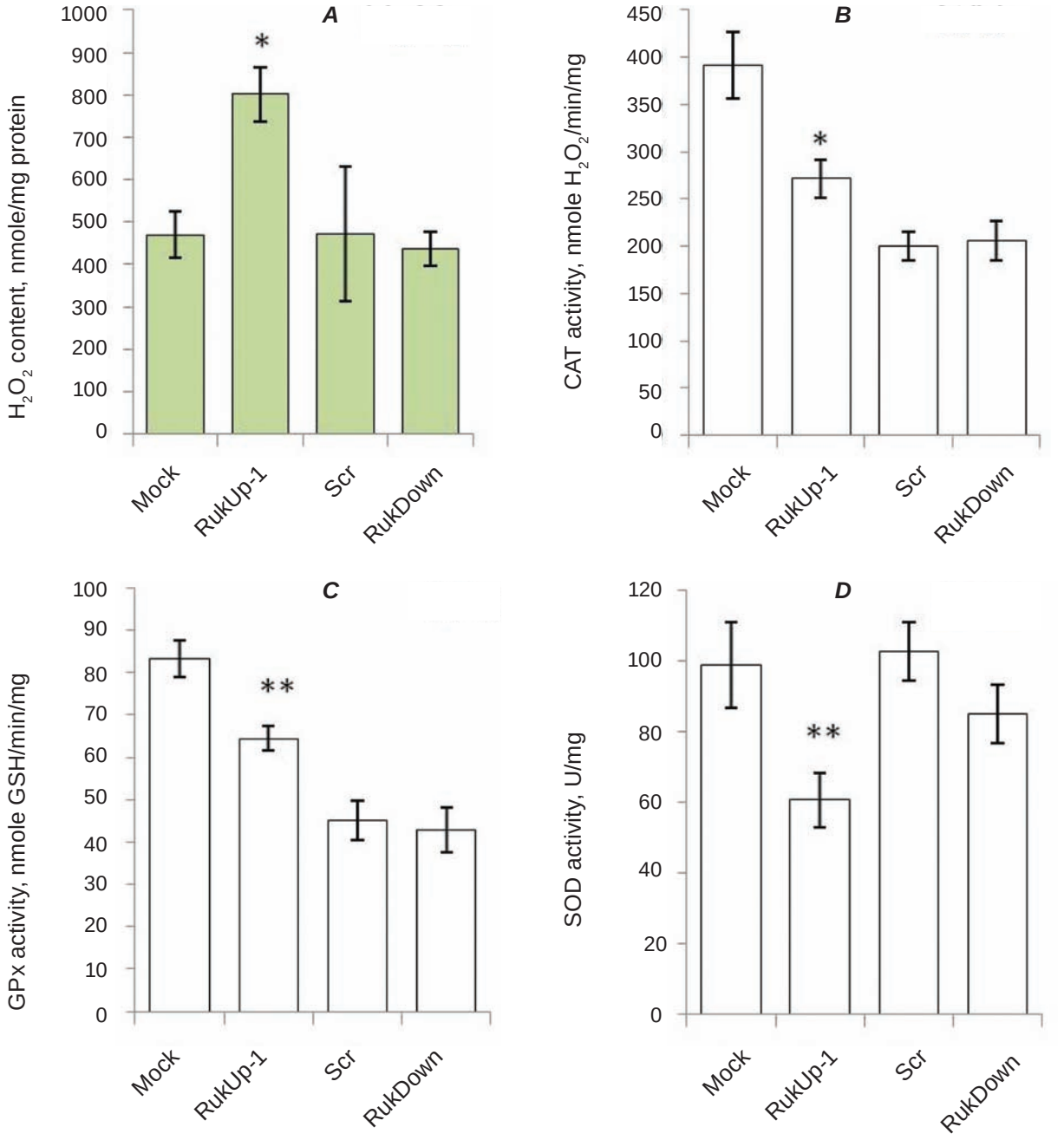

Fig. 1. Overexpression of adaptor protein Ruk/CIN85 in mouse breast adenocarcinoma 4T1 cells results in increased H_2O_2 production and reduced activities of antioxidant enzymes. ***A*** *– H_2O_2 content,* ***B*** *– catalase activity,* ***C*** *– glutathione peroxidase activity,* ***D*** *– superoxide dismutase activity; n = 4; *P < 0.05, **P < 0.01 in comparison with corresponding control*

