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ADAPTOR PROTEIN CIN85 POTENTIATES THE MOTILITY OF OSTEOSARCOMA CELLS

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Osteosarcoma (OSA) is the most common primary malignant bone tumor in children and adolescents, characterized by high metastatic potential and poor prognosis. The adaptor protein CIN85 is known to be upregulated in various cancers and is involved in cell motility and invasion.

Aim. This study was purposed to investigate the role of CIN85 in the migration of osteosarcoma cells.

Methods. *In vitro*, scratch assay, xCELLigence, and Transwell assay were used to evaluate cell migration, while RNA-seq, qPCR, and Western blotting assessed gene expression.

Results. Public datasets revealed elevated CIN85/*SH3KBP1* expression in OSA tissues compared to normal bone, with even higher levels observed in metastases. Functional studies using CIN85-overexpressing and CIN85-silenced HOS and SAOS-2 osteosarcoma cells demonstrated that CIN85 promotes OSA cell migration and invasion in both 2D and 3D models. RNA-seq analysis identified differentially expressed genes and enriched pathways related to migration, extracellular matrix, adhesion, and cell signaling. CIN85-driven motility was shown to depend on the expression of *COL3A1* and *MMP2*, Akt/mTOR signaling, and NOX activity.

Conclusion. These findings support CIN85 as a potential biomarker and therapeutic target in osteosarcoma.

Keywords: osteosarcoma, migration, metastasis, CIN85.

Osteosarcoma (OSA) represents the most prevalent primary malignant tumor of the bone, primarily affecting children and adolescents. It accounts for approximately 8.9% of all cancer-related deaths in the pediatric population. The disease most frequently arises in long bones such as the femur, tibia, and humerus, while less commonly observed in the skull, mandible, pelvis, and ribs. One of the most concerning aspects of OSA is its aggressive metastatic behavior, particularly to the lungs and, less frequently, to other bones. At the time of diagnosis, around 20% of patients already present with metastases, which significantly worsens prognosis. The 5-year survival rate for patients with metastatic OSA remains low — approximately 30%. Current treatment regimens typically involve surgical removal of the tumor in combination with multi-agent chemotherapy, which includes drugs like methotrexate, doxorubicin, and

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cisplatin. In some cases, immunotherapy is also being explored as an adjunct treatment. Despite these efforts, outcomes remain unsatisfactory, particularly for those with metastatic disease, making the identification of reliable prognostic biomarkers essential for advancing personalized therapeutic approaches [1].

High-grade OSA is characterized by a variety of genetic mutations and disruptions in several cellular signaling and metabolic pathways that control proliferation, survival, and motility. Among the most commonly observed molecular alterations are mutations in the tumor suppressor gene TP53, along with enhanced activity in pathways such as IGF1R, PI3K/Akt, and MAPK. Additionally, OSA cells exhibit metabolic reprogramming regulated by signaling cascades, including PI3K/Akt/mTOR, HIF-1, Wnt, Hippo, NF-κB, and MAPK, which contribute to the aggressive nature of the disease [1, 2].

One protein of emerging interest in cancer biology is CIN85, an adaptor protein encoded by the SH3KBP1 gene and a member of the CIN85/CMS family. CIN85 has been found to be upregulated in multiple tumor types, such as breast, prostate, cervical, colon, and esophageal cancers, as well as gliomas. Its overexpression is frequently associated with poor patient outcomes. CIN85 is known to participate in processes such as vesicle trafficking, cell adhesion, motility, and invasion. It exerts its effects through interaction with a wide range of signaling proteins (according to the BioGRID database, there are about 150 binding partners identified). In cancerous and normal cells, CIN85 is involved in multiple signaling pathways, including HIF1α, TGFβ, Src, EGFR, MAPK, PI3K-Akt, and TNF [3, 4], and in this manner affects cell behavior and gene expression.

Given these diverse roles, CIN85 is considered a significant contributor to tumor progression and a potential prognostic biomarker. Nevertheless, little is known about the specific function of CIN85 in non-epithelial tumors like osteosarcoma. Consequently, the current study aimed to examine CIN85 expression levels in OSA and to explore its functional relevance, particularly regarding the migratory behavior of osteosarcoma cells.

Methods. Public databases (TNMplot, GEO, Human Protein Atlas) were used to analyze *SH3KBP1* expression in OSA tissues and cell lines. HOS and SAOS-2 cells were transfected with pRc/CMV2-Rukl plasmid (upCIN85) or *SH3KBP1*-targeting siRNA (siCIN85). Migration was assessed using an xCELLigence real-time system, scratch assay, and collagen-embedded spheroids. The invasion was evaluated via collagen Transwell assay. The CEITEC facility at Masaryk University performed RNA-seq and DEG analysis. Statistical analysis was conducted using Origin; $P < 0.05$ was considered significant.

