CHARACTERIZATION OF TSC MUTATION-RELATED CHANGES IN THE MITOCHONDRIAL AND CELL SIGNALLING MECHANISMS OF ANGIOMYOLIPOMA MODELL SYSTEM

PhD thesis

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Introduction

In the background of numerous neoplasms known as slow-growing tumor lesions, such as lymphangioleiomyomatosis (LAM) and angiomyolipoma (AML), mutations in the Tuberous Sclerosis Complex (TSC) gene are identified. Genetic alterations affecting the TSC gene result in loss of function of the protein encoded by the TSC1 or TSC2 genes. The TSC1/TSC2 proteins play a central role in the regulation of the Rheb/mTOR/p70S6K (Ras homolog enriched in brain/ mammalian target of rapamycin/ Ribosomal protein S6 kinase beta-1) signalling pathway, which can influence cellular protein synthesis and consequently cell division. Therefore, inhibition of the mTOR signalling pathway is an important therapeutic target in the chemotherapy of LAM, AML and cancer. The drug targeting this pathway is sirolimus, or rapamycin, which inhibits signals through mTOR, so rapamycin is included in the treatment plan where possible. Rapamycin acts by inhibiting the downstream branch of the mTOR complex, including S6K1, which results in reduced protein synthesis, slower cell division, and thus slower disease progression. Unfortunately, a large group of patients experience severe side effects during treatment, including edema, diarrhea, nephrotoxicity, inadequate wound healing, thrombocytopenia, stomatitis, or hypercholesterolemia. If the patient is unable to tolerate rapamycin therapy due to its side effects, the only alternative treatment options are renal resection or angioembolization for AML and lung transplantation for LAM. A cohort study found that rapamycin at lower serum concentrations reduced the severity of side effects while slowing disease progression. This suggests that lowering the recommended therapeutic dose or combining it with other agents at this reduced dose could effectively reduce the need for subsequent invasive interventions.

The selection of the combination of drugs was based on the different mitochondrial metabolism of TSC mutant AML cells. Altered metabolic activity of the cells has been observed and described previously in several diseases. The consequence of defective mitochondrial function and overactivated mTOR signalling pathway is increased mitochondrial respiration, glycolysis, and uncontrolled cell division. In healthy cells, cell division, cell migration, regulation of cell size, maintenance of cell structure, and regulation of autophagy are all energy-consuming processes that are tightly regulated. A high ATP demand for the underlying processes characterizes the increased division potential of tumor cells. In tumor cells, a significant ratio of ATP molecules is generated during oxidative phosphorylation (OxPhos). In tumor cells, it has been observed that they can produce ATP through enhanced glycolysis independent of oxygen supply (Warburg effect). In tumor cells, metabolic dysfunction, including mitochondrial dysfunction, is a hallmark of disease development. Accordingly, many tumors have been treated experimentally with agents that act on mitochondrial function. Several studies have observed a correlation between resistance to chemotherapy and upregulation of metabolic activity, as well as between metastasis and changes in metabolism and increased aerobic glycolysis. In gastric tumors, it has been described that the development of resistance to the chemotherapeutic agent cisplatin is associated with regulatory processes related to the mTOR signalling pathway. Increased aerobic glycolysis leads to the oversaturation of electron transport chains and the

accumulation of superoxide radicals. Superoxide-binding agents used in therapy may reduce the oversaturation of electron transport chains, thereby reducing the ability of tumor cells to migrate and metastasize.

Investigation of the proliferative capacity, mitochondrial signalling and antioxidant systems of TSC (-/-) mutant S102 angiomyolipoma cells has led to identifying potential therapeutic targets that have not been used in treating TSC mutation-related diseases. The three agents we have investigated all target the cellular antioxidant system. Proxyson, a synthetic flavonoid, is a drug candidate molecule pending approval. It can effectively neutralize free reactive oxygen radicals through electron transfer/H-atom donation. The other drug molecule being investigated in combination therapy is retinoic acid (RA), approved by the FDA for treating acute myeloid leukemia. RA is a metabolite of vitamin A (retinol) with lipophilic properties, obtained from plant (carotenoids) or animal sources (retinyl esters). The third agent tested is auranofin, a gold-containing redox enzyme inhibitor that can inhibit the enzyme thioredoxin reductase (TrxR) and induce cell death in some tumor cell cultures. Auranofin has been tested in clinical cases where fast-growing tumors have not responded to treatment.