produce significantly lower amount of aldehydes (by 43%) than control Mock cells (Fig. 2, *A*). In addition, in 4T1 RukUp-1 cells activity of ALDH, one of main markers of chemoresistance involved in aldehydes catabolism, was increased by 48%. In 4T1 RukDown cells, there were no significant differences in aldehydes content, while activities of ALDH and FALDH (formaldehyde dehydrogenase) were decreased significantly, by 32 and 30%, respectively (Fig. 2). According to these results, we can conclude that high expression of Ruk/CIN85 may protect cancer cells from carbonyl stress and protein damage.

Taking into account the obtained results, we decided to analyze the public data (from transcriptomic database www.ist.medisapiens.com) for the existence of correlation between expression of Ruk/CIN85 and genes encoding antioxidant enzymes in human breast cancer samples (Table, significant, moderate and strong correlations are shown in bold). It was found significant ($P < 0.001$) moderate positive correlation between Ruk/CIN85-encoding gene *SH3KBP1* expression and catalase (*CAT*), glutathione peroxidase isoform 4 (*GPX4*), and aldehyde dehydrogenase isoform 1A1 (A*LDH1A1*), strong positive correlation between *SH3KBP1* and aldehyde dehydrogenase isoform 1L1 (*ALDH1L1*), and isoform 2 (*ALDH2*), moderate negative correlation between *SH3KBP1* and glutathione reductase (GSR), and superoxide dismutase isoform 1 (*SOD1*) in breast lobular carcinoma samples (Fig. 3). Significant, but weak correlations were found for *GPX1, SOD2* genes in breast lobular carcinoma samples, and for *CAT, GPX1, GPX2, GPX3, GPX4, GPX7, GSR, SOD1, SOD2, ALDH1A1, ALDH1L1, ALDH1L2, ALDH2, ALDH3A1* in breast ductal carcinoma samples. It is interesting that moderate or strong correlations between expression of Ruk/CIN85 and antioxidant enzymes were found only in breast lobular carcinoma samples, but not in ductal ones. Both breast lobular and ductal carcinomas belong to invasive breast cancers, but differ in genomic profiles and response to treatment [22]. Invasive lobular carcinoma (ILC) is characterized by small, noncohesive cells as a consequence of lack of intercellular adhesion, mainly due to decreased E-cadherin expression as a hallmark of ILC [22, 23].

In the present study we analyzed the effect of adaptor protein Ruk/CIN85 on the main redox balance indicators in mouse breast adenocarcinoma 4T1 cells depending of its expression levels. It was demonstrated that overexpression of Ruk/CIN85 in 4T1 cells led to increased production of H_2O_2 and reduced activities of glutathione peroxidase and superoxide dismutase. In previous studies, it was found that Ruk/CIN85 overexpression in human MCF-7 breast cancer cells and HT-29 colon cancer cells

Expression of adaptor protein Ruk/CIN85-encoding gene SH3KBP1 in human breast cancer samples correlates with antioxidant enzymes expressions

