六味地黄丸通过Wnt/β-连环蛋白通路调控细胞交互促进腱骨愈合的机制Δ

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摘　要　目的　探讨六味地黄丸通过Wnt/β-连环蛋白（β-catenin）通路调控骨髓间充质干细胞（BMSCs）与巨噬细胞交互作用促进腱骨愈合的机制。**方法**　将BMSCs与巨噬细胞按1∶4的比例进行共培养，分为对照组、10%含药血清组、20%含药血清组、Wnt/β-catenin通路抑制剂Dickkopf相关蛋白1（DKK-1）组、20%含药血清+DKK-1组。细胞共培养48 h，成骨诱导14 d后，检测巨噬细胞中CD86、CD206阳性细胞率，BMSCs中钙结节阳性染色面积占比，细胞上清液中骨钙素（OCN）、抑瘤素M（OSM）水平和碱性磷酸酶（ALP）活性，细胞中OCN、Runt相关转录因子2（RUNX2）、OSM、低密度脂蛋白受体相关蛋白5（LRP5）、β-catenin mRNA及蛋白表达水平。采用Pearson相关性分析考察LRP5、β-catenin蛋白表达分别与OCN、RUNX2、OSM蛋白表达及CD206阳性细胞率的相关性。**结果**　与对照组比较，10%含药血清组和20%含药血清组的CD86阳性细胞率均显著降低，CD206阳性细胞率、钙结节阳性染色面积占比、OCN水平、OSM水平、ALP活性和OCN、RUNX2、OSM、LRP5、β-catenin mRNA及蛋白表达水平均显著升高（$P<0.05$）。与20%含药血清组比较，20%含药血清+DKK-1组上述指标变化均显著逆转（$P<0.05$）。LRP5、β-catenin蛋白表达与OCN、RUNX2、OSM蛋白表达及CD206阳性细胞率均呈正相关。**结论**　六味地黄丸可通过激活Wnt/β-catenin通路，诱导巨噬细胞向M2型极化并分泌OSM，促进BMSCs成骨分化与基质矿化，协同促进腱骨愈合。

关键词　六味地黄丸；骨髓间充质干细胞；Wnt/β-catenin通路；腱骨愈合；巨噬细胞极化

Mechanism of Liuwei dihuang pill promoting tendon-bone healing by regulating cell crosstalk through Wnt/β-catenin pathway

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ABSTRACT　OBJECTIVE To explore the mechanism by which Liuwei dihuang pill promotes tendon-bone healing by regulating the interaction between bone marrow mesenchymal stem cells (BMSCs) and macrophages through Wnt/β-catenin pathway. **METHODS** BMSCs were co-cultured with macrophages at a ratio of 1∶4 and divided into control group, 10% drug-containing serum group, 20% drug-containing serum group, Wnt/β-catenin pathway inhibitor Dickkopf-related protein 1 (DKK-1) group, and 20% drug-containing serum+DKK-1 group. After 48 hours of cell co-culture and 14 days of osteogenic induction, the CD86 and CD206 positive cell rates in macrophages, the proportion of calcium nodule positive staining area in BMSCs, the levels of osteocalcin (OCN) and oncostatin M (OSM) and alkaline phosphatase (ALP) activity in cell supernatant, as well as the mRNA and protein expression levels of OCN, Runt-related transcription factor 2 (RUNX2), OSM, low-density lipoprotein receptor-related protein 5 (LRP5), β-catenin in cells were detected. Pearson correlation analysis was performed to assess the correlation between LRP5 and β-catenin protein expression with OCN, RUNX2, OSM protein expression, and CD206 positive cell rate, respectively. **RESULTS** Compared with the control group, the CD86 positive cell rates in the 10% drug-containing serum group and the 20% drug-containing serum group were significantly reduced, while the CD206 positive cell rate, proportion of calcium nodule positive staining area, OCN

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