Role of Tks4 scaffold protein in normal and tumor cell signaling

Theses of doctoral dissertation

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Introduction

The epithelial-mesenchymal transition (EMT) is a process that involves cells losing their epithelial phenotypic properties and acquiring mesenchymal characteristics. Three types of EMTs are known, the first type occurs during embryonic development (implantation, gastrulation, neural tube formation), the second type appears during the process of wound healing and tissue regeneration, and the third type is associated with tumor progression and metastasis [1]. Recently, the concept of a hybrid EMT, also known as partial EMT has emerged, meaning that cells co-express epithelial and mesenchymal markers. This is a form of plasticity, in which the cells oscillate freely on the epithelial-mesenchymal axis, i.e. they are able to change from one state to the other: epithelial to mesenchymal, or mesenchymal to epithelial (the latter process is called MET)[1–4]. In my work, I focused on EMT in embryonic development and EMT-like processes in tumor tissues.

The research on the Tks4 protein (gene name: SH3PXD2B) has been focused on Frank-Ter Haar syndrome (FTHS). FTHS is a rare congenital developmental disorder caused by a mutation affecting both alleles of the Tks4 gene [5]. The symptoms of the involved children include skull and bone deformities (curved and shortened bones, dental abnormalities), as well as eye disorders (glaucoma, protruding eyes), heart problems and fat differentiation problems [5–8]. The molecular processes behind this monogenic disease can be investigated using the already available Tks4-KO mouse models (our group has also generated a Tks4-KO mouse strain for this purpose [6]), however the *in vitro* human stem cell-based model, which is also a very useful tool, is not yet available. Our research group was also interested in the emerging idea that Tks4 may serve as a potential biomarker in certain cancer types, which may be related to its function in EMT and invadopodia formation [9–14].

Aims

The aim of my PhD thesis was to elucidate how the Tks4 scaffold protein regulates signaling in normal and tumor cells, and to explore the role of Tks4 in EMT, which is essential in both embryonic development and tumor biology (Figure 1).

- Generating a Tks4-deficient embryonic stem cell model to unravel the role of Tks4 protein in human embryonic development and the molecular mechanisms of FTHS.
- Investigating the role of Tks4 scaffold protein in tumor cells using a Tks4-deficient *in vitro* lung tumor model.
- Studying the EMT process in Tks4-KO cell lines in normal and lung tumor cells.

- Exploring the molecular network mediated by Tks4 in order to uncover previously unknown Tks4 partner proteins and better understand the mechanism underlying EMT.
- Investigating the expression levels of Tks4 in lung tumor tissues to explore the possibility of using Tks4 as a biomarker.

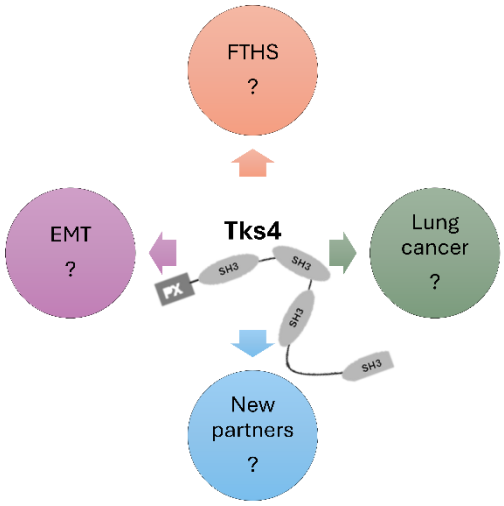

Figure 1: A comprehensive overview of this Tks4-centered study.

