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DEPARTMENT OF CELL SIGNALING RELAY RACE OF TIME: FROM STUDYING THE STRUCTURE AND FUNCTION OF INDIVIDUAL PROTEINS TO ANALYZING PROTEIN–PROTEIN INTERACTION NETWORKS

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> Life is the mode of existence of protein bodies, the essential element of which consists in *continual metabolic interchange with the natural environment outside them*, and which ceases with the cessation of this metabolism, bringing about the decomposition of the protein.
>
> Dialectics of Nature. Frederick Engels 1883

The review presents the history of establishment, key achievements, and development prospects of the Department of Cell Signaling at the Palladin Institute of Biochemistry of the NAS of Ukraine. As a structural unit of the institution, the Department was established in 2018 through the merger of two separate subdivisions: one of the oldest, the Department of Metabolic Regulation (founded in 1944), and the youngest, the Laboratory of Cell Signaling (founded in 2006). From its inception, the department's primary research focus has been the study of the structure and functions of individual animal and microbial proteins. Significant contributions to the fundamental and applied achievements during that period were made by leaders of the Department, Dr.Sci., Professor Szörényi E.T., and Academician of NASU Huliy M.F., which were recognized with State Prizes of the USSR and the Ukrainian SSR (1953, 1978, 1988). Considerable attention has been given to transforming the department's research direction to meet modern standards in molecular cell biology, a shift logically linked to the work of the unified unit under the leadership of Dr.Sci., Professor L.B. Drobot. This transformation involved not only the formal merger of two teams but also the integration of their scientific accomplishments and intellectual potential, combining traditional and innovative research approaches. The scientific paths of both subdivisions inevitably converged, necessitating joint investigations in response to contemporary challenges and societal demands. The department's central focus became the elucidation of signaling mechanisms involved in the coordinated regulation of proliferation, differentiation, and apoptosis in normal and transformed cells with the participation of adaptor/scaffold protein Ruk/CIN85. Through the efforts of the department's researchers, the pivotal role of Ruk/CIN85 in controlling the plasticity of tumor cells of various origins was convincingly demonstrated–particularly in the development of cancer stem cell traits, chemoresistance, tumor progression, and/or differentiation.

K e y w o r d s: cell signaling, metabolism, individual animal and microbial proteins, adaptor protein Ruk/CIN85, carcinogenesis.

The Department of Cell Signaling, led by Dr. Sci., Professor, and State Prize Laureate of Ukraine in Science and Technology L.B. Drobot, is the youngest division within the Palladin Institute of Biochemistry. It was established in 2018 through the merger of two structural units: one of the oldest departments in the Institute, the Department of Metabolism Regulation, and the Laboratory of Cell Signaling, founded in 2006. This union marked the convergence of classical biochemistry principles with modern approaches in cellular and molecular biology.

Head of the Department of Cell Signaling, Dr. Sci., Professor, Liudmyla Drobot, 2016

Scientific legacy and breakthroughs in the Laboratory of Tissue Proteins

The long history of the Department of Metabolism Regulation began in 1944, when the Institute returned from Ufa, Bashkir ASSR where it had been evacuated due to the Nazi occupation of Ukraine. Upon returning to Kyiv, the Institute of Biochemistry established the Laboratory of Tissue Proteins, the activities of which were closely connected with the names of prominent scientists and their disciples. The laboratory was headed by Dr. Sci., Professor E.T. Szörényi. Despite his relatively short tenure (1944–1950), Szörényi introduced progressive ideas into biochemical research and laid the scientific and methodological foundation for the department's future work.

E.T. Szörényi graduated from the Faculty of Medicine at the University of Budapest in 1927. He became passionate about biochemistry and worked for many years in leading scientific laboratories in Switzerland and Germany. In 1931, he joined the Kaiser Wilhelm Institute for Cell Physiology in Berlin-Dahlem led by Otto Heinrich Warburg, who was awarded the Nobel Prize in Physiology or Medicine in that same year for his "discovery of the nature and mode of action of the respiratory enzyme". At that time, Warburg's assistant was Hans Krebs, who would later be awarded the 1953 Nobel Prize for his "discovery of the citric acid cycle", now known as the Krebs cycle. Thus, E. Szörényi found himself at the forefront of research in the areas of cell respiration, energetics, and metabolism. However, after the Nazi came to power in 1933, young scientists from Warburg's team were forced to emigrate to the UK and Sweden. As to Szörényi E., he moved to Kyiv, Ukraine, in 1934, accepting a personal invitation from the Director of the Institute of Biochemistry, Academician O.V. Palladin.

Emerich Teodor Szörényi, 1948

E.T. Szörényi brought to the Institute the latest knowledge, experimental approaches, and, most importantly, the Warburg apparatus for measuring cellular respiration. He mastered the technical skills required to operate the device and used it extensively in his research in Kyiv, training Institute staff in its application. Naturally, the scientific focus of Szörényi's Laboratory of Tissue Proteins centered on studying the mechanisms of cellular respiration and isolating purified proteins from muscle tissue [1-4].