Aims of the study

In this thesis, I aimed to gain a deeper understanding of the molecular background of AML, a chronic proliferative disease, and to identify potential new therapeutic targets based on the molecular background processes.

The main study objectives were:

1- To investigate mitochondrial involvement associated with TSC mutation using TSC (+/+) wild-type, S103 control, and TSC (-/-) mutant S102 AML cell lines.

2- Molecular mechanisms modified by TSC mutation:

a. The p53 tumor suppressor gene

b. Vitamin A receptors and vitamin A metabolizing enzymes

c. The TrxR redox enzyme

3- Based on the molecular mechanisms identified, in vitro testing of new drugs (retinoic acid, proxyson, auranofin) with potential therapeutic potential in TSC mutation-induced diseases, using TSC (+/+) wild-type S103 control and TSC (-/-) mutant S102 AML cell lines.

Materials and Methods

1. CHARACTERISTICS OF AML CELLS, CELL CULTURES

We used AML cells 621-S103 and 621-S102 carrying mutations in both alleles of the TSC gene.

2. DRUG TREATMENTS

Cells were seeded and incubated 24 h before the planned treatments. The incubation times of the drug treatments were 3, 24, and 48 h. TSC (+/+) wild-type S103 and TSC (-/-) mutant S102 cells used in these experiments were treated with either rapamycin, auranofin, proxyson and retinoic acid alone or a combination of the latter with rapamycin (rapamycin+proxyson, rapamycin+retinoic acid and rapamycin+auranofin).

3. ELECTRONMICROSCOPY

Transmission electron microscopes Jeol 1400 and Jeol 1200 were used for the morphological analysis of mitochondria from TSC (+/+) wild-type S103 and TSC (-/-) mutant S102 cells.

4. CELL VIABILITY MEASUREMENT

1.4.1. CELL TITER GLO LUMINOMETRIC ASSAY

The CellTiter-Glo Luminescent Cell Viability Assay Kit was used to determine cell viability after treatments.

1.4.2. CRYSTAL VIOLET COLORIMETRIC ASSAY

We used the crystal violet cell viability assay to determine the dose of auranofin to be used in our experiments.

5. CELL PROLIFERATION ASSAY

Following drug treatments of 621-103 (TSC2 +/+) AML and 621-102 (TSC2-/-) AML cells, we used Click-IT EdU Cell Proliferation Kit to determine the amount of newly synthesized DNA. Images were taken using an Olympus IX 81 fluorescence microscope.

6. FUNCTIONAL STAINING OF MITOCHONDRIA

Drug-treated cells were incubated in the presence of MitoBright Green mitochondrial vital dye. Images were taken with a Zeiss LSM 710 confocal microscope.

7. IMMUNOFLUORESCENT STAINING

Following conventional staining of TSC (+/+) wild-type S103 and TSC (-/-) mutant S102 cells, images were captured using an Olympus IX-81 (Olympus Corporation, Tokyo, Japan) light and fluorescence microscope.

8. WESTERN BLOT

After the incubation period of drug treatments, cells were collected according to the western blot protocol. Following the conventional protocol, a chemiluminescent HRP substrate was used to detect the immunoreaction, and images were captured using an ImageQuant LAS-4000 imager. ImageJ software was used to determine the intensity of protein expression.

9. PROTEIN ARRAY

After incubation of the cells with the drugs, the Proteome Profiler Array, Human Cell Stress Array Kit was used to detect the protein expression patterns. Protein assays were performed according to the protocol provided by the manufacturer. The luminescence signal was detected using the ImageQuant LAS-4000 imager system. ImageJ software was used to determine the intensity of protein expression and the values obtained were normalized to reference points.