Materials and methods

In vitro and *in silico* methods were used to investigate the role of Tks4 in EMT. We generated a Tks4-deficient HUES9 embryonic stem cell line and A549 lung adenocarcinoma cell line using CRISPR-Cas9. The HUES9 cells were spontaneously differentiated via embryoid body (EB) formation in the mesenchymal stem cell direction at the Human Pluripotent Stem Cell Laboratory of TTK with the help of Dr. Ágota Apáti's group to investigate the differentiation abilities of Tks4-KO cells. The formation of invadopodia in A549 human lung adenocarcinoma cells was assessed by F-actin and cortactin staining, EMT marker levels were measured by RT-qPCR, Western blot and immunocytochemistry. Tks4 mRNA levels in human lung tumor samples were examined by bioinformatic analysis (GEPIA2) and TissueScan cDNA array (RT-qPCR). Tks4 interactome was identified by Tks4-IP mass spectrometry in different tumor cells (MCF7- breast cancer, HPAC- pancreatic cancer, N87- gastric cancer, HCT116- colon cancer and A549- lung cancer). Based on the Tks4-IP-MS results, I have identified a previously unknown Tks4 partner protein CAPZA1 (F-actin-capping protein subunit alpha-1), explored its localization and interaction with Tks4 by Duolink proximity ligation assay in three lung cancer cell lines (NCI-H460, HOP-92, A549). We also predicted the possible binding site of CAPZA1 to Tks4 with the collaboration of Dr. Rita Pancsa. Afterwards, based on network analysis,

literature data and my own experimental data, I mapped the Tks4 interactome, and those partners which are involved in EMT were investigated by Tks4-IP-Western blot in HUES9, A549, NCI-H460, HOP-92 cells.

Results

- We have produced two homozygous Tks4-KO clones and two heterozygous Tks4-KO clones from the normal karyotype HUES9 embryonic pluripotent stem cell line (with the collaboration of the Stem Cell Laboratory). Then, we tested the stem cell differentiation potential of Tks4-KO cells and found that they were able to differentiate into all three germline directions (mesoderm, endoderm, ectoderm), similarly to the original WT HUES9 cells.
- With the collaboration of the Stem Cell Laboratory, we have differentiated mesenchymal stem cells from the HUES9 embryonic pluripotent stem cell line, and we have examined the levels of various differentiation markers. Our results showed reduced levels of E-cadherin and Snai1 protein and lower expression of transcription factors involved in bone tissue formation (GATA4, GSC) in Tks4-KO cells.
- I have generated two homozygous Tks4-KO clones from A549 lung adenocarcinoma cells. I have observed that the Tks4-KO A549 clones exhibit an altered morphology with a more elongated phenotype, which I confirmed by calculating the form factor of F-actin stained cells (a value close to 1 indicates a more rounded shape [15]).
- I have shown that the number of invadopodia structures in Tks4-KO A549 cells is decreased compared to wild-type cells through cortactin and F-actin colocalization measurements. Furthermore, the results of the wound healing assay showed a slight decrease in the migratory ability of Tks4-KO cells compared to wild-type cells.
- I have examined the effect of Tks4 knockdown on the EMT process, where observed that the gene expression profile of EMT markers was altered: the expression levels of fibronectin, Snai2, N-cadherin were increased in both Tks4-KO clones, while the expression of E-cadherin was decreased. In addition, one of the Tks4-KO clones showed increased mRNA levels of the transcription factors Snai1 and Twist. Vimentin and fibronectin also showed an increased expression in Tks4-KO cells compared to wild-type cells based on ICC and WB measurements. No significant differences were observed in the protein expression levels of other EMT markers, such as N-cadherin, in Tks4-KO cells detected by Western blot and ICC. Overall, in the absence of Tks4, lung

adenocarcinoma cells were in an EMT-like state and showed a hybrid epithelial-mesenchymal phenotype.