After World War II, one of the main directions in global biomedical research was the study of the antibacterial properties of micromycetes, particularly the genus *Penicillium*. In 1945, the Nobel Prize in Physiology or Medicine was awarded to Alexander Fleming, Howard Walter Florey, and Ernst Boris Chain "for the discovery of penicillin and its curative effects on various infectious diseases". Around this time, researchers of the Institute of Microbiology of the Academy of Sciences of the Ukrainian SSR developed a new strain of fungi *Penicillium vitale*, which had a broad-spectrum antimicrobial activity. The compound derived from its culture medium was named "Microcid."

Further research on Microcid's properties was conducted at the Institute of Biochemistry in the Laboratory of Tissue Proteins, involving Dr. E. Szörényi, senior researcher M.F. Hulyi, who just returned from the front of World War II, and senior lab assistant R.H. Dehtyar. Using the Warburg apparatus and biochemical methods, E. Szörényi demonstrated that Microcid's antimicrobial activity was

due to the presence of the flavin-containing enzyme glucose oxidase (unpublished data). Given the significance of this discovery and its biomedical potential, the project received state funding and oversight.

E. Serenyi developed a protocol for purifying the antibiotic, which allowed for increased stability during long-term use and storage. Thanks to the expertise of M.F. Hulyi, P.D. Dvornikova, and R.H. Dehtyar in crystallizing tissue-derived proteins (aldolase, phosphofructokinase, ATP-arginase, myosin, etc.) glucose oxidase from Microcid was successfully crystallized, elevating the research to a new level.

In 1949, at the invitation of the Hungarian government, E.T. Szörényi moved to Budapest to establish the Institute of Biochemistry of the Hungarian Academy of Sciences, where he was elected a full member. Nevertheless, study on Microcid continued. E. Szörényi returned to Kyiv periodically to complete research and documentation. In 1952, for the creation of the Microcid, E.T. Szörényi, M.F. Hulyi, and microbiologists from the Institute of Microbiology M.M. Pidoplichko and V.Y. Bilai were awarded the USSR State Prize, 2nd degree. Those who worked with E.T. Szörényi in Kyiv retained deep respect for this outstanding biochemist and individual who passed on his experience to future scientists and left a lasting mark in history.

The researchers of the Laboratory of Tissue Proteins in 1950: A.H. Sabaldyr, M.F.Hulyi, P.D. Dvornikova, R.H. Dehtyar working Warburg apparatus

The Era of Maksym Fedotovych Hulyi

Starting from 1950 and for nearly four decades thereafter, the scientific and organizational management of the Laboratory of Tissue Proteins, later transformed into a department was led by Academician of the National Academy of Sciences of Ukraine M.F. Hulyi. A remarkable and unique figure, M.F. Hulyi was a pioneer of Ukrainian biochemistry, an unrivaled expert in biosynthetic and metabolic processes and a long-lived scientist centenarian (102 years old). His innovative developments consistently led to practical applications, which remained the hallmark of his scientific mission.

The contributions of M.F. Hulyi to Ukrainian biochemistry science are immeasurable. The talented young scientists he mentored achieved significant scientific success. Among them were Academician of NAS of Ukraine, Director of the Institute of Molecular Biology and Genetics of NASU (1973–2003) H.Kh. Matsuka; Academician of NAS and NAMS of Ukraine, Director of the Palladin Institute of Biochemistry of NASU since 1998 S.V. Komisarenko; Academician of NAS and NAAS, Rector of the Kyiv Agricultural University of NAAS of Ukraine (1984–2008) D.O. Melnychuk.

M.F. Hulyi was inspired by a new area, molecular biology, which in the 1950s–1960s became a focus of the international biochemical community, and established the Laboratory of Nucleic Acids within the Department. The Lab was headed by Hulyi's longtime colleague and co-author, Dr.Sci. O.P. Chepinoha.

At this time, M.F. Hulyi published two monographs on protein biosynthesis [5, 6]. In 1964, the Department of Tissue Proteins was renamed the Department of Protein Biosynthesis and Biological Properties. The Nucleic Acids Laboratory, which was later headed by Doctor of Biological Sciences G.H. Matsuka, worked successfully for almost ten years, and in 1973 was transferred as a full-fledged

M.F. Hulyi, 1975

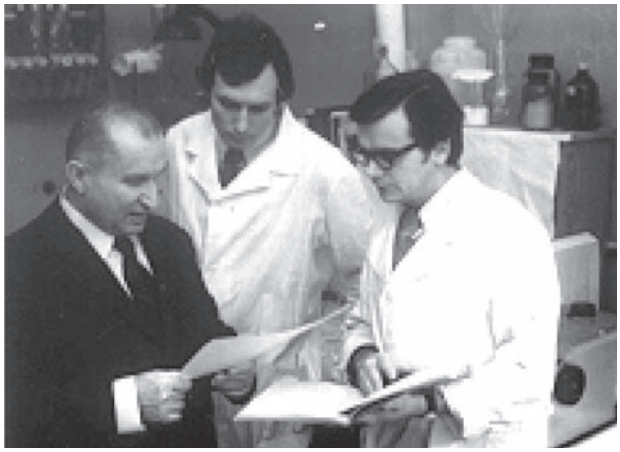
department to the newly established Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine.

