OLIVE LEAF EXTRACT INHIBITS CELLULAR AND CYSTIC GROWTH IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE: AN IN VITRO MODEL.

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INTRODUCTION AND OBJECTIVES

Mutations in polycystin-1 (PKD1) and polycystin-2 (PKD2) genes cause Autosomal Dominant Polycystic Kidney Disease (ADPKD), a genetic disorder characterized by enlarged cysts that subverts the renal architecture, leading to end-stage renal disease in about 50% of cases. To date, there are no specific pharmacological treatments to counteract the cystic invasion of the renal parenchyma.

Recent accumulated in vitro and in vivo evidences demonstrated that the natural polyphenolic compounds possess therapeutic properties. Therefore, the present study aimed to analyze the effect of polyphenols extracted from olive leaf in epithelial cells bearing mutation in PKD1 gene with the goal of establish whether and how these polyphenols were able to mitigate cystogenesis.

METHODS

Immortalized proximal tubular renal cells obtained from human autosomal dominant polycystic kidney cysts, WT9-12; immortalized human tubular cells HK2; MTT assay; western blotting (WB); Real Time PCR; DNA Laddering; 3D cellular cultures; transient trasfections.

RESULTS AND CONCLUSIONS

From the evaluation of the growth rate of WT 9-12 versus HK2, which do not exhibit PKD1 gene mutations, it emerges that the number of cells doubles after 48h in WT 9-12 confirming that this mutation gives them a higher growth rate compared to HK2 already in basal conditions (Fig 1). In monolayer culture, untreated or treated with Olive Leaf Extract (OLE), we evaluated cell viability by MTT assay and protein levels influenced by the PC1/PC2 complex by western blotting. Our in vitro studies revealed that OLE exposure decreased, in a dose-dependent manner, WT9-12 cells viability (Fig 2). Concomitantly we observed that, after 72h, OLE exposure induced a protein down-regulation of Cyclin D1 and a strong trascriptional protein up-regulation of the well-known cell-cycle inhibitor, p21 (Fig 3). Transient transfection assays, performed using a construct containing wild-type p21 promoter region, have shown that OLE causes an important transcriptional activation of p21 promoter resulting in cell cycle blockade (Fig 4).

It is well known that cAMP, which levels are elevated in ADPKD cells, plays a crucial role in the pathophysiology of the disease removing the inhibition of the axis PKA-B-Raf-MEK-ERK AKT-mediated; therefore, the inhibition of the MAPK is lost leading to an excessive cell proliferation. Time course assays show that OLE decreases PKA levels, increases AKT phosphorilation, that in turn phosphorilates and inhibits B-Raf leading to ERK down-regolation. The inhibitor of PI3K, LY294002, mitigates the up-regolation of p21 OLE-induced showing the crucial role of AKT as key mediator of growth inhibition. Moreover, OLE exposure reduces the nuclear traslocation of phosphorilated CREB decreasing its binding to the cAMP responsive elements (Fig 5).

Literature data reported that elevated basal apoptotic events are directly involved in cystogenesis. Therefore, we tested different apototic markers after OLE exposure. Results obtained show that OLE reduces the DNA fragmentation, BAD and p53 protein levels, while the content of Bcl2 is increased (Fig 6).

Three-dimensional studies leading to cyst formation show, after six days of OLE exposure, significantly inhibited growth and decreased size of cysts respect to untreated cells (30%) (Fig 7).

Our results demonstrate, for the first time, that treatment with OLE slows the growth of WT9-12 cells and decreases cyst size via p21, suggesting that OLE could represent an alternative therapeutic strategy for ADPKD.

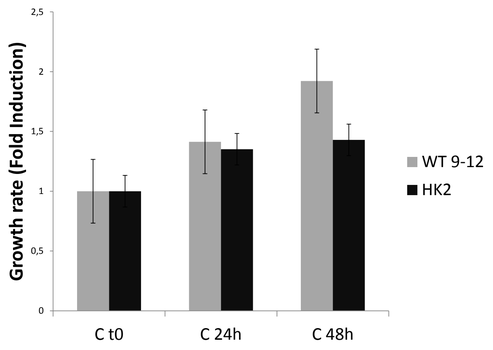

Fig. 1: The number of cells doubles after 48h in WT 9-12 vs HK2 cells.