- I have investigated the potential use of Tks4 as a biomarker by database analysis (GEPIA2), where a significant decrease in Tks4 mRNA expression was detected in lung tumor tissues compared to normal lung tissues.
- Similarly, in the lung tumor cDNA array from patients, I have also measured the Tks4 mRNA levels and confirmed the results of the database analysis, as Tks4 expression was reduced in lung cancer samples compared to normal samples. It was observed that reduced levels of Tks4 mRNA correlate with poorer survival in lung cancer patients.
- I have identified new partner proteins in the Tks4-IP-MS analysis from which I focused on CAPZA1, since CAPZA1 was present in all five analyzed cell lines as a potential Tks4 partner molecule.
- To confirm the interaction of Tks4 and CAPZA1, I have examined its localization in three lung cancer cell lines (A549, HOP-92 and NCI-H460), which showed that both Tks4 and CAPZA1 are present in the submembrane structures and cytoplasm of the cells. We also identified a short linear motif within Tks4 (between 636-654), that could be considered as a potential "capping protein interacting (CPI) motif" to which CAPZA1 could bind.
- In addition, I have also performed a proximity ligation assay (PLA) to confirm the interaction of the two proteins in cells (Tks4 and CAPZA1), demonstrating the close proximity and binding of the two proteins in the cytoplasm in all three lung cancer cell lines.
- I have investigated the effect of low oxygen concentration condition on the EMT-like phenotype in A549 lung cancer cells and found that Tks4-KO cell lines exhibited a more pronounced EMT-like state than wild-type cells. We have also detected a faster response to EMT-inducing low oxygen environment: Snai2 and Twist mRNA levels showed a more significant increase, and E-cadherin mRNA level showed a decrease in Tks4-KO clones. Furthermore, we detected decreased CAPZA1 levels in Tks4-deficient cells under low oxygen conditions.
- I have also studied the interaction network of Tks4 in HUES9 embryonic stem cells and lung tumor cells (A549, HOP-92, NCI-H460). I generated a Tks4-based protein interaction network with the STRING database incorporating our experimental data, from which I selected the proteins involved in EMT and confirmed that these selected

proteins (Grb2, Cortactin, CD2AP, CAPZA1) are Tks4 partners in these cell lines by Tks4-IP western blot experiments.

Conclusion

In the embryonic stem cells, Tks4 deficiency did not disrupt the basic germ cell differentiation process, which is also the case in FTHS and Tks4-KO mice, as the viability of the affected children is preserved despite the disease. Therefore, it is not surprising that Tks4-KO HUES9 cells also retained their broad differentiation ability. Thus, the absence of Tks4 does not cause pronounced problems at this level of embryonic development. Next, we differentiated mesenchymal stem cells from pluripotent stem cells and examined the levels of differentiation markers, where the expression levels of the major EMT markers (E-cadherin and Snai1) were altered. These data suggest that Tks4 is not a key regulator of EMT but plays a role in the fine-tuning process. Also, the lower levels of transcription factors involved in bone tissue formation in Tks4-KO MSCs (GATA4, GSC [16,17]) suggest that the kinetics of the differentiation process are impaired. These data are consistent with the observation that Tks4 has a role in bone tissue formation [5,7], which may be related to the bone phenotype of FTHS patients.

The role of Tks4 in tumor biology is also being investigated by research groups, since its use as a biomarker has been demonstrated in gastric cancer, hepatocellular carcinoma and melanoma [10,11,18], but its role in lung cancer has not yet been studied. Therefore, I performed database analysis and qPCR measurements of human lung cancer tissue samples. The results showed that Tks4 mRNA levels are lower in lung cancer tissues than in healthy tissues. This suggests that Tks4 may serve as a potential biomarker to distinguish between healthy and cancerous lung tissues. Moreover, the lowest Tks4 expression levels were measured in the most advanced stage of lung cancer. Accordingly, measurement of Tks4 mRNA expression levels by qPCR in surgically removed lung tissue samples may help to determine the possible prognosis of the disease.

Furthermore, I have also studied Tks4 deficiency in lung adenocarcinoma cells (A549) *in vitro*, resulting in an elongated mesenchymal-like phenotype. This is similar to that previously observed in Tks4-KO colon cancer cells by our group, where we discovered elongated and less circular cell shapes [12]. In addition, our results in Tks4-KO lung cancer cells suggest the presence of an epithelial/mesenchymal hybrid phenotype characterized by increased gene expression of mesenchymal markers such as fibronectin, N-cadherin and transcription factor Snai2 and decreased expression of the epithelial marker E-cadherin in Tks4-KO clones, which