At the next stage of investigation, the team led by Dr.Sci. R.H. Dehtyar (PhD L.V. Hudkova, PhD M.O. Dolhii, PhD N.V. Latyshko, PhD N.M. Mironenko) extracted from the culture fluid of *P. vitale* another protein, the enzyme catalase, and studied its structure [7]. Hulyi's team developed also a method for obtaining two industrially and medically important enzymes in a highly pure and stable crystalline form - glucose oxidase (used as an agent with antimicrobial activity, and for testing glucose and monosaccharides content in biological fluids) [8] and catalase (used in the peroxide-catalase method for producing decolorized animal blood for food industry) [9]. For these achievements, M.F. Hulyi and R.H. Dehtyar were awarded the State Prize of the USSR in Science and Technology in 1978.

During the 1970s–1980s, the Department's studies focused on the role of carbon dioxide in the energy and biosynthetic metabolism regulation in heterotrophic organisms. It was identified that protein modification through carboxylation–decarboxylation reactions is a mechanism for regulating the structural and functional properties of various enzymes.

It was shown that stimulation of carboxylation reactions activated the tricarboxylic acid cycle. This enabled the development of methods for enhancing biosynthetic processes, as well as for preventing diseases related to acid–base imbalance [10]. Based on these findings, the drug "Carboxylin" was created to improve animal productivity. Its creators, Academicians M.F. Hulyi and D.O. Melnychuk, were awarded the State Prize of the Ukrainian SSR in Sciene and Technology in 1985.

After the death in 1972 of Academician V.O. Palladin, the founder and long-time director of the Institute of Biochemistry, M.F. Huliy held the position of advisor to the Institute's directorate until the end of his life. In 2005, on the day of the 100th anniversary, in honor of his outstanding contribution to Ukrainian biochemical science, M.F. Hulyi was awarded the title of Hero of Ukraine. In two years, the heart of this outstanding scientific luminary stopped beating.



M.F. Hulyi with his graduate students S.V. Komisarenko and V.O. Mykhailovsky

Department of metabolism regulation: implementation of Hulyi's ideas and new horizons

In 1988, the Department was renamed again, this time to the Department of Metabolism Regulation, headed by Huly's students, Academician D.O. Melnychuk and PhD V.O. Mikhailovsky. Over the next 20 years, they continued to implement the visionary ideas of their mentor.

D.O. Melnychuk formulated an original concept of the metabolic system of acid–base homeostasis, focused on maintaining stable concentrations of key buffer components, H^+, CO_2, and HCO_3^-, essential for supporting the function of the overall organism. Two resulting drugs were developed and patented: a modified version of "Carboxylin" and the adaptogenic agent "Namatsyt," recognized for its antacid properties [11, 12].

Another important research direction of the Department was the study of ethanol impact on human metabolism. It was demonstrated that this compound suppresses redox processes and protein biosynthesis, whereas formate, in combination with other low-molecular-weight compounds, exhibits a corrective effect. Based on these findings, a new drug called "Medichronal" was developed by PhD N.A. Stohnii, PhD Sushkova, PhD N.V. Silonova, N.F. Shevtsova, N.M. Kasianiva [13]. The drug is still produced by the pharmaceutical company "Darnytsia" for the prevention of alcoholism, detoxification, and relief from withdrawal syndrome.

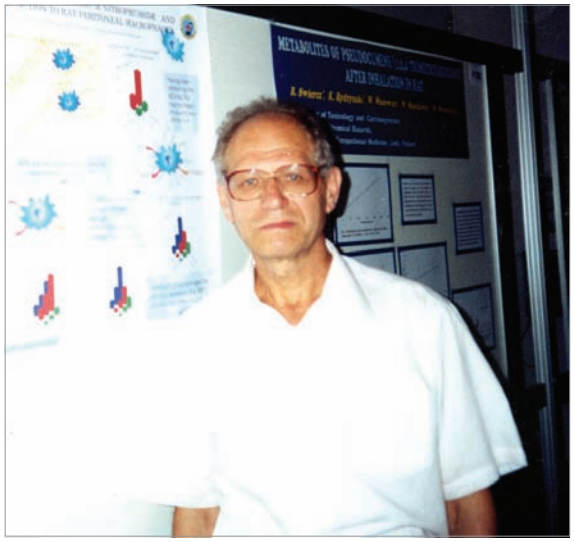
In the 1980s–1990s, PhDs T.T. Volodina and T.M. Pechenova, focused on the structural and functional state of the main component of the connective tissue matrix, collagen, under leukemia conditions. Based on the analysis of changes in type I collagen and its role in leukemogenesis, studied clinical samples and animal models, a therapeutic drug, "Correctin" [14], was developed for treating musculoskeletal complications in children with acute leukemia [15].