10. PRDX5 PROTEIN CONCENTRATION MEASUREMENT WITH ELISA

Following the drug treatments, a Human Peroxiredoxin 5 ELISA Kit was used to determine the concentration of Prdx5 protein. A Perkin Elmer Enspire Multiplate Reader was used to detect absorbance.

11. ALDH AND ADH ENZYME ACTIVITY MEASUREMENT

ALDH Activity Assay Kit and Alcohol Dehydrogenase Assay Kit were used to quantify ALDH and ADH activity of TSC (-/-) mutant S102 cells compared to pre- and post-treatment controls. Color changes based on the enzyme activity were measured using a Perkin Elmer EnSpire Multimode Plate Reader at OD450 nm.

12. METABOLIC ENZYME RT2 ARRAY

Following cDNA synthesis, the Human Drug Metabolism: Phase I Enzyme array was used to examine mRNA expression levels of metabolic enzymes. The array was run on a Quantstudio 12k flex device.

13. RNS ISOLATION

For total RNA extraction from cells, the MN NucleoSpin RNA isolation kit was used, following the manufacturer's protocol. For cDNA synthesis, we used the High capacity RNA to cDNA kit. For reverse transcription, a random hexamer primer was used.

14. TAQMAN ASSAY

The Taqman assays were run on a Quantstudio 12K Flex Real-Time PCR system. Gene expression was normalized to the GAPDH housekeeping gene. Data were evaluated by the comparative ddCt method.

15. INTRACELLULAR ROS PRODUCTION

To determine the level of reactive oxygen species, a green Fluorometric Intracellular Ros Kit was used according to the manufacturer's instructions. Cellular ROS production after treatments was determined using a Perkin Elmer EnSpire Multimode Plate Reader.

16. TrxR ENZYME ACTIVITY

17. Cell homogenate was used to measure the activity of the TrxR enzyme. TrxR enzyme activity was performed as described in the kit.

18. FLOW CYTOMETRY

BD FACSCanto™ II (three lasers: blue 488-nm, red 633-nm, and violet 405-nm) and BD FACS DIVA software version V6 were used to detect cells stained with Annexin V and 7-AAD, and FCS Express V3 software was used to analyze the data.

19. INGENUITY PATHWAY ANALYSIS

I used Ingenuity Pathway Analysis software for molecular pathway analysis. Interaction network analysis and MAP (Molecule Activity Predictor) analysis filter were applied on the available data.

20. STATISTICAL ANALYSIS

SPSS version 20 and GraphPad Prism version 8 were used to statistically analyze the results. The data plotted represent the mean of the measurements and the SEM resulting from the three replicates. For statistical analysis, we used two-way ANOVA and a two-sample t-test, respectively, and the results are marked as significant below $p < 0.05$ (*). Additional notations are $p < 0.0021$ (**), $p < 0.0002$ (***) and $p < 0.0001$(****).

