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 growth¹. 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.

Results
Cell viability and proliferation

Figure 3: Viability of PHTS (LipP1, 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 LipP1 cells.

Cell death

Figure 4: Cell death after alpelisib treatment of PHTS (LipP1, 2 and 3) and PROS (Lip3 and 4) patients lipoma cells. (A) Annexin V/PI apoptosis assay showed no apoptosis induction in PHTS or 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.

Proliferation markers and metabolism

Figure 5: Alpelisib treatment of PTEN-haploinsufficient LipP1 cells. (A) Ki67 immunofluorescence staining (green) and Hoechst nuclei staining (blue) of LipP1 cells after 48 h treatment. Proliferation marker Ki67 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 < 0.05, ** p < 0.01

Signaling

Figure 6: PI3K pathway deactivation after alpelisib treatment in LipP1 cells. (A) Phosphorylated ribosomal protein S6 (pS6) immunofluorescence staining (green) and Hoechst nuclei staining (blue) of LipP1 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 < 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 patients², this drug could be a potential new treatment option for PHTS-related adipose tissue hyperplasia.

REFERENCES

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