No	Gene	Breast lobular carcinoma, $n = 80$		Breast ductal carcinoma, $n = 557$	
		R	*P*	R	*P*
1	*CAT*	**0.479**	**<0.001**	0.101	<0.001
2	*GPX1*	0.112	<0.001	0.089	<0.001
3	*GPX2*	-0.059	0.60	0.077	<0.001
4	*GPX4*	**0.345**	**<0.001**	-0.128	<0.001
5	*GPX5*	-0.052	0.65	0.013	0.75
6	*GPX7*	0.011	0.92	0.217	<0.001
7	*GSR*	**-0.321**	**<0.001**	-0.162	<0.001
8	*SOD1*	**-0.345**	**<0.001**	-0.186	<0.001
9	*SOD2*	0.220	<0.001	0.176	<0.001
10	*ALDH1A1*	**0.312**	**<0.001**	0.109	<0.001
11	*ALDH1L1*	**0.569**	**<0.001**	0.132	<0.001
12	*ALDH1L2*	0.065	0.57	0.072	<0.001
13	*ALDH2*	**0.654**	**<0.001**	0.037	<0.001
14	*ALDH3A1*	-0.01	0.93	0.095	<0.001

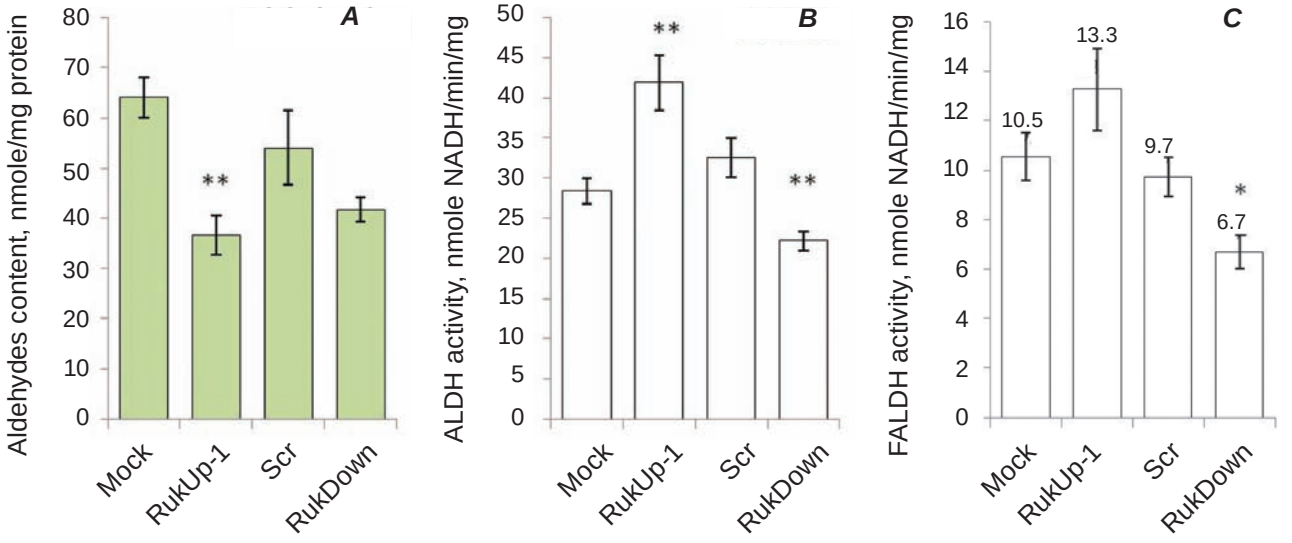

Fig. 2. Adaptor protein Ruk/CIN85 modulates production of aldehydes and activity of aldehydes-neutralizing enzymes in 4T1 breast cancer cells. ***A*** *– aldehydes content,* ***B*** *– aldehyde dehydrogenase (ALDH) activity,* ***C*** *– formaldehyde dehydrogenase (FALDH) activity; n = 4; *P < 0.05, **P < 0.01 in comparison with corresponding control*