In 2007, the head of the Department became Dr. Sci, Professor M.P. Dmytrenko. He enthusiastically continued the traditions established by Academician M.F. Hulyi.

At this period, the studies focused on the metabolism of such low-molecular-weight highly reactive compounds as reactive oxygen and nitrogen species, as well as aldehydes, and their roles in oxidative, nitrosative, and carbonyl stresses that accompany the development of various pathological conditions and disease complications. These findings led to the development of preparation for reducing alcohol intoxication, hangover syndrome and drug addiction upon alcohol abuse [16]. Besides, the approaches were developed for the diagnostics of *Helicobacter pylori* infection [17].

In 2011 the Department was headed by PhD S.H. Shandrenko. The research study during that period focused on uncovering the interplay between oxidative, nitrosative, and carbonyl stress [18, 19]. Shandrenko's group demonstrated that oxidative deamination of biogenic amines and polyamines by amine oxidases is the intersection point in the pathways of pathological conditions development [20-22]. The studies on animal models (glycerol-induced rhabdomyolysis, streptozotocin-induced diabetes, ovalbumin-induced asthma, Lewis lung carcinoma) showed therapeutic effects of reactive compounds scavengers and amine oxidase inhibitors [22, 23].

PhD S.H. Shandrenko, 2013



Dr.Sci., Professor M.P. Dmytrenko

Shandrenko's team (I.P. Krysiuk, I.M. Panas, D.S. Heraschenko) successfully worked according to the scientific and technical program of NAS of Ukraine on the project "Implementation of sensor devices for diagnosing *Helicobacter pylori* infection, lactose intolerance, and bronchial asthma". The project resulted in the creation of Helicotester for diagnosing *Helicobacter pylori* infection, Lactosotester for detecting lactose intolerance and Astmatester for assessing asthma severity.

The integration of the scientific paths of the two subdivisions unification of scientific fields

In 2018, the Department of Metabolic Regulation and the Laboratory of Cell Signaling, which was founded within the Institute of Biochemistry in 2006, were merged into one Department of Cell Signaling. This process led not only to the formal unification of the teams but also to the integration of their scientific achievements, intellectual potential, and the convergence of traditional and innovative research directions. The study of intracellular metabolic changes, as well as signaling mechanisms involved in the regulation of proliferation, differentiation, and apoptosis of normal and malignantly transformed cells, has become the subject of research within the framework of the joint department. It is important to emphasize the societal demand for effective solutions to cancer treatment has reached a critical peak, especially in Ukraine, where the situ-

ation is further complicated by the rise of post-traumatic stress disorder under conditions of martial law.

The feature of the study in this area lies in the fact that dynamic control of sequential events in space and time is primarily mediated by protein–protein interactions and reciprocal regulation of individual subnetworks, many of which are governed by adaptor proteins. Given the critical role of adaptor proteins in cellular signal processing, it is highly plausible that dysfunctions in adaptor protein activity may contribute to carcinogenesis [24, 25].

The adaptor protein Ruk/CIN85: the beginning of a new direction of research in the field of cell signaling

Adaptor protein Ruk/CIN85, which includes three SH3 domains, Pro- and Ser-rich sequences, and a C-terminal coiled-coil region, was cloned and characterized in 2000 with the participation of L.B. Drobot within the framework of international collaboration with colleagues from the United Kingdom (Ivan Gout, Michael Waterfield from the Ludwig Institute for Cancer Research, and Vladimir Buchman from the University of St Andrews). It was identified as a protein capable of inhibiting PI3-kinase activity through interaction with its regulatory p85 subunit [26], initiating a new direction in cell signaling research.

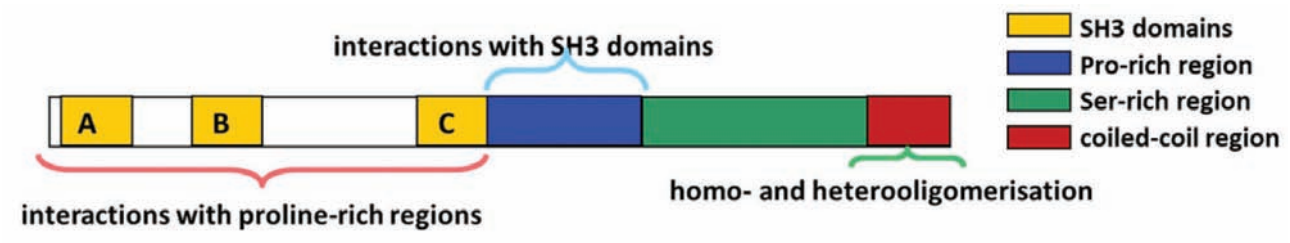
Preliminary data obtained by L. Drobot and colleagues demonstrated a significant increase in the expression of the full-length form of Ruk/CIN85 (85 kDa) in samples of primary tumors of various tissue origins [27-30]. It was hypothesized that this adaptor protein may serve as a tissue-specific marker of tumor progression, a prognostic factor, and a potential target for the development of targeted pharmacological agents.

