Projekt záró szakmai beszámoló (K124045)

The aim of the OTKA research work was to evaluate the significance of Tks4 (tyrosine kinase substrate with four SH3 domains) scaffold protein in tissue homeostasis. As the knock-out of Tks4 gene resulted in severe adipogenic and osteogenic phenotype in human and in mice, it was reasonable to study the role of Tks4 in brown and white adipocyte and osteocyte biology.

Initially, the focus of research work was to describe the role of Tks4 scaffold protein in **adipose tissue homeostasis**. We have collected data concerning the effect of Tks4 on brown and white adipocytes. The histology and the gene expression profile of the adipogenic depots of Tks4-KO and wild type mice were compared and the differences in cell size and the presence of beige adipocytes were analysed. We have also isolated the adipocyte stem cell-enriched (ASC) population from mouse fat depots and challenged the Tks4-knock-out ASCs differentiation potential. Subsequently, we used an Adipogenesis Profiler PCR Array to measure the gene expressional changes regulated by white, brown and beige adipocyte-related transcription factors in Tks4 knock out and wild type adipose tissues. This analysis revealed that in the Tks4-KO white adipose tissue, the expression levels of adipogenesis-supporting genes are downregulated, while the expression levels of browning-selective genes tended to be upregulated. Moreover, the beigeing process-related signalling pathways were more pronounced in these animals than in the WT animals. We have also shown that the differentially expressed genes form a network and that PPARγ is the central regulator of the altered signalling pathways. Based on our results, we proposed that the loss of Tks4 leads to changes in multiple cellular signalling pathways that contribute to the fine-tuning of PPARγ-regulated transcription in adipocytes. This result helps to better understand the mechanism by which beige adipocytes differentiate in white adipose tissues and might serve potential targets of intervention relevant in the fight against obesity. As the adipocyte homeostasis regulating function of Tks4 was not described earlier the publication process was relatively fast and the accepted article was published in Cells, 2019 [1].

Furthermore, we have also revealed that the Tks4 protein is involved in **osteoporotic processes** via the modulation of osteoblast cell differentiation. During this part of the project, we have also analysed the osteogenic phenotype of the Tks4-KO mice and a Tks4 gene-mutant Frank-ter Haar syndrome patient. These results were performed in collaboration with Prof. Dr. Csaba Dobó-Nagy (Department of Oral Diagnostics, SOTE) and were also published in Scientific Reports, 2019 [2].

The possibility that the Tks4 protein can have **a regulative role in cancer biology** was raised recently. To study the possible cancer cell-related function of Tks4, we knocked the Tks4 gene out of a colon cancer cell line (HCT116) with the CRISPR/Cas9 system and investigated the morphological, phenotypic and transcriptional changes which developed at the absence of Tks4. Our results showed that loss of Tks4 initiated epithelial mesenchymal transition (EMT)–like features in the cancer cell line. Without the Tks4 protein, the cancer cells had increased motility, spreading and altered expression of EMT-related markers (fibronectin, E-cadherin, Snail1, Twist). As these results were quite surprising and scientifically interesting, we could rapidly publish our study describing the role of Tks4 in the process of EMT in Cells, 2019 [3] and disseminate the new findings also 'BIOKÉMIA' [4] (in quarterly bulletin of the Hungarian Biochemical Society) and in Oncoplatform conference (2019).

In the fourth year of the project, the summarised results of the first three years were incorporated in an article according to the workplan and published a comprehensive

review about the OTKA-project-facilitated results about the newly described regulative role of Tks4 proteins [5]**.**