was associated with increased ROS production [24]. H_2O_2 can be generated in the reaction of superoxide anion $O_2^{-\bullet}$ dismutation by SOD, or by NADPH oxidases (NOXs). It should be noted that Ruk/CIN85 is a binding partner of NOX1 adaptor TKS4 [25], and Ruk/CIN85 overexpression in MCF-7 breast cancer cells led to increased expression of several NOXs: NOX1, NOX2, and DUOX2 [24]. In low doses (nanomolar range of concentrations) H_2O_2 oxidizes thiolate anion Cys residues in proteins (Cys-S-) to sulfenic state (Cys-SOH), which cause changes in proteins structure and alter their functions. This process is reversible due to the action of thioredoxin and glutaredoxin-dependent disulfide reductases, and is mainly involved in signaling initiation. At high concentrations, H_2O_2 may irreversibly oxidize Cys residues to sulfinic (SO_2H) or sulfonic (SO_3H) forms that cause protein damage and oxidative stress [26, 27].

We demonstrated earlier that overexpression of Ruk/CIN85 in MCF-7 cells resulted in constitutive activation of ERK1/2, Akt and Src kinases , attenuation of cell proliferation as well as alterations in redox balance [25, 28]. The abovementioned signaling pathways control both ROS-producing and ROS-scavenging mechanisms that depend on cellular context [29]. It is also known that H_2O_2 can promote the activation of Ras and in this way indirectly activate PI3K/Akt/mTOR and MAPK/Erk1/2 signaling cascades [30]. So, it could not be excluded that the same regulatory interrelations exist in 4T1 cells overexpressing adaptor protein Ruk/CIN85.

There are data that H_2O_2 may activate NF-κB signaling pathway by inducing the degreadation of NF-κB inhibitory subunit, IκBα [3, 30]. As well, H_2O_2 is able to activate other transcription factors, especially HIF-1α [31], STAT3 [32], p53 etc [33]. By activation of protein kinase C, H_2O_2 controls cell cycle, proliferation, and exocytosis [30]. Also, ROS content is increased dramatically in response to activation of a number of receptor tyrosine kinases (RTKs), such as EGFR, PDGFR etc [2, 34]. Produced in such way, H_2O_2 may oxidize Cys residues in the catalytic centers of protein phosphatases, thereby activating RTKs-dependent signaling [35]. Similar mechanism was demonstrated also for MAP kinases activation [36].

In this study we also demonstrated, that aldehydes content in Ruk/CIN85-overexpressing 4T1 cells is decreased due to activation of ALDH and FALDH. It is interesting to note that ALDH serves not only as aldehydes-eliminating enzyme, but also as a marker of breast cancer stem cells (CSCs) [37]. There are two types of CSCs: epithelial-like proliferative E-CSCs, with high expression of ALDH, low percent of $CD24^-/CD44^+$ cells, and high ROS content; and mesenchymal-like migratory M-CSCs, with low ALDH expression, high $CD24^-/CD44^+$ percent and low ROS content. So, effective antitumor