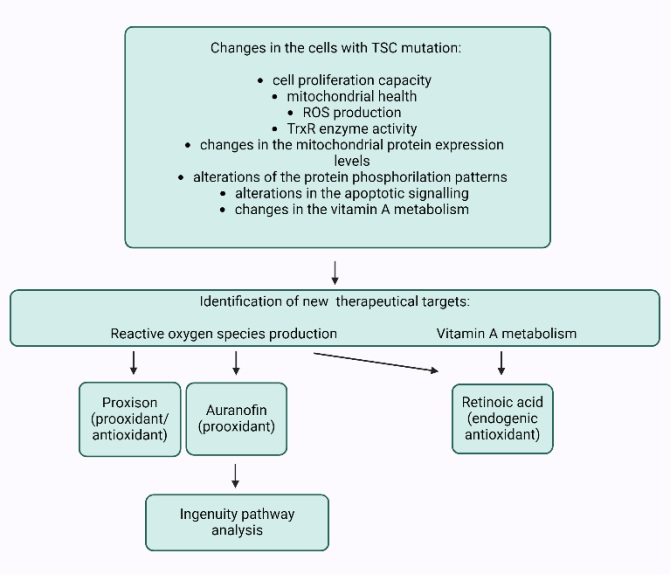
Results and conclusions

1. CHARACTERISATION OF AML CELLS

TSC (-/-) mutant S102 cells are, in many cases, smaller than healthy cells, and they have an increased proliferative capacity. Their mitochondria are smaller, darker and so electrodense that inner membrane cristae are not recognizable. Compared to the healthy control cells TSC mutant cells showed reduced mitochondrial membrane potential, increased expression of VDAC, a protein that influences mitochondrial membrane potential, and decreased levels of prohibitin, a protein that plays a central role in maintaining mitochondrial integrity. The changes in TSC mutant cells also affect signalling processes that regulate cell apoptosis. Despite our results showing that phosphorylation of p53 protein was detected at three major serine phosphorylation sites (S15, S392, and S46), TSC (-/-) cells proliferated faster than TSC (+/+) wild-type control cells. To learn more about the complex regulatory processes that characterize pro- and anti-apoptotic signalling pathways, we examined the amount of ROS generated in cells. The metabolic processes in healthy cells are also characterized by ROS generation. However, due to damage to mitochondria, large amounts of ROS accumulating in cells can lead to cell death. Protection against the reactive radicals generated is provided by the antioxidant enzyme systems in the cells, which include TrxR and glutathione reductase enzymes. In TSC (-/-) mutant cells, an increase in TrxR enzyme activity was observed compared to TSC (+/+) wild-type cells, while ROS concentrations were decreased.

Vitamin A is a cellular endogenous non-enzymatic antioxidant molecule, acting by binding to RARß receptors. Our study demonstrated that vitamin A metabolism and RARß expression of TSC mutant cells also differ from wild-type TSC cells. The levels of four enzymes of the alcohol dehydrogenase enzyme family (ADH1A, ADH1B, ADH1C, and ADH6) were significantly increased, and the levels of one enzyme were decreased (ADH4) in the mutant cell line. In the aldehyde dehydrogenase family, three enzymes (ALDH1A2, ALDH1A3, and ALDH3A1) showed increased levels, while five enzymes showed decreased expression (ALDH1A1, ALDH3B1, ALDH3B2, ALDH4A1, and ALDH5A1).

2. ALTERNATIVE THERAPEUTIC OPTIONS BASED ON THE MOLECULAR MECHANISMS IDENTIFIED



3. THE EFFECT ON CELLS OF RAPAMYCIN TREATMENT IN COMBINATION WITH RETINOIC ACID AT REDUCED CONCENTRATIONS

Since patients with AML are treated with rapamycin, which is well known to downregulate RARβ levels, we measured RARβ protein expression levels in TSC (-/-) mutant S102 angiomyolipoma cell line after 10 nM rapamycin treatment and combined rapamycin treatment with 2 μM RA. While 10 nM rapamycin mono-treatment did not affect RARβ levels, 2 μM RA combined with rapamycin increased RARβ expression in TSC2-/- cell lines. To test whether normalization of enzyme levels and vitamin A metabolism activity, as well as inhibition of mTOR activity, affected cell proliferation and migration, we performed a BrdU (EdU) assay after mono- and combination treatment with 2 μM RA and 10 nM rapamycin. Combined treatment of TSC mutant (-/-) cell lines with rapamycin (10 nM) and RA (2 μM) significantly reduced the proliferative capacity detected in the BrdU assay compared to rapamycin mono treatment.