are hallmarks of the EMT process. It is important to highlight that not all EMT-related markers showed a uniform change in protein levels, as N-cadherin levels were maintained in Tks4-KO cells, similar to the wild-type cells. In general, the ability to migrate and the development of invadopodia are characteristics of invasive cancer cells. Interestingly, the migratory ability of Tks4-KO cancer cells is slightly reduced, and these cells also show reduced levels of invadopodia formation. We hypothesized that the mechanism behind the different effects (decreased invadopodia formation, increased EMT marker expression, decreased migratory ability) may be due to the fact that Tks4 partners can affect EMT in several ways. **Thus, the current level, ratio, binding and thus downstream signaling of Tks4 partner molecules may have different effects on the outcome of EMT processes.** To describe the Tks4 interactome, Tks4 IP mass spectrometry analysis was performed, where I identified new Tks4 partners, for example: the CAPZA1 protein. The Tks4-CAPZA1 interaction was validated by western blot, Duolink proximity ligation assay and prediction analysis with Dr. Rita Pancsa. In hepatocellular carcinoma cells it was also shown that decreased expression of CAPZA1 drives the EMT process induced by low oxygen levels [19], and another research group has shown that a decrease in oxygen levels in the A549 cell line induces the EMT process [20]. Thus, I also tested the effect of low oxygen conditions in the Tks4-deficient lung cancer cell line. Tks4-KO cells responded with a more pronounced EMT-like phenotypic change than wild-type A549 cells to the low oxygen stress. This result may indicate that, knocking out Tks4 results in a more plastic cell type that responds more rapidly to EMT induction.

Based on our hypothesis, the presence of signal transduction molecules associated with the Tks4 scaffold protein at a given moment and the binding relationships between them may affect downstream Tks4 signaling in different ways. We performed additional Tks4 interactome studies to identify Tks4 partner molecules. In these experimental sets, I included three lung cancer cell lines (A549, NCI-H460, HOP-92) and the HUES9 embryonic cell line to perform Tks4-IP-WB experiments. To select the target proteins for this experiment, firstly I created a protein-protein interaction network based on the STRING database, literature data and my own research data (Figure 2), from which I selected those proteins that are involved in the EMT process (Grb2 [21], CAPZA1 [19], CD2AP [13], Cortactin [22]). The results show that these partner proteins are present in the Tks4 interactome in embryonic mesenchymal stem cells and in all the three lung cancer cell lines as well.

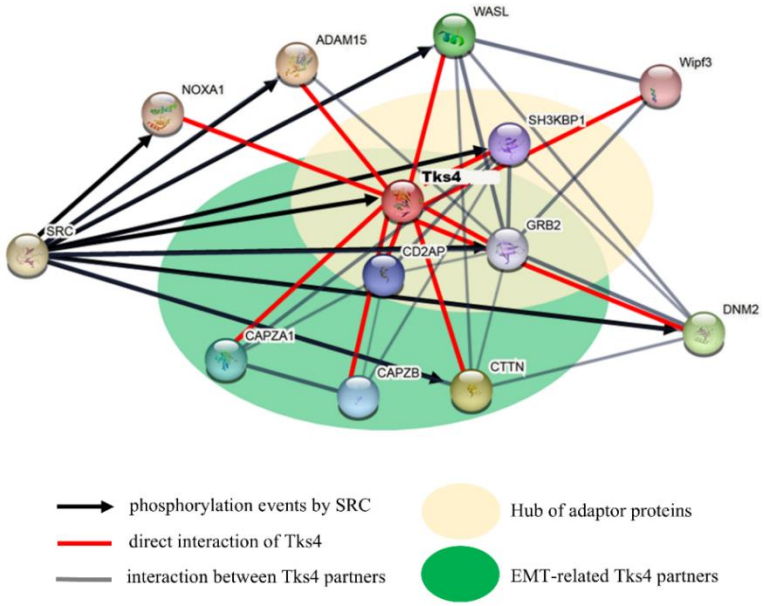

Figure 2: Analysis of protein-protein interactions of Tks4 partner molecules: the interaction network of Tks4. The interaction analysis was visualized using STRING (https://string-db.org/), incorporating experimental and literature data.

Overall, the *in vitro* experiments I have presented indicate that the absence of Tks4 induces a hybrid EMT process in tumor cells, while it slightly slows down this process in embryonic HUES9 MSC cells and may further inhibit differentiation/commitment steps. This suggests that normal and tumor cells respond differently to the absence of Tks4. These results show that Tks4 (Figure 2) and the molecules involved in its network contribute together to EMT-like processes, invadopodia formation and regulation of differentiation. In conclusion, this PhD research significantly extends our knowledge of the Tks4 interactome and its role in the regulation of EMT-like processes in embryonic stem cells and lung cancer cells (Figure 3).