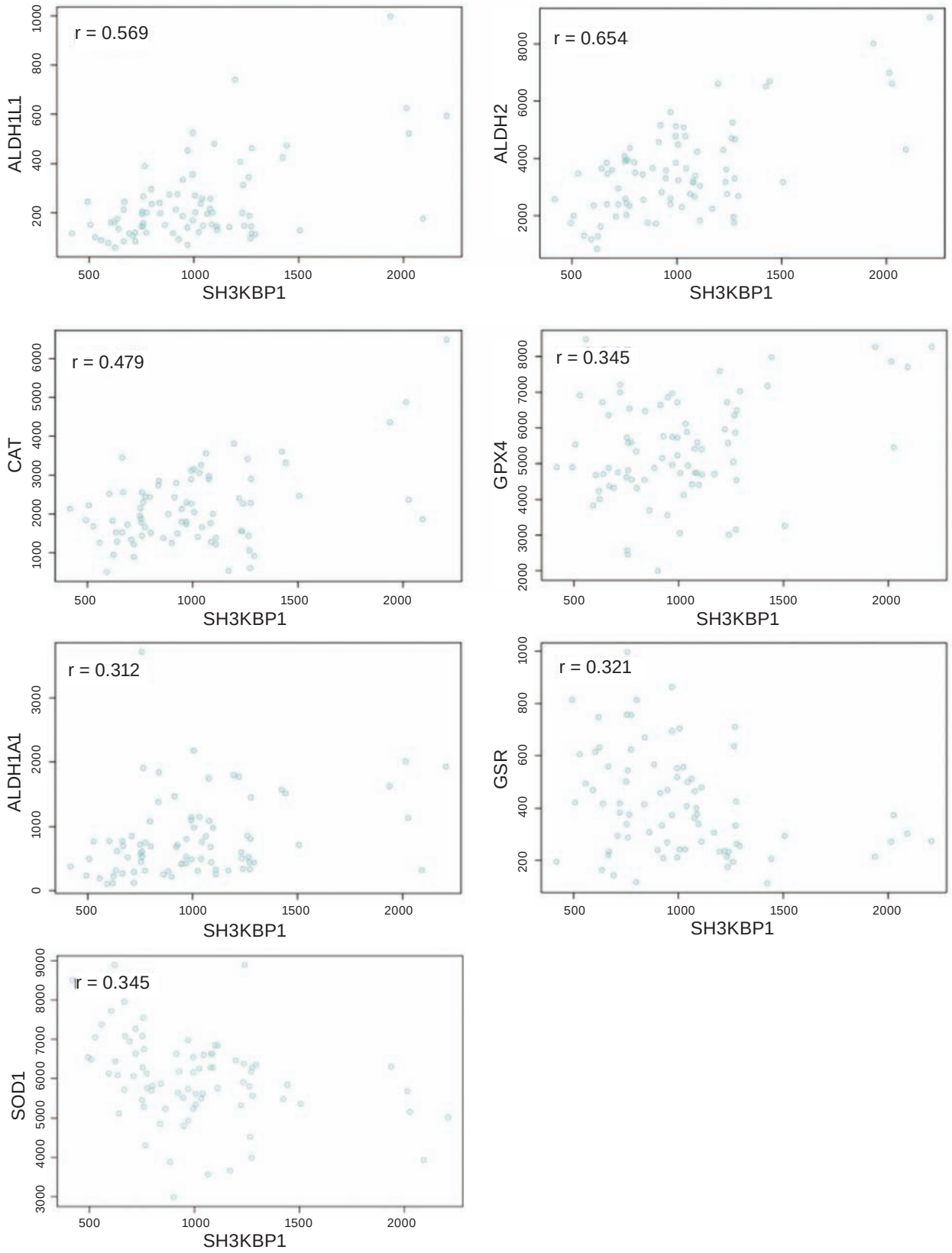

Fig. 3. Expression of adaptor protein Ruk/CIN85-encoding gene SH3KBP1 correlates with expression of genes, encoding antioxidant enzymes, in human breast lobular carcinoma samples (according to database ist. medisapiens.com). n = 80, P < 0.001

therapy should combine approaches, aimed on both E- and M-CSCs [38]. Our results showed that Ruk/CIN85-overexpressing cells have increased ALDH activity and CD44 expression [13], suggesting Ruk/CIN85 as a potential regulator of CSCs metabolic reprogramming.

Tumor cells with high ALDH expression levels possess resistance to some chemotherapeutic drugs [39] and radiation [40]. Among others ALDH isoforms, ALDH1A1 makes the largest contribution to cancer chemoresistance, including breast, lung and colon cancers [41]. In present sudy we found that SH3KBP1 gene expression in breast cancer samples correlates with expression of this ALDH isoform, which confirms our previous results about Ruk/CIN85 role in breast cancers cells' chemoresistance [13].