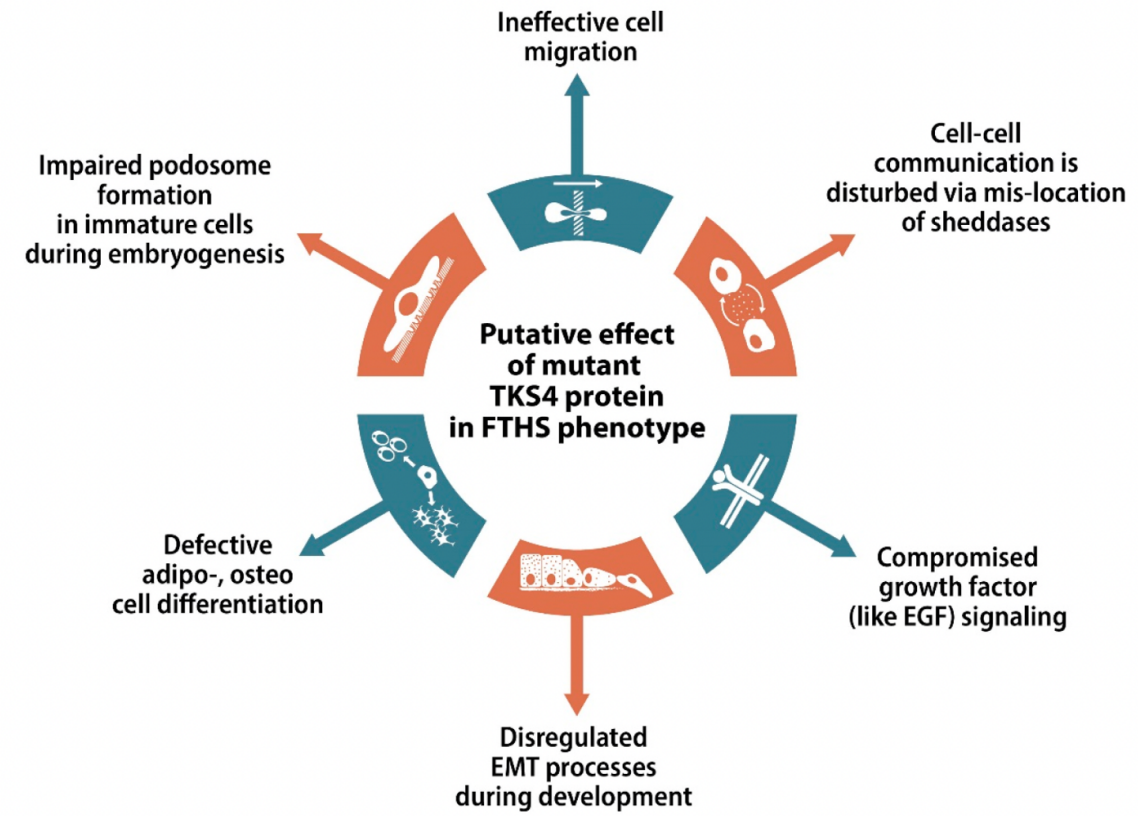

Figure 1.: Different roles of the lack of Tks4 protein in Frank-ter Haar syndrome - summarizing our data. [5] Our results shed light to experimentally described role of Tks4 in several steps of FTHS formation: in adipocyte and osteocyte development, in growth factor signals as EGFR, during differentiations the immature cells migrate via podosome machinery to the determined position in the embryo, degrade the extracellular matrix (ECMŰ) during their migration, send extracellular signalling molecules to other cells, and occasionally undergo epithelial-mesenchymal transition (EMT). In all of this processes Tks4 plays a determining role and in case of the lack of Tks4 in FTHS the EGFR signalling, the cell differentiations are dysregulated leading to the damage of the FTHS-affected tissues.

During the pandemic period of 2020, our lab members worked mostly in home office. This forced brake in lab work was very productive in the view of publications. We took part in the production of a special issue of Cancer and Metastasis Reviews (2020), and wrote three reviews [6–8]. Meanwhile, as we became involved in cancer cell research due to the Tks4-goverend EMT processes, we have written a review about the aberrant tumour cell signalling [9].

