CELLULAR SENESCENCE ILK DEPENDENT IS INDUCED BY THE CONSTITUTIVE ACTIVATION OF THE INSULINE-LIKE GROWTH FACTOR-1 (IGF-1) PATHWAY

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Introduction and objectives

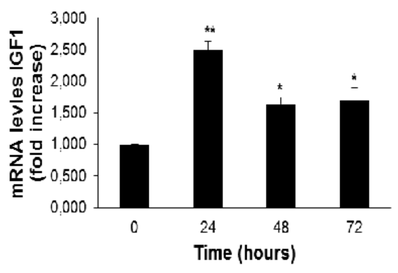
Integrin linked kinase (ILK) is a component of the multiprotein transmembrane complex which is mediating the integrin-dependent signaling. ILK plays a central role in cellular senescence induced by stressfull stimuli. ILK is up-regulated after stimulation with glycated albumin (GA), oxidants or high phosphate and increases the expression of senescent genes p53 and p16. However, the mechanisms involved in this ILK over-expression remain unclarified. On the other side, the IGF-1 axis, a nutritient sensing pathway, and its downstream intracellular effectors AKT, and FOXO, also has an essential role in the aging process. The aim of the present work was to analyze the role of IGF-1-AKT pathway stimulation in the cellular senescence induced by ILK over-expression.

Methods

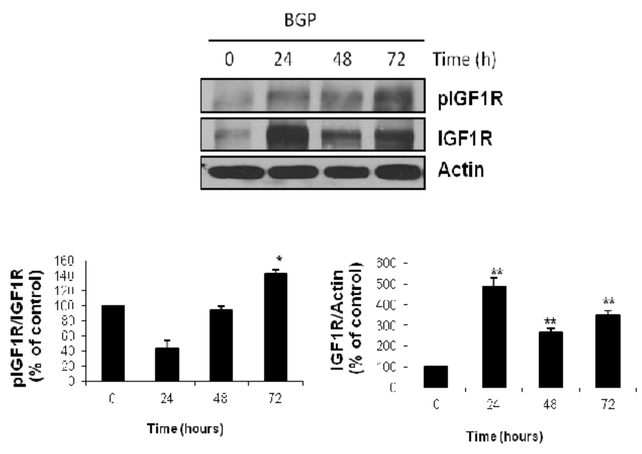
Human vascular smooth muscle cells (HVSMC) were treated with the phosphate donor beta-glycerophosphate (BGP, 10mM) for 24, 48 and 72 hours. After treatment senescence associated β-galactosisase activity (SA-B-GAL) was assessed by confocal microscopy using a fluorescent probe, and by the expression of p16 and p53 by western blot. ILK expression was analyzed by western blot. IGF-1 R activation was determined by western blot using specific phospho-IGF-1 receptor and IGF-1 receptor antibodies. To analyze the involvement of IGF-1 receptor in ILK over-expression and senescence we used a pharmacological inhibitor of IGF-1R activation, IGF-1R inhibitor 12μM, and a stable transfection of cells with a vector encoding for Klotho protein, an endogenous inhibitor of the IGF-1 receptor. IGF-1 expression was analyzed by qRT-PCR. AKT and FOXO activity and expression were assessed by western blot.

Results

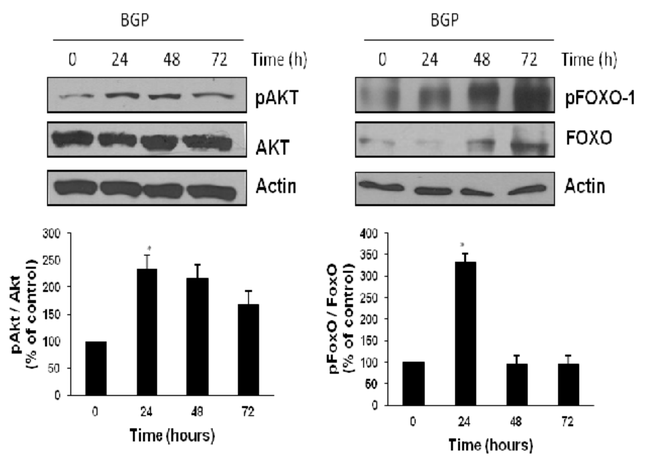
The treatment with BGP increases the mRNA expression of IGF-1



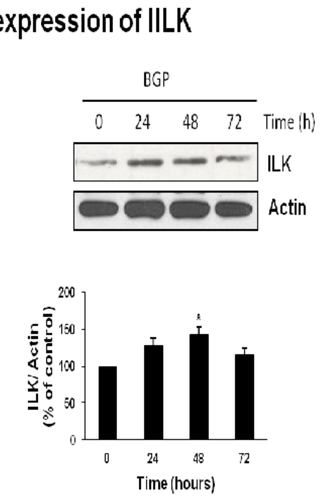
The treatment with BGP increases the expression and activity of IGF-1 receptor