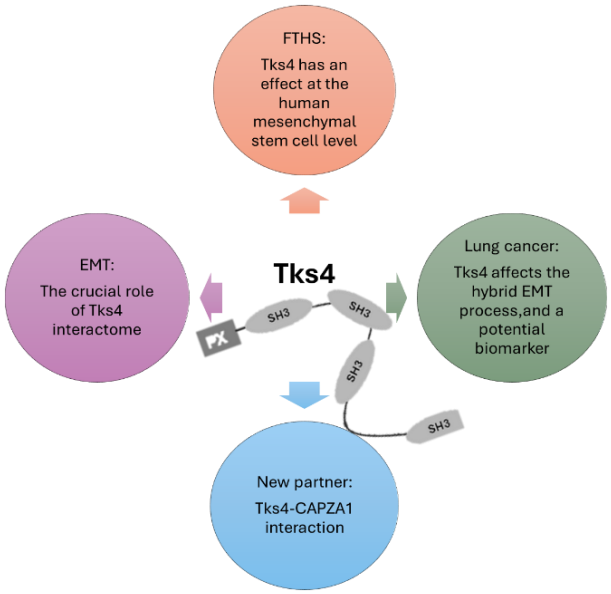

Figure 3: Summary of the results shown in the PhD thesis.

List of relevant publications:

László, L.; Maczelka, H.; Takács, T.; Kurilla, A.; Tilajka, Á.; Buday, L.; Vas, V.; Apáti, Á. A Novel Cell-Based Model for a Rare Disease: The Tks4-KO Human Embryonic Stem Cell Line as a Frank-Ter Haar Syndrome Model System. Int. J. Mol. Sci. 2022, 23, doi:10.3390/ijms23158803.

László, L.; Kurilla, A.; Tilajka, Á.; Pancsa, R.; Takács, T.; Novák, J.; Buday, L.; Vas, V. Unveiling Epithelial Plasticity Regulation in Lung Cancer: Exploring the Crosstalk Among Tks4 Scaffold Protein Partners. Mol. Biol. Cell 2024, 1–19, doi:10.1091/mbc.e24-03-0103.

Other publications during the PhD program:

László, L.; Kurilla, A.; Takács, T.; Kudlik, G.; Koprivanacz, K.; Buday, L.; Vas, V. Recent Updates on the Significance of KRAS Mutations in Colorectal Cancer Biology. Cells 2021, 10, 1–19, doi:10.3390/CELLS10030667.

Sipeki, S.; Koprivanacz, K.; Takács, T.; Kurilla, A.; **László, L.**; Vas, V.; Buday, L. Novel Roles of SH2 and SH3 Domains in Lipid Binding. Cells 2021, 10, doi:10.3390/CELLS10051191.

Kurilla, A.; **László, L**.; Takács, T.; Tilajka, Á.; Lukács, L.; Novák, J.; Pancsa, R.; Buday, L.; Vas, V. Studying the Association of TKS4 and CD2AP Scaffold Proteins and Their Implications in the Partial Epithelial-Mesenchymal Transition (EMT) Process. Int. J. Mol. Sci. 2023, 24, doi:10.3390/IJMS242015136.

Tilajka, Á.; Kurilla, A.; **László, L**.; Lovrics, A.; Novák, J.; Takács, T.; Buday, L.; Vas, V. Predictive value analysis of the interaction network of Tks4 scaffold protein in colon cancer. Front. Mol. Biosci. 2024, 11, 1–15, doi:10.3389/fmolb.2024.1414805.

Takács, T.; **László, L**.; Tilajka, Á.; Novák, J.; Buday, L.; Vas, V. Insulin receptor substrate 1 is a novel member of EGFR signaling in pancreatic cells. Eur. J. Cell Biol. 2024, 103, doi:10.1016/j.ejcb.2024.151457.

Other publication written before the PhD program:

Iván P. Uray and **Loretta László** Translation of Effects of Retinoids and Rexinoids: Extraction and Quality Assessment of RNA from Formalin-Fixed Tissues. Retin. Rexinoid Signal. Methods Protoc. Methods Mol. Biol. 2019, 2019, 225–236, doi:10.1007/978-1-4939-9585-1.

