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Effect of $1,25(OH)_2D_3$ in Proliferation of Human Glomerular Mesangial Cells and Expression of Ki67

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Objective: To investigate the effects of 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] impact on the expression of Ki67 in human glomerular mesangial cells and its proliferation.
Methods: Cultured human mesangial cells, taking the subcultured cells, which were randomly divided into four groups: normal control group; epidermal growth factor group (EGF group); $1,25(OH)_2D_3$ group; and combined group of EGF and $1,25(OH)_2D_3$, treatment 48 h. The expression of Ki67 were detected by immunofluorescene and fluorescence quantitative PCR (RT-PCR).
Results: Compared with the normal control group, EGF group had a higher Ki67 expression ($P < 0.05$); Ki67 expression of $1,25(OH)_2D_3$ group was significantly reduced ($P < 0.05$); compared with EGF group, Ki67 expression in $1,25(OH)_2D_3$ group and combined group of EGF and $1,25(OH)_2D_3$ was low ($P < 0.05$), there was no significant difference between the normal control group and combined group of EGF and $1,25(OH)_2D_3$ ($P > 0.05$).
Conclusion: 1,25-dihydroxyvitamin D_3 can inhibit the expression of Ki67 and the proliferation of human glomerular mesangial cells.

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Elevated Concentrations of Free Fatty Acids (Linoleic Acids) May Inhibit Mesangial Cell Proliferation and Induce Cell Cycle Arrest and Apoptosis by Lipotoxicity

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Objective: This study was to investigate the effects of free fatty acids (linoleic acids) on the mesangial cells.
Methods: Rat mesangial cells (HBZY-1) were treated with various concentrations (250 μM, 500 μM, 1000 μM, 2000 μM) of linoleic acids. Non-treated cells served as controls. 24 h, 48 h and 72 h after the stimulation, cell proliferation activity, cell cycle, cell apoptosis and intracellular lipid deposition of the cells were assessed by MTT, flow cytometry and Oil Red O staining respectively. One-way ANOVA was used to do statistical analysis and $P < 0.05$ considered as significant.
Results: Compared to the controls, the cells treated by linoleic acids (500 μM, 1000 μM, 2000 μM) were decreased in the cell proliferation activity and increased in the percentage of cells in G0/G1 phase, the apoptotic rate and the intracellular lipid deposition significantly ($P < 0.05$ or $P < 0.01$).
Conclusion: Elevated concentrations of free fatty acids (linoleic acids) may inhibit mesangial cell proliferation and induce cell cycle arrest and apoptosis by lipotoxicity.

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Effects of $1,25(OH)_2D_3$ on Proliferation and Expression of mTOR/p70s6K of Human Glomerular Mesangial Cells

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Objective: To investigate the effects of 1,25-dihydroxyvitamin D_3[$1,25(OH)_2D_3$] on cell proliferation in human glomerular mesangial cells and its effects in the regulation of glomerular mesangial cells of the mTOR/p70s6K signaling pathways.
Methods: The cultured human mesangial cells, which was subcul-tured 3～7 generations, were divided into four groups:normal control group, 1,25-dihydroxyvitamin D_3 (10^{-8} mol/L) group, rapamycin (5 μg/mL) group and rapamycin combined 1,25-dihydroxyvitamin D_3 group for treatment of 48 h. The effects of mesangial cell proliferation were measured by CCK-8 colorimetric assay. The cell cycles were measured by flow cytometry. The expression of mTOR and p70s6K were detected by immunofluorescence.
Results: (1) CCK -8 assay detects the cells proliferation and flow cytometry detects the cell cycles, compared with normal control group, the human glomerular mesangial cells of 1,25-dihydroxyvitamin D_3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D_3 group were significantly inhibited and cell cycle were blocked in G_1 phase ($p < 0.01$); compared with 1,25-dihydroxyvitamin D_3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D_3 group were significantly inhibited and cell cycle were blocked in G_1 phase ($p < 0.01$); compared with rapamycin group, the mesangial cells proliferation and cell cycles of rapamycin combined 1,25-dihydroxyvitamin D_3 group were inhibited and cell cycle were blocked in G_1 phase ($p < 0.05$). (2) Immunofluorescence detects the expression of mTOR and p70s6K Compared with normal control group, the expression of mTOR and p70s6K in 1,25-dihydroxyvitamin D_3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D_3 group were significantly recudced ($p < 0.01$); compared with 1,25-dihydroxyvitamin D_3 group, the expression of mTOR and p70s6K in rapamycin group have no obvious difference ($p > 0.05$), rapamycin combined 1,25-dihydroxyvitamin D_3 group were significantly recudced ($p < 0.01$); compared with rapamycin group, the expression of mTOR and p70s6K in rapamycin combined 1,25-dihydroxyvitamin D_3 group were reduced ($p < 0.05$).
Conclusion: 1,25-dihydroxyvitamin D_3 can inhibit mesangial cell proliferation significantly, and 1,25-dihydroxyvitamin D_3 may regulate the glomerular mesangial cell proliferation through the mTOR/p70s6K signaling pathways.

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1,25-Dihydroxyvitamin D_3 Modulated Human Mesangial Cell Proliferation via PI3K/Akt/mTOR Pathway

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Objective: While the serine/threonine protein kinase (Akt) has attracted attention as a mediator of survival (anti-apoptotic) signal, the PI3-kinase-Akt-mammalian target of rapamycin pathway (PI3K/Akt/mTOR) is critical for cellular growth and survival in varied cells, but the regulation and function of it in mesangial cells is not well known. In this study, we evaluated the role of PI3K/Akt/mTOR signaling pathway in inhibiting the survival of human glomerular mesangial cells (HMC) induced to differentiate with 1,25-dihydroxyvitamin D_3 (1,25D).
Methods: To explore the effects of 1,25D and the significance of the PI3K/Akt/mTOR pathway, we selected 1,25D and PI3-kinase inhibitor (LY294002) intervened HMC for 48 hours, its mechanisms were examined in cultured rat mesangial cells by cell counting kit-8 (CCK-8) assay, flow cytometry, real-time fluorescence quota PCR and western blot.
Results: 1,25D, LY294002 and 1,25D combined LY294002 inhibited mesangial cells proliferation and blocked cell cycle into G_1 phase, resulted in increased levels of Akt mRNA expression but decreased mTOR levels of mRNA expression, the phosphorylation of Akt and mTOR were decreased after the exposure to 1,25D and LY294002.
Conclusion: These results demonstrate that 1,25D can inhibit mesangial cells proliferation and blocks G_1 to S phase cell cycle significantly, and 1,25D may regulate the glomerular mesangial cells proliferation through mTOR signaling pathway.

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