0282
Effect of 1,25(OH)2D3 in Proliferation of Human Glomerular Mesangial Cells and Expression of Ki67
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Objective: To investigate the effects of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] on cell proliferation in human glomerular mesangial cells and its expression of Ki67.

Methods: Cultured human mesangial cells were randomly divided into four groups: normal control group, EGF group, 1,25(OH)2D3 group and combined group of EGF and 1,25(OH)2D3 (10-8 mol/L) for 48 h. The expression of Ki67 were detected by immunofluorescence and fluorescence quantitative PCR (RT-PCR).

Results: Compared with the normal control group, the EGF group had a higher Ki67 expression (P < 0.05); Ki67 expression of 1,25(OH)2D3 group was significantly reduced (P < 0.05); compared with EGF group, Ki67 expression in 1,25(OH)2D3 group and combined group of EGF and 1,25(OH)2D3 was low (P < 0.05), there was no significant difference between the normal control group and combined group of EGF and 1,25(OH)2D3 (P > 0.05).

Conclusion: 1,25-dihydroxyvitamin D3 can inhibit the expression of Ki67 and the proliferation of human glomerular mesangial cells.

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0284
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. Nontreated 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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0290
Effects of 1,25(OH)2D3 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 D3 [1,25(OH)2D3] 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 D3 group (10-8 mol/L) group, rapamycin (5 μg/mL) group and rapamycin combined 1,25-dihydroxyvitamin D3 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 D3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D3 group were significantly inhibited and cell cycle were blocked in G1 phase (p < 0.01); compared with 1,25-dihydroxyvitamin D3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D3 group were significantly inhibited and cell cycle were blocked in G1 phase (p < 0.01); compared with rapamycin group, the mesangial cells proliferation and cell cycles of rapamycin combined 1,25-dihydroxyvitamin D3 group were inhibited and cell cycle were blocked in G1 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 D3 group, rapamycin group and rapamycin combined 1,25-dihydroxyvitamin D3 group were significantly reduced (p < 0.01); compared with 1,25-dihydroxyvitamin D3 group, the expression of mTOR and p70s6K in rapamycin group have no obvious difference (p > 0.05), rapamycin combined 1,25-dihydroxyvitamin D3 group were significantly reduced (p < 0.01); compared with rapamycin group, the expression of mTOR and p70s6K in rapamycin combined 1,25-dihydroxyvitamin D3 group were reduced (p < 0.05).

Conclusion: 1,25-dihydroxyvitamin D3 can inhibit mesangial cell proliferation significantly, and 1,25-dihydroxyvitamin D3 may regulate the glomerular mesangial cell proliferation through the mTOR/p70s6K signaling pathways.

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0293
1,25-Dihydroxyvitamin D3 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 D3 (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 (LY29402) 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, LY29402 and 1,25D combined LY29402 inhibited mesangial cells proliferation and blocked cell cycle into G1 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 LY29402.

Conclusion: These results demonstrate that 1,25D can inhibit mesangial cells proliferation and blocks G1 to S phase cell cycle significantly, and 1,25D may regulate the glomerular mesangial cells proliferation through mTOR signaling pathway.

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