Accumulated experimental data have confirmed the integrative role of Ruk/CIN85 in regulating actin cytoskeleton architecture, cell migration, and adhesion, apoptosis, mitogenic signaling, ligand-mediated endocytosis of receptor tyrosine kinases. Nevertheless, further investigation of the mechanisms underlying the biological activity of Ruk/CIN85 remained relevant and required new ideas and approaches.

In light of this, significant efforts by L. Drobot and her colleagues were directed at identifying new binding partners of Ruk/CIN85 and elucidating the biological roles of these interactions. Notably, it was first discovered that affinity-purified recombinant and endogenous Ruk/CIN85 proteins are capable of binding to and hydrolyzing DNA [31]. Mapping of the C-terminal coiled-coil region of the signaling protein identified it as the domain responsible for interaction with nuclease(s). In collaboration with colleagues from the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine (Prof. A.V. Rynditch) and the Nencki Institute of Experimental Biology of the Polish Academy of Sciences (Prof. Jolanta Rędowicz), it was established that the adaptor/scaffold proteins intersectin [32] and Tks4, an organizer of the NADPH oxidase complex, are among the novel binding partners of Ruk/CIN85. Mass spectrometry analysis (LC-MS/MS) of proteins interacting with the SH3 domains of Ruk/CIN85 confirmed its ability to form complexes with nucleases and Tks4 [33].

Ser/Thr-specific protein kinase D (PKD) was also identified as a novel binding partner of Ruk/CIN85. This kinase not only interacted with Ruk/CIN85 but also phosphorylated it, thereby regulating the functional activity of the adaptor protein [34]. The research findings suggest a potential functional role for the Ruk/CIN85–PKD complex in the regulation of cell migration and invasion.

Intratumoral hypoxia is a driving force in the progression of cancer, playing a key role in metastasis and angiogenesis. At the molecular level, transcription factors stimulated by hypoxia, known as hypoxia-inducible factors (HIFs), are crucial for cellular adaptation to low oxygen conditions. Intracellu-



Schematic structure of the adaptor/scaffold protein Ruk/CIN85 [26]

lar hypoxia serves as a major trigger for the expression of plasminogen activator inhibitor-1 (PAI-1). Further studies of the signaling networks regulating PAI-1 expression, conducted under the supervision of Senior Research Fellow A.A. Samoylenko, revealed that the transcription factor HIF-1α and the hypoxia response element (HRE) in the PAI-1 promoter mediate insulin-induced stimulation of this gene expression [35]. Studies by L. Drobot, A. Samoylenko and PhD student N. Kozlova provide the first evidence that transient or stable overexpression of Ruk/CIN85 prevented proline hydroxylation-dependent destabilization of the HIF-1α protein while induced PAI-1 expression [36]. Moreover, neither an increase in HIF-1α levels nor enhanced PAI-1 expression was observed following RNA interference-mediated suppression of elevated Ruk/CIN85 expression [37]. Given that both PAI-1 and HIF-1α are components of regulatory networks involved in cancer development, it was hypothesized that up-regulation of Ruk/CIN85 contributes to malignant transformation of cells. Based on these findings, a model was proposed for the regulation of PAI-1 expression under the influence of hypoxia, hydrogen peroxide, insulin, and Ruk/CIN85 [35].

In collaboration with the University of Oulu, Finland (Prof. Thomas Kietzmann), researchers identified Ruk/CIN85 as a direct inhibitory binding partner of prolyl hydroxylase 2 (PHD2), leading to stabilization of the transcription factor HIF-1α and HIF-1α-dependent enhancement of malignancy in breast adenocarcinoma cells [38]. These experimental findings significantly contributed to the broader understanding of hypoxia-independent molecular mechanisms regulating HIF-1α activity and HIF-1α-dependent signaling.

Stable transfectants/infectants of human breast adenocarcinoma cell line MCF-7, mouse breast adenocarcinoma cell line 4T1, human lung adenocarcinoma cell line A549, and rat pheochromocytoma cell line PC12 with up/down regulation of recombinant forms of Ruk/CIN85 were developed and characterized in the laboratory. Sub-lines of PC12 cells with tetracycline-inducible expression of full-length and intermediate forms of Ruk/CIN85 were also established by PhD N. Byts. The experimental workflow incorporated gene silencing of Ruk/CIN85 using shRNA (small hairpin RNA) and gene expression analysis via quantitative PCR.