Finally, we analysed the correlation between *SH3KBP1* gene, that encode adaptor protein Ruk/CIN85, and antioxidant enzymes genes in breast cancer samples. It was found that *SH3KBP1* expression correlates positively with expression of *CAT, GPX4, ALDH1A1, ALDH1L1, ALDH2,* and negatively – with *GSR* and *SOD1.* Many of these genes are involved in tumor development and may be considered as markers of poor prognosis. According to Leone et al. analysis, high expression of *GPX1-7, NOX4, DUOX2, SOD3, CAT* genes is associated with poor prognosis for patients with breast cancer [42]. Together with ALDH1A1 isoform, other ALDHs are widely present in cancer cells. Namely, ALDH isoforms 1A2, 1B1, 1L1, 1L2, 2, and 3A1 are associated with tumor growth [43]. Catalase is an essential enzyme in the protecting cells from oxidative stress and together with p53 controls the transition between oxidative stress defense and apoptosis strategies [44]. As well, catalase may play a role in acquisition of resistance to pro-oxidant therapy [45]. SOD activity in cancer cells has dual role: it is critical for elimination of toxic superoxide anion, but, as a side effect, it generates H_2O_2, which may promote cancer growth, motility and angiogenesis [45]. The inconsistency between the experimental data on 4T1 cells and expression correlations in primary samples can be explained by specific Ruk/CIN85-mediated cellular context, that presumably rely on H_2O_2 production for signaling purposes, while maintaining resistance to apoptosis.

Taken together, the obtained results demonstrate that ROS signaling mechanisms may be involved in Ruk/CIN85-dependent control of breast cancer cells proliferation, motility, and resistance to chemotherapy. However, future investigations are needed in order to elucidate the networking between Ruk/CIN85 and redox balance in cancer cells, and to develop new strategies in anticancer therapy.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ АДАПТЕРНОГО ПРОТЕЇНУ Ruk/CIN85 НА ОКИСНО-ВІДНОВНИЙ ПОТЕНЦІАЛ КЛІТИН РАКУ ГРУДНОЇ ЗАЛОЗИ

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Посилене генерування активних форм кисню (АФК) може призводити до пошкодження клітинних протеїнів, ліпідів і ДНК і навіть спричинити загибель клітин. Наші попередні дослідження показали, що підвищений вміст адаптерного протеїну Ruk/CIN85 призводить до посилення злоякісності клітин раку грудної залози. Метою роботи було дослідити роль Ruk/CIN85 у підтриманні окисно-відновного балансу в ракових клітинах. Як модель було використано клітини аденокарциноми грудної залози миші лінії 4T1 із різними рівнями експресії Ruk/CIN85. Активність каталази (CAT), глутатіонпероксидази (GPx), супероксиддисмутази (SOD), альдегіддегідрогенази (ALDH) та формальдегіддегідрогенази (FALDH), а також вміст H_2O_2 та альдегідів визначали за допомогою флуорометричних тестів. Кореляції між рівнями експресії Ruk/CIN85 та антиоксидантних ензимів у зраз-

ках раку грудної залози аналізували за допомогою транскриптомної бази даних ist.medisapiens. Показано, що клітини 4T1 із надекспресією Ruk/CIN85 характеризуються підвищеним продукуванням H_2O_2 та пригніченням активності CAT, GPx і SOD. Надекспресія Ruk/CIN85 супроводжувалась зниженням вмісту альдегідів разом із підвищенням активності ALDH, тоді як у клітинах 4T1 із пригніченням експресії Ruk/CIN85 знижувалась активність ALDH та FALDH. Дані транскриптомного аналізу виявили кореляцію між експресією *SH3KBP1* і *CAT, GPX4, ALDH1A1, ALDH1L1, ALDH2, GSR, SOD1* у первинних зразках карцином молочної залози людини. Одержані результати свідчать про те, що адаптерний протеїн Ruk/CIN85 бере участь у контролі окисно-відновного балансу клітин аденокарциноми грудної залози миші лінії 4T1.

Ключові слова: рак грудної залози, адаптерний протеїн Ruk/CIN85, пероксид водню, АФК, антиоксидантні ензими, альдегіддегідрогеназа.

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