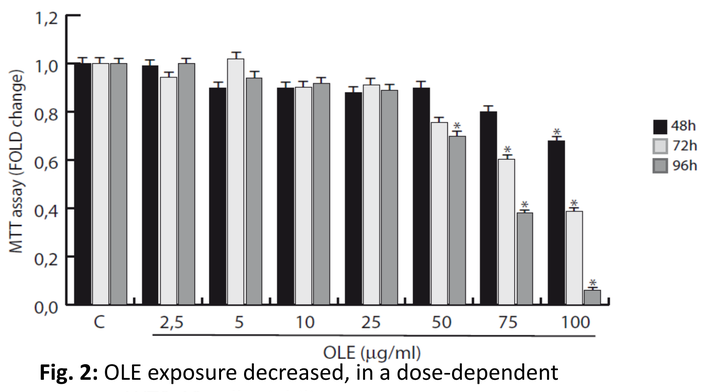

Fig. 2: OLE exposure decreased, in a dose-dependent manner, WT9-12 cells viability.

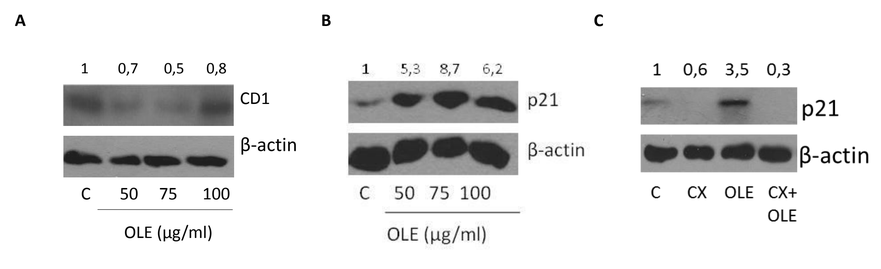

Fig. 3: After 72h, OLE exposure induced a protein down-regulation of Cyclin D1 **(A)** and a strong trascriptional protein up-regulation of the well-known cell-cycle inhibitor, p21 **(B,C).**

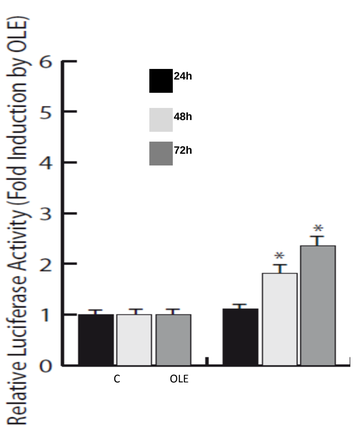

Fig. 4: OLE causes an important transcriptional activation of p21 promoter.