Laboratory staff under the leadership of Prof. L. Drobot demonstrated that human breast adenocarcinoma MCF-7 cells with stable overexpression of the adaptor protein Ruk/CIN85 acquired a fibroblast-like morphology, exhibited reduced growth intensity and adhesiveness, and showed increased survival, colony-forming ability, and motility. The observed changes in the biological properties of MCF-7 cells overexpressing Ruk/CIN85 correlated with alterations in both the dynamics and intensity of Erk1/2 and Akt-dependent signaling induced by epidermal growth factor [39].

Recent studies have revealed that tumor/cancer stem cells (CSCs), due to their relative resistance to radiation and cytotoxic drugs, contribute to the development of therapeutic resistance and disease recurrence [40]. It is worth noting that the current literature lacks data on the contribution of adaptor proteins to the regulation of CSCs biological properties.

Experimental studies conducted by us on human MCF-7 and murine 4T1 breast adenocarcinoma cell models with stable Ruk/CIN85 overexpression confirmed for the first time the involvement of this adaptor in the development of functional and molecular CSCs' traits, such as reduced proliferative activity, mammosphere formation, increased $CD44^+/CD24^-$ cell populations, resistance to drugs including doxorubicin, cisplatin, tamoxifen, and etoposide, as well as elevated activity of the detoxification enzyme ALDH and ATP-binding cassette membrane transporters. Cell technologies developed in our laboratory are capable of maintaining CSCs activity *in vitro* over extended periods. That is why these technologies can be recommended for implementation by biotechnology companies to evaluate the efficacy of novel anticancer drugs and for large-scale screening of small molecules and siRNA libraries aimed at eliminating cancer stem cells [41-44].

The existing data suggest that H_2O_2-signaling, in terms of its mechanisms and biological significance, is comparable to the major paradigms established for the well-studied signaling mechanism of post-translational protein modification via phosphorylation [45]. Therefore, the laboratory set out to investigate the role of the adaptor protein Ruk/CIN85 in regulating the functional activity of NADPH oxidases and redox-dependent signaling in tumor cells. As a result of comprehensive research, it was established for the first time that the production of reactive oxygen species (ROS) by tumor cells, mediated by the activity of membrane-bound NADPH oxidase complexes, positively correlated with Ruk/CIN85

expression level. A mammalian plasmid vector encoding an intracellular hydrogen peroxide biosensor, HyPer, fused with Ruk/CIN85 in mammalian cells was constructed, designated Ruk/CIN85-HyPer-N1 [46]. Using live-cell fluorescence microscopy, it was demonstrated that Ruk/CIN85 content and H_2O_2 generation are localized within dot-like vesicular structures in human breast adenocarcinoma MCF-7 cells. Furthermore, it was shown that elevated ROS production in MCF-7 cells overexpressing the adaptor protein correlates with differential systemic changes in NOX genes expression [47]. Experimental data obtained showed that pretreatment of MCF-7 cells overexpressing Ruk/CIN85 with apocynin (an inhibitor of NADPH oxidase complex assembly) and NAC led to a change in the long-term activation of Akt kinase induced by EGF to a temporal dynamics. This is accompanied by enhanced autophosphorylation of the EGF receptor and inhibition of cell migration [48]. The experimental data obtained allowed us to conclude that there is regulatory relationship between Ruk/CIN85 expression in tumor cells, the intensity and compartmentalization of ROS production, the dynamics of redox-dependent signaling and the biological responses of tumor cells highlighting their critical importance for the development of novel redox-related pharmacological agents.

A new stage of research: the role of Ruk/CIN85 in nuclear and metabolic reprogramming of tumor cells

It is now well established that the development of a more aggressive tumor phenotype is initiated by the reprogramming of tumor cells during the reverse process of epithelial-to-mesenchymal-to-amoeboid transition (EMT-MAT). The ability of tumor cells to undergo reciprocal metabolic and nuclear reprogramming/transdifferentiation during EMT-MAT/AMT-MET without the need for additional genetic changes has become known as epithelial-mesenchymal plasticity (EMP) [49-57]. Recent studies conducted by scientists of the department have demonstrated for the first time that the adaptor protein Ruk/CIN85 is one of the key regulators of EMP in breast adenocarcinoma cells, depending on the level of its expression. Specifically, it was found that overexpression of Ruk/CIN85 in murine 4T1 breast adenocarcinoma cells led to suppressed proliferative activity, reduced adhesiveness, enhanced anchorage-independent growth, increased motility, invasiveness, chemoresistance, and the emergence of cancer stem cell (CSCs) traits. Conversely, down-regulation of Ruk/CIN85 in 4T1 cells resulted in a loss of plasticity due to induced differentiation and the formation of a stable epithelial phenotype. It was also revealed that overexpression of Ruk/CIN85 in 4T1 cells increased mRNA levels of several key transcription factors – "master regulators of EMP" – such as Snai1, Slug, Zeb1, Zeb2, and Twist1, which promote mesenchymal and suppress epithelial markers. Down-regulation of Ruk/CIN85 produced the opposite effect. The expression levels of transcription factors Klf17, Myb, Stat3, Nrf2, transcriptional/splicing regulator Yb1, epithelial marker Cdh1, mesenchymal marker Vimentin, cell adhesion regulators, CSCs markers, reprogramming genes, extracellular matrix-degrading enzymes, tight junction proteins, a set of cytokines as well as the components of plasminogen activation system in 4T1 cells with up/down regulation of Ruk/CIN85 were estimated and analyzed. It was also shown that overexpression of Ruk/CIN85 in murine (4T1) and human (MCF-7) breast adenocarcinoma cells disrupted mRNA synthesis of key components of the vitamin D_3 auto/paracrine system (CYP27B1, CYP24A1, VDR, and RXR). Meanwhile, down-regulation of Ruk/CIN85 restored the balance between components of the receptor complex responsible for intracellular vitamin D_3 signaling resulting in equilibrium between calcitriol synthesis and its catabolism. For the first time, it was shown that elevated expression and activity of MMP-2 and MMP-9 in 4T1 cells with down-regulated Ruk/CIN85 correlated with increased production of angiostatins and reduced invasive potential.