4. THE EFFECT OF RAPAMYCIN TREATMENT WITH PROXYSON AT REDUCED CONCENTRATIONS ON CELLS

We investigated the possibility that the regulation of apoptosis could be restored. To this end, we performed a combination treatment with FDA-approved rapamycin and the drug candidate proxyson, a flavonoid-based drug candidate molecule shown to restore mitochondrial morphology and function in our previous experiments. On this basis, we investigated the effect of rapamycin at 3 μM proxyson and/or standard (20 nM) or reduced (10 nM) concentrations on TSC (-/-) S102 mutant and TSC (+/+) wild-type S103 control cell lines. Fluorescence staining of cells showed increased cellular presence of p53 (S15) protein after combined treatment with 3 μM proxyson or 10 nM rapamycin and 3 μM proxyson. To determine the effect of mono- and combined treatments of proxyson on cell viability, ATP level measurements, ROS concentration measurements, and AnnexinV staining were performed on TSC (-/-) mutant S102 and TSC (+/+) control S103 cells. While 20 nM rapamycin significantly increased ROS production in TSC (-/-) mutant cells, ROS levels in the 10 nM rapamycin-treated sample were not different from the control. The combination of 10 nM rapamycin and 3 μM proxyson showed a similar result as proxyson treatment alone. Both significantly increased ROS production decreased ATP levels and resulted in similarly increased AnnexinV positivity.

5. A CSÖKKENTETT KONCENTRÁCIÓBAN ALKALMAZOTT, AURANOFINNAL KOMBINÁLT RAPAMYCIN KEZELÉS HATÁSA A SEJTEKRE

The cytostatic rapamycin in combination with the redox enzyme inhibitor auranofin significantly reduced the proliferative capacity of TSC (-/-) cells, improved their mitochondrial function, reduced the activity of the enzyme TrxR and the level of the enzyme protein Prdx5 in the cells. The levels of these antioxidant enzymes are strongly correlated with the production of ROS by the affected cells, which in turn is associated with ER stress. In search of a link between Prdx5 and ER stress signalling, we detected elevated levels of HSP70 expression following combined treatment with rapamycin (10 nM) and auranofin (0.75 μM) in both TSC (-/-) mutant S102 and TSC (+/+) wild-type S103 cell lines. HSP70 is well known for preventing protein aggregate formation under increased intracellular stress. Properly folded proteins enter the Golgi complex, while misfolded proteins accumulate in the ER, coupled to the HSP70 chaperone molecule. The complex between the unfolded or misfolded protein and the HSP70 molecule triggers the UPR-mediated cell death process. Prxd5 can protect cells from such death, indicating that Prdx5 has a dual role in these complex biochemical mechanisms.

Previous studies and our results support that TSC mutation causes an imbalance in the antioxidant system of cancer cells, and increased activity of TrxR and Prdx5 enzymes compensates for increased ROS production and maintains increased proliferative capacity, protecting cells from ER stress-induced death. Auranofin can inhibit this preserved antioxidant status, leaving cancer cells unprotected against increased intracellular ROS levels and cell death.

Discussion

Our experiments have uncovered molecular mechanisms associated with TSC mutations that help to understand the pathomechanisms of these mutation-associated diseases and to get closer to targeted therapies for the treatment of the disease.

In many tumor types, the activity of the RARβ receptor itself is inhibited through various signalling pathways, which inhibition leads to mTOR activation. The results of my work confirm that downregulation of RARβ is also present in S102 angiomyolipoma cells with TSC mutations. Our study shows that the ability to metabolize retinol in TSC-deficient cells is severely impaired, with levels of several enzymes (aldehyde and alcohol dehydrogenases), differing from those measured in wild-type controls. A typical function of aldehyde dehydrogenases is the oxidation of aldehydes, which otherwise minimize ROS production and are involved in signalling pathways and RA signalling cascades. Variations in the levels of alcohol dehydrogenases affect retinoic acid biosynthesis in the liver. In particular, it is important to highlight that the co-expression of vitamin A metabolic enzymes and the physical interactions between them are tightly regulated and play an important role in regulating cell differentiation, proliferation, and migration. Our experiments have highlighted that patients may benefit from a combination of RA and routine rapamycin treatment in diseases affected by TSC mutations. Such treatment with reduced doses of rapamycin and normalized levels of enzyme activity in vitamin A metabolism may lead to beneficial physiological effects, including reduced cell proliferation and cell migration.