Results and Discussion. SH3KBP1 expression was significantly elevated in OSA vs. normal bone (TNMplot) and higher in metastases than in primary tumors (GEO datasets GSE220538, GSE237033, GSE87624, GSE32981). It was shown that a high level of CIN85 expression was associated with enhanced OSA cell motility in 2D and 3D assays.

Transcriptomic analysis identified hundreds of differentially expressed genes dependent on CIN85. Eight genes (*HCLS1, PLPP2, ETS1, ESM1, DHRS2, ARHGDIB, ADAM19, PHLDA2*) were inversely regulated between upCIN85 and siCIN85 cells. Enrichment analysis revealed involvement in proliferation, apoptosis, adhesion, ECM remodeling, and migration. Key pathways included NF-κB, TNF, MAPK, Wnt, PI3K/Akt/mTOR, TGF, and VEGF.

Functional assays confirmed that CIN85-driven migration involves the expression of *COL3A1* and *MMP2* genes, Akt/mTOR signaling, and NADPH oxidase (NOX) activity.

This study highlights the adaptor protein CIN85 as a key regulator of osteosarcoma (OSA) cell migration and collagen invasion. Elevated expression of CIN85 has previously been observed in several cancers and is frequently linked to poor clinical outcomes. Consistent with these findings, our analysis shows that CIN85 is significantly upregulated in osteosarcoma tissues compared to normal bone and in metastasis compared to primary tumors. Furthermore, CIN85 upregulation leads to increased migration and invasiveness of osteosarcoma cells. The main highlights of this study are shown in Fig. 1.

Conclusion. Our results demonstrate that CIN85 is overexpressed in osteosarcoma and is associated with increased metastatic potential. Functional studies confirmed that CIN85 promotes cell migration and invasion in 2D and 3D conditions. Notably, CIN85-induced motility depends on *COL3A1* and *MMP2* expression, as well as on activation of the Akt/mTOR signaling and NOX. These findings provide a basis for further investigation of CIN85 as a potential prognostic marker and therapeutic target in OSA.

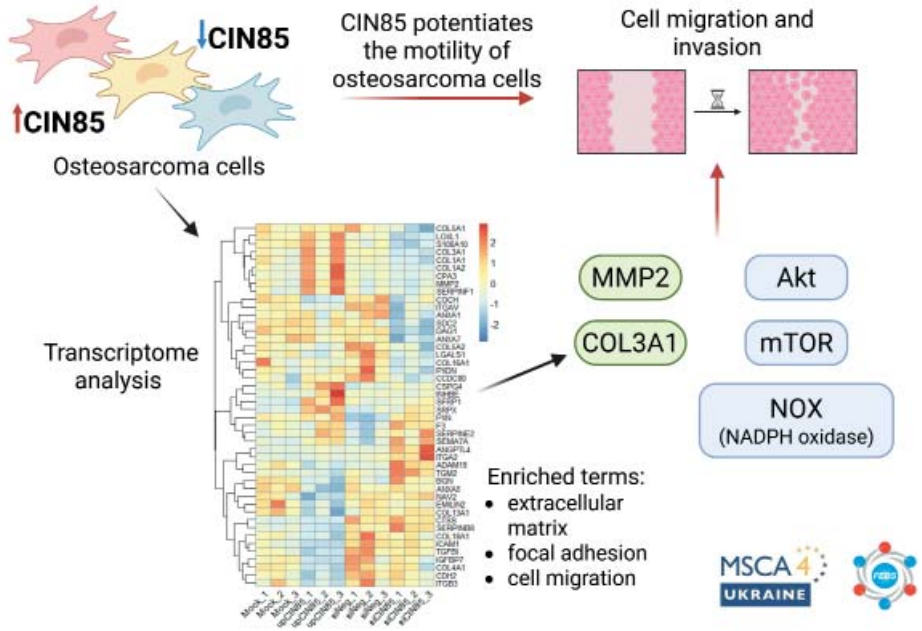

Fig. 1. **Adaptor protein CIN85 potentiated the motility of osteosarcoma cells**
Image created with BioRender [https://www.biorender.com]

Authors' contribution

IH performed migration/invasion assays, RNA isolation, transcriptomic analysis, and statistical analysis. TS studied the effect of Apocynin on cell motility. MP and JN contributed to 3D spheroid assays. IH, LK, PB, JS, and LB conceptualized the study. All authors contributed to manuscript preparation and revision.

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