The merger of the two departments allowed us to expand the scope of research and conduct systematic metabolomic analysis. This included evaluating the activity/expression levels of key enzymes and components of glycolysis (glucose transporter Glut1, glucokinase, aldolase, lactate dehydrogenase, and lactate content), oxidative phosphorylation (NAD-dependent isocitrate dehydrogenase and malate dehydrogenase), enzymes involved in extracellular matrix remodeling (lysyl oxidase), regulators of invasive potential (semicarbazide-sensitive amine oxidase), redox state regulators (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, NAD^+/NADH ratio, and H_2O_2 content), and mitochondrial membrane potential in 4T1 and MCF-7 cell models with up- and down-regulated Ruk/CIN85 expression. The observed metabolic shifts in breast adenocarcinoma cells revealed for

the first time the involvement of the adaptor protein Ruk/CIN85 in regulating the Warburg effect. The results of these studies led to the conclusion that Ruk/CIN85 functions as a concentration-dependent switch of transcriptional and metabolic programs involved in controlling EMP in murine 4T1 breast adenocarcinoma cells.

An exceptionally important phase of the department's experimental research was the implementation of *in vivo* experiments to validate the results obtained *in vitro.* A murine model was introduced to analyze both experimental and spontaneous metastasis *in vivo.* It has been demonstrated that overexpression of the adaptor protein Ruk/CIN85 in 4T1 cells enhances their ability to extravasate and metastasize to the lung, whereas down-regulation of Ruk/CIN85 results in an almost complete inhibition of these processes. It was further established that in pulmonary metastases, Ruk/CIN85 induces a mesenchymal-to-epithelial transition (MET), which is associated with increased proliferative activity of tumor cells. The identified regulatory patterns were also confirmed in human and mouse lung adenocarcinoma cell models [58-61].

Extracellular vesicles and the regulatory role of Ruk/CIN85 in tumor cell communication and carcinogenesis

In recent years, it has become increasingly evident that one of the primary mechanisms of intercellular communication involves the transfer of signaling molecules via extracellular vesicles (EVs), which are formed through exocytosis. EVs serve as a valuable source of information about numerous cellular processes and dysfunctions under both normal and pathological conditions, including cancer. They offer potential as a non-invasive clinical resource for differential diagnostics and the development of personalized treatment protocols.

According to published data, EVs marker proteins Alix and Tsg101, as well as cortactin, are binding partners of the adaptor protein Ruk/CIN85. Based on this information, we isolated and characterized EVs produced by murine renal carcinoma Renca cells under normoxic and hypoxic conditions, depending on Ruk/CIN85 expression levels. It was found that the concentration of EVs produced by Renca cells overexpressing Ruk/CIN85 was significantly higher under hypoxic conditions compared to normoxia. Under normoxia, the content of Ruk/CIN85 and EVs markers Alix and CD81 increased in vesicles from RukUp cells compared to control cells. In contrast, under hypoxia, the levels of these markers in EVs from Renca-RukUp cells decreased by more than two orders of magnitude [62]. Thus, we demonstrated that Ruk/CIN85 is a novel component of EVs produced by tumor cells and may play a regulatory role in controlling EV composition under normoxic and hypoxic conditions. Moreover, we confirmed that Ruk/CIN85 is not only a constitutive component of the protein cargo of tumor-derived EVs but also plays an active role in regulating carcinogenesis, depending on its content within EVs. [63].

Targeted anticancer therapy

Among pharmacologically active plant-derived compounds traditionally used in medicine, alkaloids deserve special attention due to their general therapeutic effects and notable anticancer properties.