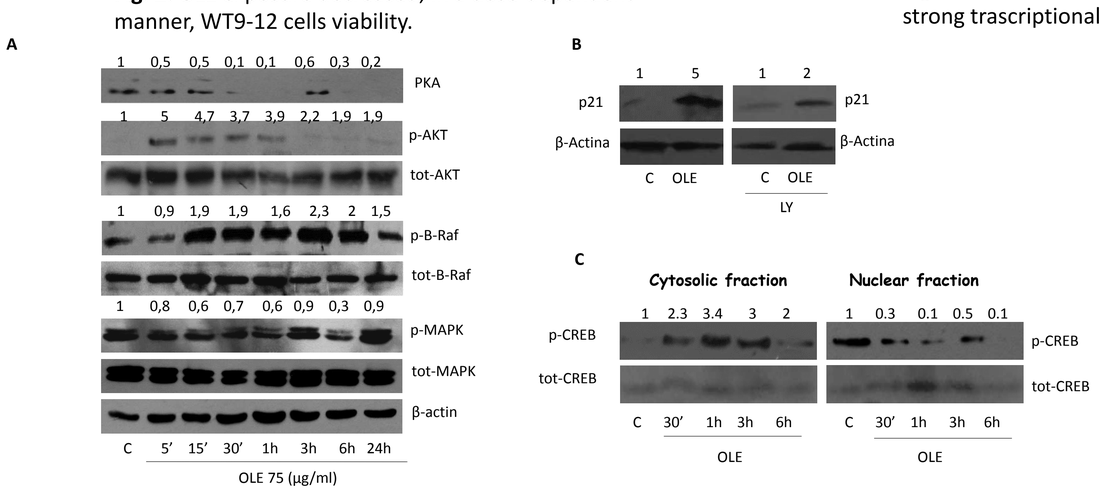

Fig. 5: A) OLE decrease PKA levels, increases AKT phosphorilation, that in turn phosphorilates and inhibits B-Raf leading to ERK downregolation. **B)** The inhibitor of PI3K, LY294002, mitigates the up-regolation of p21 OLE-induced. **C)** OLE exposure reduces the nuclear traslocation of phosphorilated CREB decreasing its binding to the cAMP responsive elements.

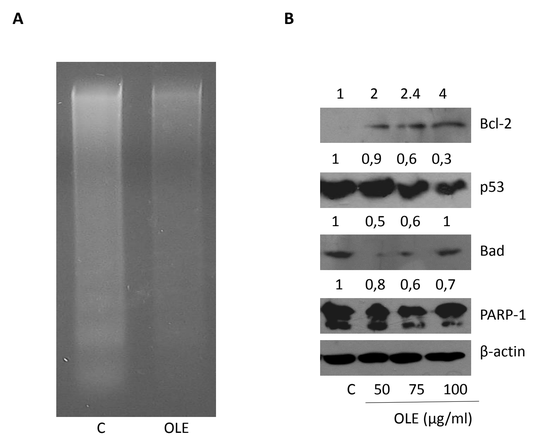

Fig. 6: OLE reduces the DNA fragmentation **(A)**, BAD and p53 protein levels, while the content of Bcl2 is increased **(B).**

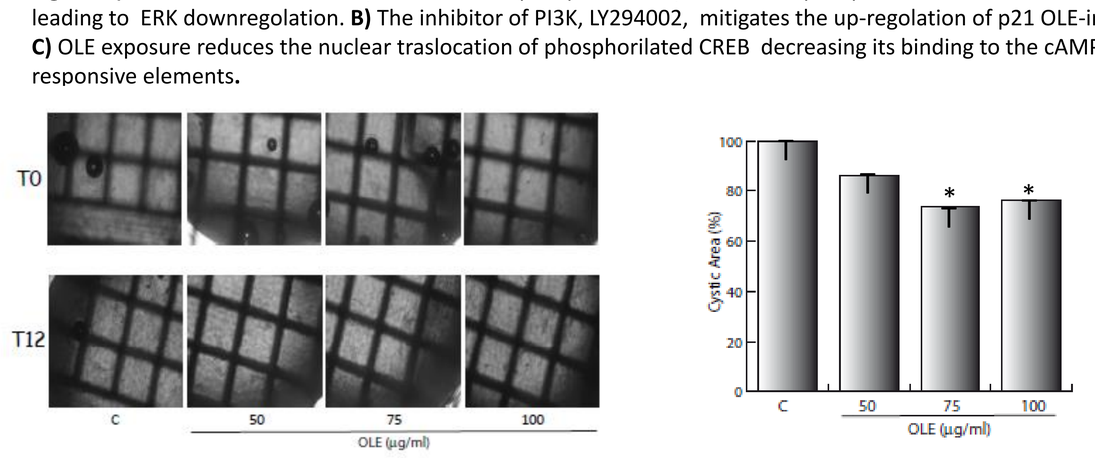

Fig. 7: After six days of OLE exposure, significantly inhibited growth and decreased size of cysts respect to untreated cells (30%)

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