Bibliography

1. Kim, D.H.; Xing, T.; Yang, Z.; Dudek, R.; Lu, Q.; Chen, Y.H. Epithelial Mesenchymal Transition in Embryonic Development, Tissue Repair and Cancer: A Comprehensive Overview. *J. Clin. Med. 2018, Vol. 7, Page 1* **2018**, *7*, 1, doi:10.3390/JCM7010001.
2. Saitoh, M. Involvement of partial EMT in cancer progression. *J. Biochem.* **2018**, *164*, 257–264, doi:10.1093/JB/MVY047.
3. Bornes, L.; Belthier, G.; van Rheenen, J. Epithelial-to-mesenchymal transition in the light of plasticity and hybrid e/m states. *J. Clin. Med.* **2021**, *10*, doi:10.3390/jcm10112403.
4. Brabletz, T.; Kalluri, R.; Nieto, M.A.; Weinberg, R.A. EMT in cancer. *Nat. Rev. Cancer* **2018**, *18*, 128–134, doi:10.1038/nrc.2017.118.
5. Iqbal, Z.; Cejudo-Martin, P.; de Brouwer, A.; van der Zwaag, B.; Ruiz-Lozano, P.; Scimia, M.C.; Lindsey, J.D.; Weinreb, R.; Albrecht, B.; Megarbane, A.; et al. Disruption of the Podosome Adaptor Protein TKS4 (SH3PXD2B) Causes the Skeletal Dysplasia, Eye, and Cardiac Abnormalities of Frank-Ter Haar Syndrome. *Am. J. Hum. Genet.* **2010**, *86*, 254–261, doi:10.1016/j.ajhg.2010.01.009.
6. Dülk, M.; Kudlik, G.; Fekete, A.; Ernszt, D.; Kvell, K.; Pongrácz, J.E.; Mero, B.L.; Szeder, B.; Radnai, L.; Geiszt, M.; et al. The scaffold protein Tks4 is required for the differentiation of mesenchymal stromal cells (MSCs) into adipogenic and osteogenic lineages. *Sci. Reports 2016 61* **2016**, *6*, 1–9, doi:10.1038/srep34280.
7. Vas, V.; Kovács, T.; Körmendi, S.; Bródy, A.; Kudlik, G.; Szeder, B.; Mező, D.; Kállai, D.;