Under the leadership of Prof. L. Drobot and PhD I. Horak, in collaboration with Chinese colleagues, the department researchers evaluated the effects of the alkaloid berberine on molecular markers of EMT and CSCs in breast cancer models, specifically in triple-negative human adenocarcinoma MDA-MB-231 cells and estrogen-sensitive human adenocarcinoma MCF-7 cells. For the first time, it was demonstrated that berberine suppressed tumor cell proliferation, migration, and invasion via the Wnt/β-catenin signaling pathway, inhibited EMT by reducing N-cadherin and increasing E-cadherin levels, and induced apoptosis through both intrinsic (caspase-9) and extrinsic (caspase-8) pathways. These mechanisms confirmed the therapeutic potential of berberine in combinatorial therapy for human breast cancer [64].

However, the anticancer effect of berberine has several limitations for active clinical use, prompting researchers to explore strategies to enhance its efficacy. This challenge was addressed through a long-term research initiative titled "Innovative Nanobiotechnologies for Early Diagnosis and Chemotherapy of Pathological Conditions," led by Prof. L.B. Drobot and Prof. O.P. Matyshevska, for which the authors were awarded the State Prize of Ukraine in Science and Technology in 2020. This research cycle demonstrated the effectiveness of the carbon nanostructure fullerene C_{60} as a nanoplatform for targeted drug delivery. Consequently, a decision was made to use C_{60} for the targeted delivery of alkaloids in anticancer therapy. In collaboration with colleagues from

the Kyiv Institute of Biology and Medicine at Taras Shevchenko National University, molecular design of C_{60} nanocomplexes with berberine and piperlongumine was carried out, and their anticancer activity against Lewis lung carcinoma was confirmed both *in vivo* and *in vitro*. Unexpectedly, Western blot analysis revealed that the studied alkaloids could reduce Ruk/CIN85 expression levels in LLC cells [65-67]. The high citation index of these publications in Scopus reflects the broad resonance of the research findings, strong interest from the scientific community, and the urgent societal demand for new low-toxicity agents for cancer treatment at low doses.

Thus, based on years of research by the Department of Cell Signaling, the key role of the adaptor protein Ruk/CIN85 in regulating epithelial-mesenchymal plasticity has been unequivocally demonstrated. The achievements of the Department of Cell Signaling demonstrate the continuity of generations of scientists who pass on accumulated knowledge and experience, ensuring continuous scientific progress.

Conflct 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.

«ЕСТАФЕТА ЧАСУ: ВІД ВИВЧЕННЯ СТРУКТУРИ І ФУНКЦІЙ ОКРЕМИХ ПРОТЕЇНІВ ДО АНАЛІЗУ МЕРЕЖ ПРОТЕЇНО-ПРОТЕЇНОВИХ ВЗАЄМОДІЙ»

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В огляді представлено історію створення, основні здобутки та перспективи розвитку відділу сигнальних механізмів клітини Інституту біохімії ім. О. В. Палладіна НАН України. Як підрозділ установи, відділ створено у 2018 році з двох окремих структурних одиниць, однієї з найстаріших, відділу регуляції обміну речовин (1944 рік заснування), та наймолодшої, лабораторії сигнальних механізмів клітини (2006 рік заснування). Основним напрямком досліджень відділу від початку заснування стало вивчення структури та функцій окремих протеїнів тваринного та мікробіологічного походження. Визначний внесок до фундаментальних і прикладних досягнень підрозділу того періоду було зроблено його керівниками, д.б.н., професором Е.Т. Сорені та академіком НАН України М.Ф. Гулим, що увінчалось присудженням Державних премій СРСР та УРСР (1953 р., 1978 р., 1988 р). Значну увагу приділено трансформуванню напрямку досліджень відділу до сучасного рівня в галузі молекулярної клітинної біології, що логічно було пов'язано з роботою об'єднаного підрозділу під керівництвом д.б.н., професора Л.Б. Дробот. Відбулось не тільки формальне об'єднання двох колективів, але й поєднання їх наукових досягнень та інтелектуального потенціалу, традиційного та новаторського напрямів досліджень, оскільки науково-пошукові шляхи обох підрозділів неминуче перетнулись й стали потребувати сумісних досліджень відповідно до викликів часу та соціального запиту. В центрі уваги стало з'ясування сигнальних механізмів, залучених до узгодженого контролю процесів проліферації, диференціювання та апоптозу нормальних і трансформованих клітин за участі адаптерного/риштувального протеїну Ruk/CIN85. Завдяки зусиллям співробітників відділу переконливо продемонстровано ключову роль Ruk/CIN85 у контролі пластичності пухлинних клітин різного ґенезу, зокрема у розвитку ознак ракових стовбурових клітин, хіміорезистентності, прогресії пухлинного росту та/або диференціювання.

Ключові слова: внутрішньоклітинне сигналювання, метаболізм, протеїни тваринного та мікробіологічного походження, адаптерний протеїн Ruk/CIN85, канцерогенез.

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