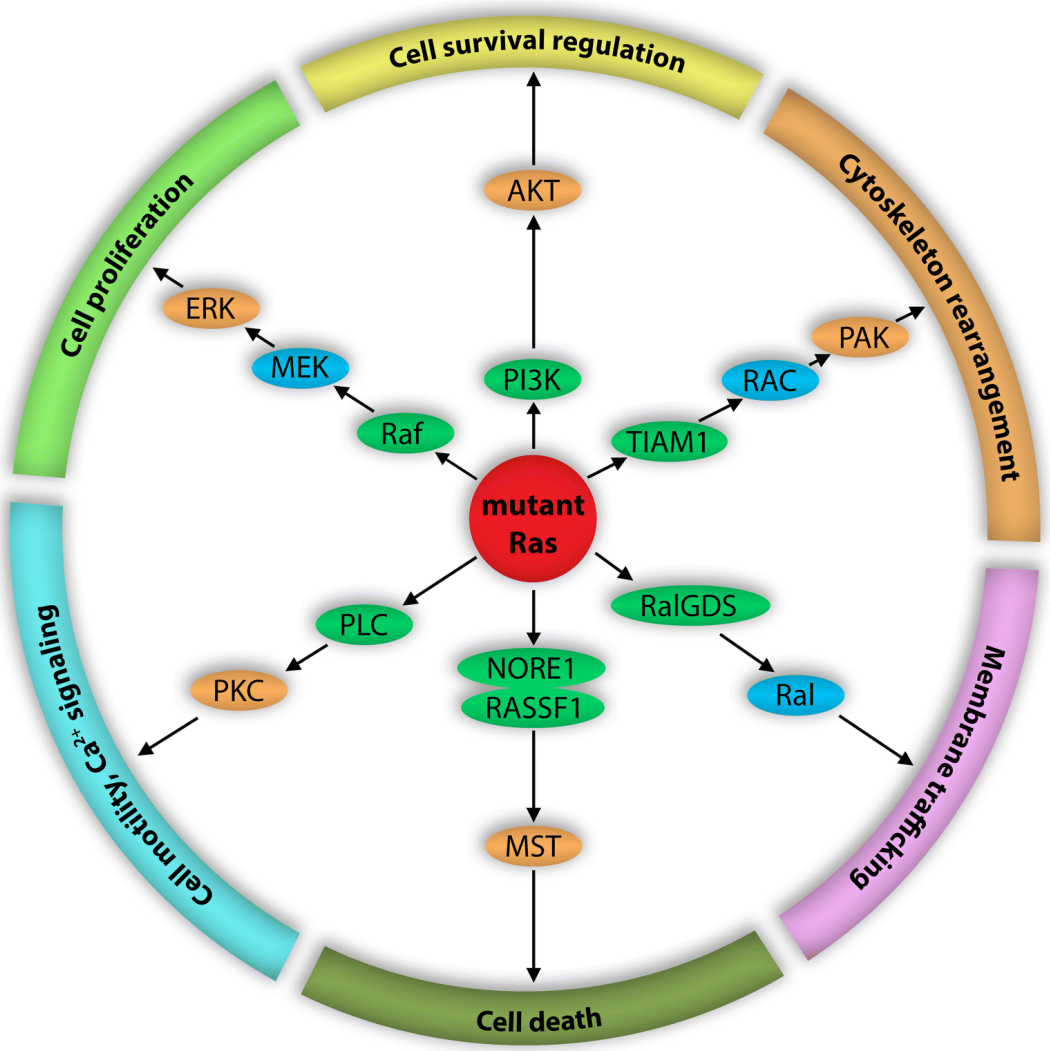

Figure 1.: This figure was published in our review [7] **and selected for the cover of the Cancer and Metastasis Reviews. This figure shows the core members of the signalling pathways radiating from mutant Ras.** The two robust Ras-driven signalling routes are the RAF/MEK/ERK and PI3K/AKT pathways, which regulate diverse cellular processes, particularly cell proliferation and cell survival regulation, respectively. Other Ras activation-dependent signalling routes are less studied. The TIAM1/RAC/PAK pathway primarily controls cytoskeleton rearrangement in certain cells, and the RalGDS/Ral pathway mostly influences membrane trafficking. The NORE1/RASSF1/MST signalling pathway is a regulator of cell death processes. Mutant Ras can also mediate signalling via PLC/PKC molecules to influence Ca+-dependent signalling in cancer cells

Beside the description of the novel function of Tks4 in adipocyte cells, bone biology, and cancer cell development, we have also been focusing on **molecular aspects of SH3 domain regulating processes.**

1., We could describe a widespread mechanism regulating a significant part of the SH3-interactom in cells using protein crystallization method, SAXS experiments and NMR spectroscopy. We have shown a novel tyrosine phosphorylation site in the ligand binding groove of the SH3 domains of Abl1 and Abl2, and demonstrated that the tyrosine phosphorylation of the SH3 domain strongly inhibits it's interaction with proline-rich ligands

due to steric blocking. The results of the structural and biochemical analysis of the SH3 domain-regulated processes are published in 2019 in JBC [10].