Based on our results, the combined treatment of retinoic acid with rapamycin provided not only encouraging results, but the results of the other two drugs used in combination (proxyson and auranofin) may be the subject of further studies shortly. We examined the apoptotic signalling, which revealed significantly elevated levels of p53 protein in TSC (-/-) mutant S102 cells, indicating a disturbance in the balance between pro- and anti-apoptotic regulation. In TSC mutant cells, cell death initiation mechanisms are inhibited, and apoptosis induction does not occur despite p53 activation, largely due to defective regulation between mTOR and p53 protein. The AKT-AMPK-mTOR-P70S6K signalling pathway plays a central role in regulating p53 protein activity. Inhibitory processes associated with p53 are transmitted to mTOR via the dysfunctional AMPK-TSC pathway, which disrupts the feedback loop when mutations in the TSC genes persist. We also revealed that in the presence of TSC mutations, anti-ROS reductive mechanisms show higher activity, which does not allow the initiation of mitophagy and autophagy. Our knowledge of p53 suggests that the accumulation and increased phosphorylation of the protein at serine 15, 20 and/or 46 sites promotes the transactivation of pro-apoptotic target genes. Phosphorylation of p53 alone is not sufficient to induce any of the signalling pathways required to induce apoptosis. However, targeting deregulated phosphorylation cascades, mitochondrial function or metabolic enzyme levels may be possible ways to normalise cell death mechanisms.

TSC (-/-) mutant S102 cells show, in addition to abnormalities in p53 protein, increased proliferation, reduced mitochondrial membrane potential, increased TrxR activity, and abnormal mitochondrial biogenesis. mTOR regulates mitochondrial activity and plays a central role in setting the delicate relative balance determining whether ATP is produced from mitochondrial or non-mitochondrial sources. In several cases, cancer cells produce

ATP by anaerobic fermentation with higher glycolysis rates, even in the presence of oxygen (Warburg effect). The Warburg effect has been demonstrated in TSC-deficient cell lines as well as in neoplasms driven by TSC mutations, such as LAM and AML. The 'Warburg effect' leads to increased production of ROS during respiration and a reduction in the antioxidative capacity of affected cells, resulting in mitochondrial damage. A cell in a state of increased ROS production tries to increase its ROS scavenging capacity through NADPH oxidation and activation of the enzyme TrxR. Redox homeostasis, which regulates the predominance of ROS, is a critical component of cell survival. Several antioxidant molecules and cascades are involved, including PrdX proteins. The Prdx family of enzymes consists of thiol-dependent peroxidase enzymes that play a central role in cellular redox reactions. Prdx5, a thioredoxin peroxidase enzyme that does not require cofactors, is found in mitochondria, peroxisomes, cytosol, and the nucleus. Its main function is to act as a cytoprotective antioxidant. Since ROS-induced cytotoxicity is a central component of chemo- and radiotherapies, increased levels and activity of antioxidant enzymes, including Prdx5, play an important role in developing chemo- and radioresistance in cancer cells. While the cellular response depends on the antioxidant status of the cell, depletion of the antioxidant molecule Prdx5 can make cells sensitive to chemotherapy. The regulation of Prdx5 is complex, and its levels are significantly increased in TSC (-/-) mutant S102 cells. Elevated Prdx5 levels can be reduced by combination treatment with rapamycin and auranofin, approaching the levels detected in TSC (+/+) wild-type cells. Prdx5 is a target of both rapamycin and auranofin. As Prdx5 is a protein that communicates between mitochondria and the ER, the significant upregulation of the ER stress protein HSP70 following auranofin and rapamycin combination treatment suggests that the ER and UPR play an important role in ROS-induced cell death. HSP70 is well known for preventing protein aggregate formation under increased intracellular stress. Properly folded proteins enter the Golgi complex, while misfolded proteins accumulate in the ER, coupled to the HSP70 chaperone molecule. The complex between the unfolded or misfolded protein and the HSP70 molecule triggers the UPR-mediated cell death process. Prxd5 can protect cells from such death, indicating that Prdx5 has a dual role in these complex biochemical mechanisms.