Koprivanacz, K.; Merő, B.L.; et al. Significance of the Tks4 scaffold protein in bone tissue homeostasis. *Sci. Reports 2019 91* **2019**, *9*, 1–10, doi:10.1038/s41598-019-42250-6.
8. Durand, B.; Stoetzel, C.; Schaefer, E.; Calmels, N.; Scheidecker, S.; Kempf, N.; De Melo, C.; Guilbert, A.S.; Timbolschi, D.; Donato, L.; et al. A severe case of Frank-ter Haar syndrome and literature review: Further delineation of the phenotypical spectrum. *Eur. J. Med. Genet.* **2020**, *63*, 103857, doi:10.1016/J.EJMG.2020.103857.
9. Kui, X.; Wang, Y.; Zhang, C.; Li, H.; Li, Q.; Ke, Y.; Wang, L. Prognostic value of SH3PXD2B (Tks4) in human hepatocellular carcinoma: a combined multi-omics and experimental study. *BMC Med. Genomics* **2021**, *14*, 1–10, doi:10.1186/S12920-021-00963-6/FIGURES/3.
10. Zhu, Y.; Hu, Y.; Wang, P.; Dai, X.; Fu, Y.; Xia, Y.; Sun, L.; Ruan, S. Comprehensive bioinformatics and experimental analysis of SH3PXD2B reveals its carcinogenic effect in gastric carcinoma. *Life Sci.* **2023**, *326*, 121792, doi:10.1016/J.LFS.2023.121792.
11. Iizuka, S.; Abdullah, C.; Buschman, M.D.; Diaz, B.; Courtneidge, S.A. The role of Tks adaptor proteins in invadopodia formation, growth and metastasis of melanoma. *Oncotarget* **2016**, *7*, 78473, doi:10.18632/ONCOTARGET.12954.
12. Szeder, B.; Tárnoki-Zách, J.; Lakatos, D.; Vas, V.; Kudlik, G.; Merő, B.; Koprivanacz, K.; Bányai, L.; Hámori, L.; Róna, G.; et al. Absence of the Tks4 Scaffold Protein Induces Epithelial-Mesenchymal Transition-Like Changes in Human Colon Cancer Cells. *Cells 2019, Vol. 8, Page 1343* **2019**, *8*, 1343, doi:10.3390/CELLS8111343.
13. Kurilla, A.; László, L.; Takács, T.; Tilajka, Á.; Lukács, L.; Novák, J.; Pancsa, R.; Buday, L.; Vas, V. Studying the Association of TKS4 and CD2AP Scaffold Proteins and Their Implications in the Partial Epithelial-Mesenchymal Transition (EMT) Process. *Int. J. Mol. Sci.* **2023**, *24*, doi:10.3390/IJMS242015136.
14. Jacksi, M.; Schad, E.; Buday, L.; Tantos, A. Absence of Scaffold Protein Tks4 Disrupts Several Signaling Pathways in Colon Cancer Cells. *Int. J. Mol. Sci.* **2023**, *24*, 1310, doi:10.3390/IJMS24021310/S1.
15. Szebényi, K.; Barrio-Hernandez, I.; Gibbons, G.M.; Biasetti, L.; Troakes, C.; Beltrao, P.; Lakatos, A. A human proteogenomic-cellular framework identifies KIF5A as a modulator of astrocyte process integrity with relevance to ALS. *Commun. Biol.* **2023**, *6*, doi:10.1038/s42003-023-05041-4.
16. Khalid, A.B.; Pence, J.; Suthon, S.; Lin, J.; Miranda-Carboni, G.A.; Krum, S.A. GATA4 Regulates Mesenchymal Stem Cells via Direct Transcriptional Regulation of the WNT Signalosome. *Bone* **2021**, *144*, 115819, doi:10.1016/J.BONE.2020.115819.
17. Rivera-Pérez, J.A.; Wakamiya, M.; Behringer, R.R. Goosecoid acts cell autonomously in mesenchyme-derived tissues during craniofacial development. *Development* **1999**, *126*, 3811–3821, doi:10.1242/DEV.126.17.3811.
18. Kui, X.; Wang, Y.; Zhang, C.; Li, H.; Li, Q.; Ke, Y.; Wang, L. Prognostic value of SH3PXD2B (Tks4) in human hepatocellular carcinoma: a combined multi-omics and experimental study. *BMC Med. Genomics* **2021**, *14*, 1–10, doi:10.1186/S12920-021-00963-6/FIGURES/3.
19. Huang, D.; Cao, L.; Xiao, L.; Song, J. xian; Zhang, Y. jun; Zheng, P.; Zheng, S. guo Hypoxia induces actin cytoskeleton remodeling by regulating the binding of CAPZA1 to F-actin via PIP2 to drive EMT in hepatocellular carcinoma. *Cancer Lett.* **2019**, *448*, 117–127, doi:10.1016/J.CANLET.2019.01.042.
20. Liu, K.H.; Tsai, Y.T.; Chin, S.Y.; Lee, W.R.; Chen, Y.C.; Shen, S.C. Hypoxia Stimulates the Epithelial–to–Mesenchymal Transition in Lung Cancer Cells Through Accumulation of Nuclear β-Catenin. *Anticancer Res.* **2018**, *38*, 6299–6308, doi:10.21873/ANTICANRES.12986.
21. Mitra, P.; Kalailingam, P.; Tan, H.B.; Thanabalu, T. Overexpression of GRB2 Enhances Epithelial to Mesenchymal Transition of A549 Cells by Upregulating SNAIL Expression. *Cells 2018, Vol. 7, Page 97* **2018**, *7*, 97, doi:10.3390/CELLS7080097.
22. Ji, R.; Zhu, X.J.; Wang, Z.R.; Huang, L.Q. Cortactin in Epithelial–Mesenchymal Transition. *Front. Cell Dev. Biol.* **2020**, *8*, 585619, doi:10.3389/FCELL.2020.585619/BIBTEX.