2., Furthermore, the molecular structure and the roles of each SH3 domain of Tks4 are not fully identified yet, therefore we aimed also to analyse the SH3 domain regulated intra-molecular interactions within the Tks4 molecule. We have showed via fluorescence-based titration, MST, ITC and SAXS measurements, that the tandem SH3 domain of Tks4 binds the proline rich region, and simultaneously the PX domain binds the third SH3 domain of Tks4. We have published in 2021 the Tks4 intra-molecular interaction analysis results [11].

3., Another SH3 domain-governed mechanism was also revealed by us with the support of the OTKA project. We have elucidated the details of the molecular mechanism of Tks4 and Src kinase complex formation. It turned out that the SH3 and the SH2 domains of Src kinase binds to a PRR and a phosphotyrosine (Tyr508) motif of Tks4, this interaction results in the long term stabilization of the kinase leading prolonged Src activity following EGFR stimulation [12].

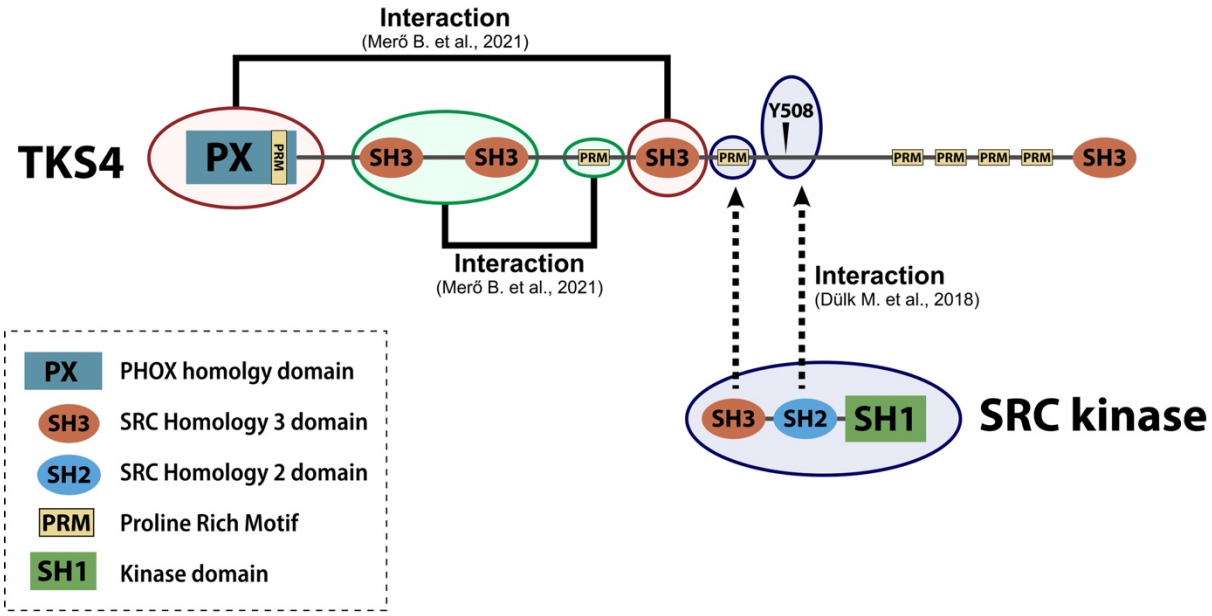

Figure 3.: Model of the Tks4 protein and the newly revealed interacting surfaces in the molecule.

A comprehensive review paper has been also written by us about our current knowledge about the traditional and the novel functions of the SH3 domain [13].

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