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SFB 1052 Obesity Mechanisms

The novel phosphatidylinositol-3-kinase (PI3K) inhibitor alpelisib effectively inhibits growth of PTEN haploinsufficient lipoma cells

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Background

Germline mutations in the tumor suppressor gene PTEN cause PTEN Hamartoma Tumor Syndrome (PHTS). Pediatric patients frequently develop lipomas, for which there is no current treatment option except surgery. Treatment attempts with the mTORC1 inhibitor rapamycin could not reverse lipoma growth[1]. Recently, lipomas associated with a related syndrome caused by mosaic activating PI3K mutations (PIK3CA-related overgrowth syndrome, PROS) were successfully treated with the novel PI3K inhibitor alpelisib.[2] Here we tested whether alpelisib has growth-restrictive effects and induces apoptosis in lipoma cells from pediatric patients with PHTS and PROS.

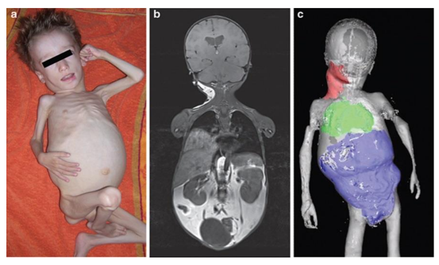

Figure 1: Patient with heterozygous PTEN mutation and abdominal lipoma.[2]

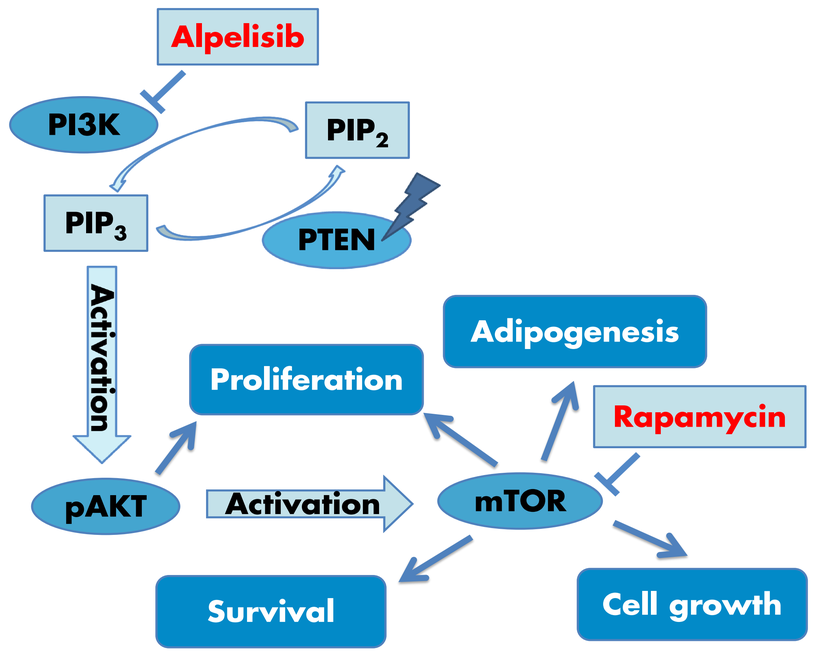

Figure 2: PI3K/AKT/mTOR pathway. PIP2: Phosphatidyl-inositol 4,5-bisphosphate, PIP3: Phosphatidylinositol (3,4,5)-trisphosphate

Results

Cell viability and proliferation

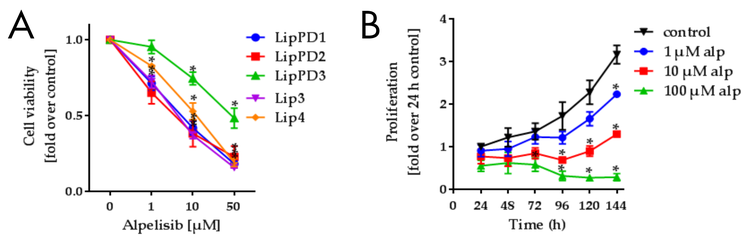

Figure 3: Viability of PHTS (LipPD1, 2 and 3) and PROS (Lip3 and 4) patients lipoma cells during alpelisib treatment. (A) WST-1 assays after 72 h treatment demonstrated a concentration dependent reduction in cell viability for all lipoma cells. (B) 100 µM alpelisib (alp) completely inhibited proliferation of LipPD1 cells. n=3, * $p \geq 0.05$