The third molecule tested, proxyson, also used in combination therapy, reduces TrxR activity, increases ROS production, and reduces cell viability. The results suggest that the mechanism of action of proxyson, similar to auranofin, is based on an upset of the cellular antioxidant balance, leading to increased oxidative burdens and cell death in TSC (-/-) mutant S102 cells.

In summary, our experiments suggest that some patients affected by TSC mutation-regulated neoplasms may benefit from auranofin and rapamycin or proxyson and rapamycin combination therapy, which could potentially prolong remission or slow disease progression, improving patients' quality of life.

Short summary of the new results

1. The results of our experiments allowed us to characterize the morphological abnormalities and increased proliferative capacity of AML cells with TSC mutations. The results showed that the mitochondria of cells with TSC mutations are smaller than healthy controls, their cristae are not recognizable, and mitochondrial function is impaired. The latter was demonstrated by showing a decrease in mitochondrial membrane potential levels and changes in the levels of mitochondrial proteins prohibitin and VDAC.

2. We have identified several molecular mechanisms associated with TSC mutations, which, based on the results presented here, may serve as potential new therapeutic targets in diseases with TSC mutations. In our work, we have characterized the expression and phosphorylation changes of proteins involved in apoptosis in TSC mutant cells (tumor suppressor protein p53), the alterations of their vitamin A metabolism (RARß, ADH, and ALDH enzymes), and the involvement of their antioxidant system (TrxR).

   Based on the literature, reducing the therapeutic dose of rapamycin would be desirable for patients with AML and LAM. One of the reasons for this is the serious side effects it causes, which significantly impair the quality of life of patients and the adverse changes observed, for example, in vitamin A metabolism or the antioxidant capacity of cells following rapamycin mono treatment. Vitamin A metabolism can be favorably influenced by the use of retinoic acid, while antioxidant capacity can be effectively regulated through the inhibition of the TrxR enzyme.

3. Our results demonstrate that several enzymes involved in vitamin A metabolism and the nuclear receptor RARβ may also be potential therapeutic targets in TSC mutant tumors. In our experiments, by reducing the level of rapamycin, the inhibitory effect of rapamycin on ALDH could be reversed, allowing for a more balanced vitamin A metabolism and RARβ activity. Increased expression and activity of RARβ may lead to inhibition of cell migration and proliferation, which is associated with improved patient outcomes.

4. Our results suggest that upsetting the antioxidant balance through inhibition of the TrxR enzyme is a possible pathway to modulate p53-associated signalling pathways in the right direction. Currently, the potent anti-apoptotic microenvironment of TSC mutant cells cannot be fully overcome by the FDA-approved drug rapamycin. Our study shows that a lower dose of rapamycin (10 nM) in combination with the drug candidate Proxyson (3 μM) can result in cell death. Although further studies are needed, co-inhibiting the mTOR pathway and TrxR could potentially result in more effective therapy.

5. The FDA-approved auranofin, tested in our experiments in mono and combination treatments, also targets the cellular antioxidant system, including TrxR and Prdx5 enzymes. Used alone, auranofin is a less-tolerated drug with severe side effects and a strong cytotoxic effect. Our experiments have demonstrated that auranofin, in combination with rapamycin, significantly reduces the proliferative activity of cells with TSC mutations and provides better tolerability for healthy cells without mutations. Its reuse in combination therapy for

treating tumors with TSC mutations requires further investigation. As both drugs also have immunosuppressive activity, the timing of such combination therapy in some cancer patients may require increased attention to avoid interference with otherwise used immunotherapies.

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Publications

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- **Bovari-Biri, J**; Abdelwahab, EMM; Garai, K; Pongracz, JE: **Prdx5 in the regulation of Tuberous Sclerosis Complex mutation-induced signalling mechanisms.**

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- **Bovari-Biri, J**.; Garai, K. ; Banfai, K. ; Csongei, V. ; Pongracz, J.E. **miRNAs as Predictors of Barrier Integrity**

  2023. Biosensors, 13 : 4 Paper: 422 , 21 p., https://doi.org/10.3390/bios13040422 **(IF: 5,743) Q1**

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