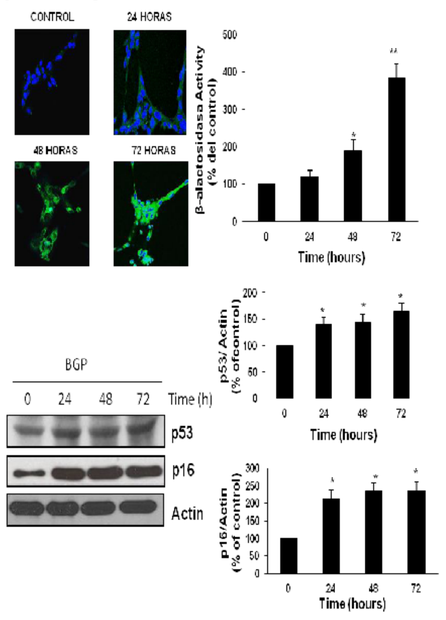
AKT activity was increases after BGP addition and FOXO-1 was inactivated in the same conditions.



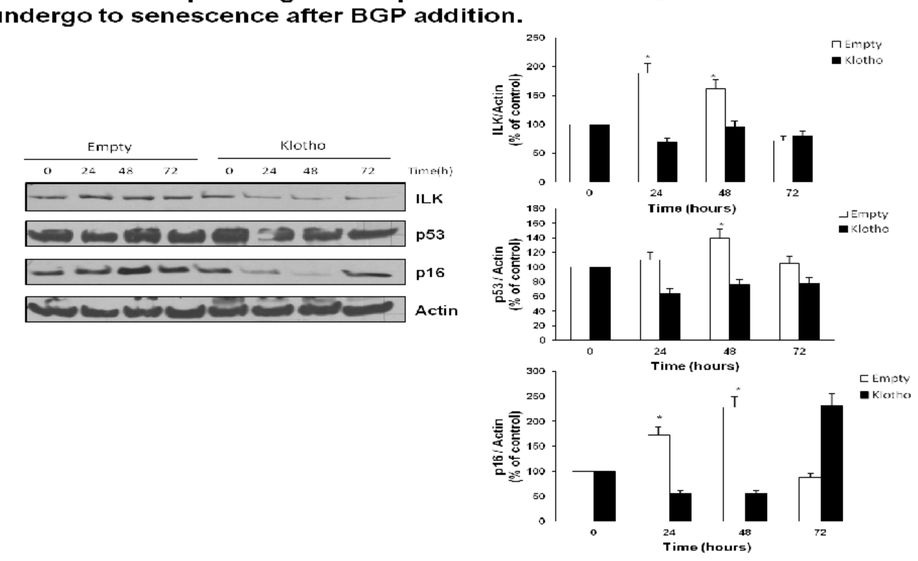
The treatment with BGP increases the expression of IILK



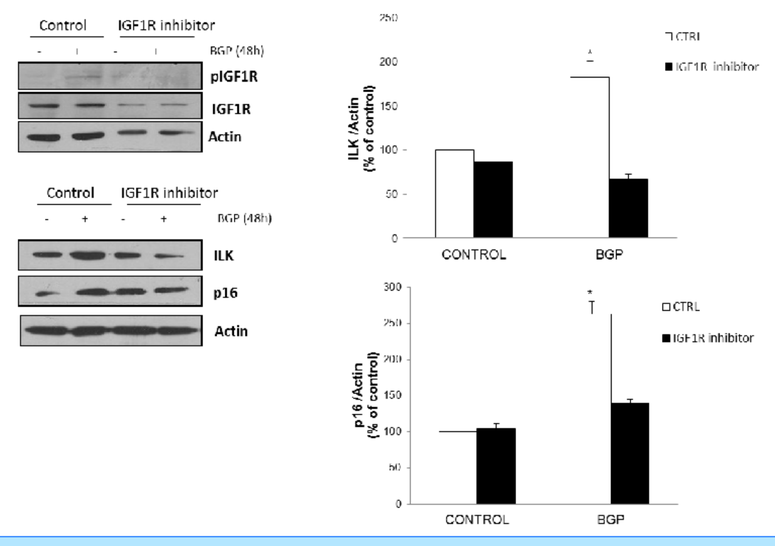
BGP promoted cellular senescence in HVSMC increasing the expression levels of p53 and p16



HVSMC over-expressing klotho protein not shown ILK increase and did not undergo to senescence after BGP addition.



The inhbition of IGF-1R with a specific inhibitor suppressed ILK expresion and senescence induced by BGP.



Conclusions

We proposed that activation of IGF-1 receptor pathway by stressful stimuli, such as high phosphate levels, is responsible for the overexpression of ILK in senescent cell.

References

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B) Cell signalling. Cell growth control and related alterations (hypertrophy, hyperplasia and apoptosis) including neoplasia.
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