Cell death

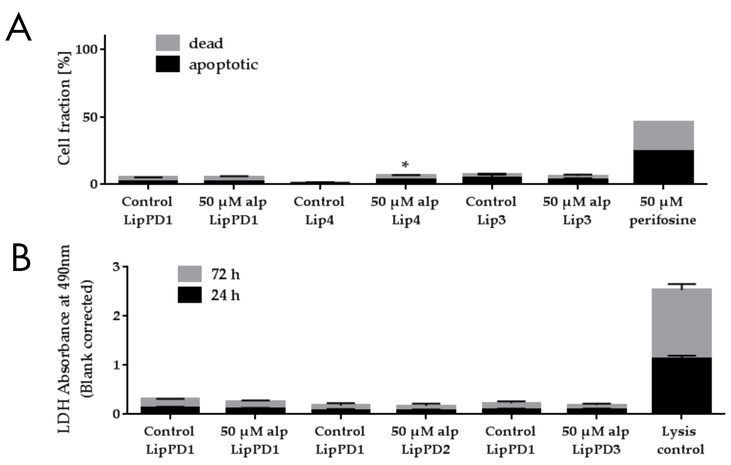

Figure 4: Cell death after alpelisib treatment of PHTS (LipPD1, 2 and 3) and PROS (Lip3 and 4) patients lipoma cells. (A) Annexin V/PI apoptosis assay showed no apoptosis induction in PHTS or in PROS patient lipoma cells after 72 h 50 µM alpelisib treatment, positive control: AKT inhibitor perifosine (50 µM). (B) LDH cytotoxicity assays showed no cell death after 24 h or 72 h 50 µM alpelisib treatment for PHTS lipoma cells. n=3, * $p \geq 0.05$

Proliferation markers and metabolism

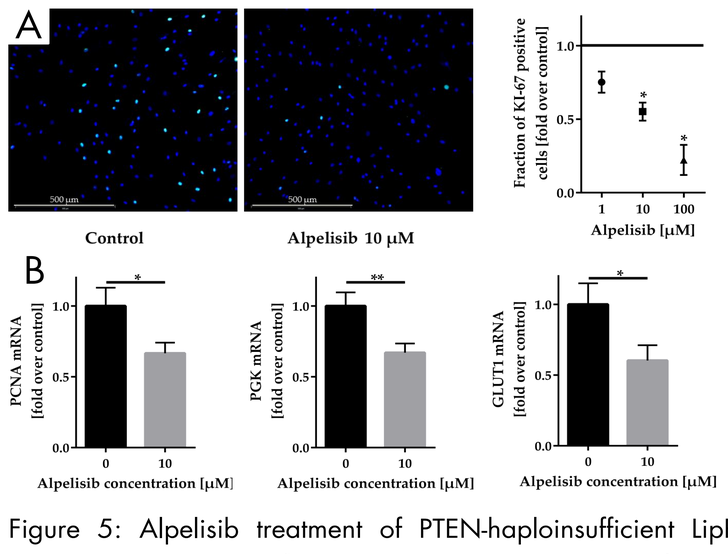

Figure 5: Alpelisib treatment of PTEN-haploinsufficient LipPD1 cells. (A) Ki-67 immunofluorescence staining (green) and Hoechst nuclei staining (blue) of LipPD1 cells after 48 h treatment. Proliferation marker Ki-67 positive cell fraction decreased in a concentration dependent manner. (B) qPCR of proliferation marker *PCNA*, glycolysis enzyme *PGK* and glucose transporter *GLUT1* genes showed reduction after 24 h 10 µM alpelisib treatment. n=3, * $p \geq 0.05$, ** $p \geq 0.01$

