mTOR INHIBITORS PROMOTE AUTOPHAGIC NOT APOPTOTIC CELL DEATH IN PROXIMAL TUBULAR RENAL CELLS, via p75^{NTR} ACTIVATION

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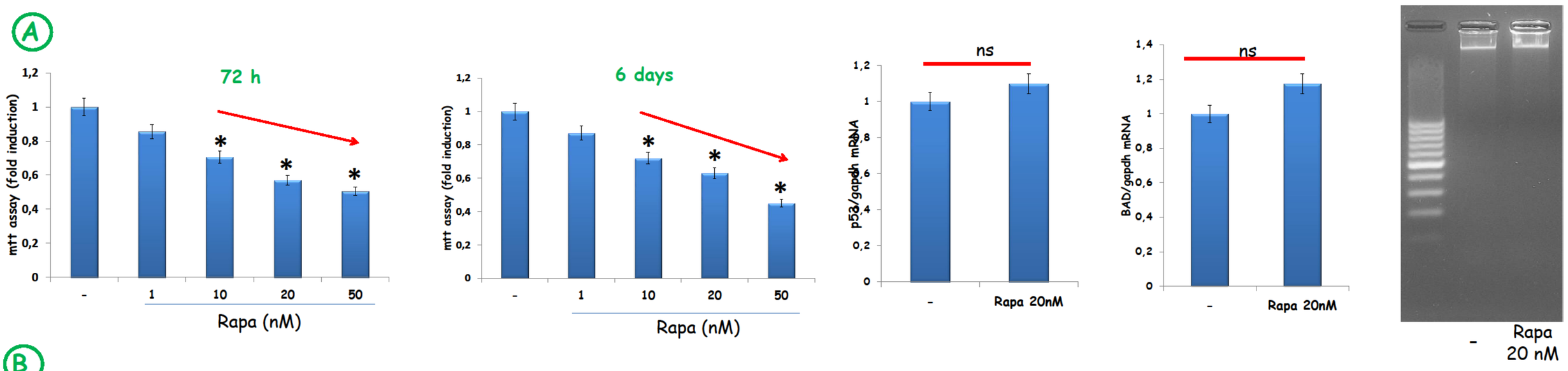
BACKGROUND AND AIM

Autophagy is a highly conserved process that entails the degradation of intracellular components through the lysosomal machinery to regenerate metabolites for energy and growth. In many contexts, autophagy promotes cell survival under stressful conditions such as nutrient and growth factor deprivation. However, excess or prolonged autophagy could promote an alternative mechanism that leads to programmed cell death. Recently some authors explored autophagy as a therapeutic strategy for kidney diseases, although the precise role of autophagy in kidney injury remains to be elucidated. Therefore pharmacological approaches to modulate autophagy are currently receiving considerable attention. One class of candidate drugs that upregulates autophagy is the mTOR inhibitors, as rapamycin and its derivates. In this study we investigated the biological mechanisms by which rapamycin reduces HK-2 cells vitality.

MATERIALS AND METHODS

Immortalized human proximal tubular renal cells, HK-2; MTT assay; DNA laddering assay, mono-dansyl-cadaverine staining, Real-time RT-PCR assays, Western Blot Analysis, RNA interference (RNAi), Transient transfection assay, Immunoprecipitation studies, Statistical analysis.



* p < 0,05 treated vs untreated; ns = not significant; ** p < 0,05 p75siRNA treated vs scramble p75 treated

RESULTS

(A) We found that in proximal tubular renal cells, HK-2, pharmacological doses of rapamycin reduced cells vitality inducing autophagic not apoptotic cell death, as we did not observed a genic and/or protein up-regulation of keys pro-apoptotic markers, p53 and BA. In addition, DNA laddering assay showed the absence of DNA fragmentation. (B) On the contrary, real-time PCR and western blot analysis showed an increase of beclin-1 and LC3 expression. Interestingly we observed for the first time that in our experimental model the autophagic process was mediated by the receptor p75^{NTR}, a death receptor belonging to the tumor necrosis receptor superfamily involved in mediating part of the biological effects exerted by the neurotrophin Nerve Growth Factor (NGF). (C) Indeed, by real time RT-PCR, western blot analysis and transiently transfection assay performed using a plasmid containing the wt-promoter p75^{NTR} gene, we observed that in HK-2 cells rapamycin exposure transcriptionally increased the expression of p75^{NTR}, which chemical inhibition, performed by silencing approaches, reversed the autophagy rapalog-induced. On the contrary, rapamycin treatment did not modulate the expression levels of NGF as well as its pro-survival receptor TrKA.

CONCLUSIONS

Collectively our results highlight a new molecular mechanism by which mTOR inhibitors, at pharmacological doses, promoted autophagy in renal cells expressing p75^{NTR} receptor, reducing cells vitality.

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