Signaling

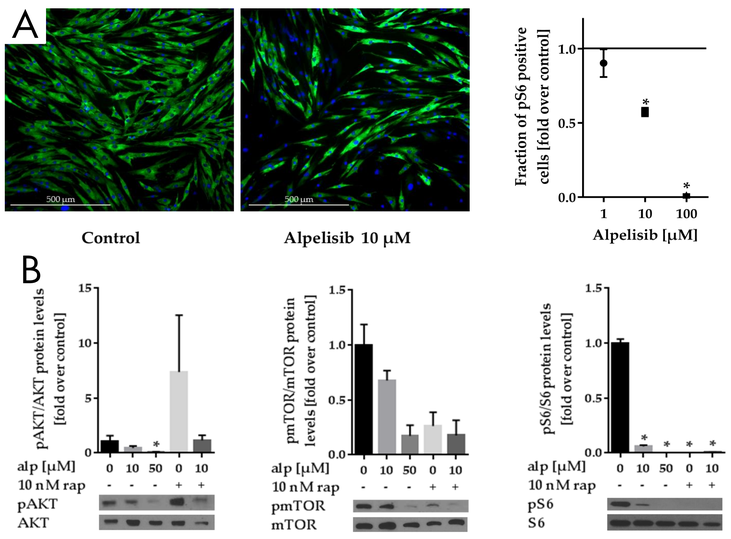

Figure 6: PI3K pathway deactivation after alpelisib treatment in LipPD1 cells. (A) Phosphorylated ribosomal protein S6 (pS6) immunofluorescence staining (green) and Hoechst nuclei staining (blue) of LipPD1 cells after 48 h 10 µM alpelisib treatment. pS6 positive cell fraction decreased in a concentration dependent manner. (B) Western blots showed reduced activation of AKT, mTOR and S6 after 24 h alpelisib treatment. n=3, * $p \geq 0.05$

Differentiation and senescence

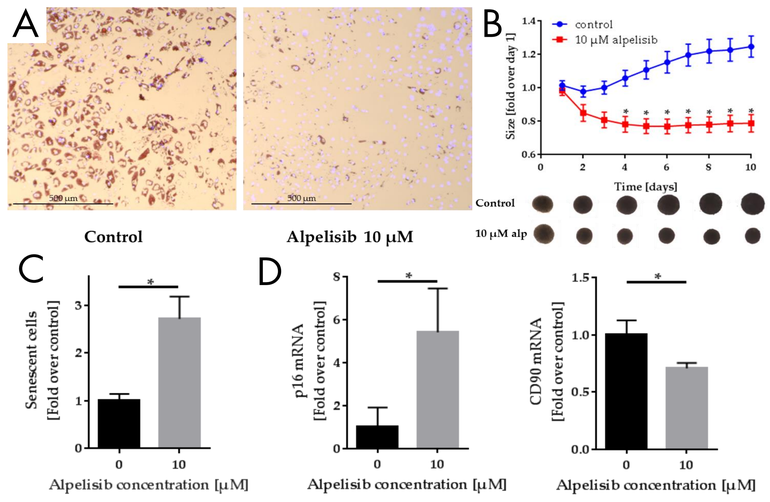

Figure 7: Adipogenesis and senescence under alpelisib treatment of LipPD1 cells (A) Oil-Red O lipid (red) and Hoechst nuclei (blue) staining after 10 days of adipocyte differentiation showed reduced adipogenesis using 10 µM alpelisib. (B) Size of 3D lipoma spheroids during 10 days in differentiation medium decreased with 10 µM alpelisib. (C) β-Galactosidase staining after 72 h alpelisib treatment showed elevated number of senescent cells. (D) Senescence marker *p16* mRNA was elevated after 10 days of differentiation in 10 µM alpelisib while the mesenchymal stem cell marker *CD90* mRNA was reduced. n=3, * $p \geq 0.05$

Conclusion

The attenuated activation of AKT through inhibition of PI3K with alpelisib reduced cell viability and proliferation of *PTEN* mutant lipoma cells in vitro without induction of cell death. Alpelisib prevented adipogenesis and induced senescence of preadipocytes, thereby reducing the size of lipoma cell spheroids. Since alpelisib was well tolerated in first clinical trials also for pediatric PROS patients[2], this drug could be a potential new treatment option for PHTS-related adipose tissue hyperplasia.

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REFERENCES

1 Sirolimus treatment of severe PTEN hamartoma tumor syndrome: case report and *in vitro* studies (Schmid et al. 2014)
2 Targeted therapy in patients with PIK3CA-related overgrowth syndrome. (Venot et. al. 2018)

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