[1]李红丽,申利红,芦永良,等.Wnt3a对小鼠破骨细胞分化调控的影响[J].第三军医大学学报,2013,35(05):412-415.

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Wnt3a对小鼠破骨细胞分化调控的影响(PDF)

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Title: Effect of Wnt3a on regulation of mouse osteoclast differentiation

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摘要: 目的 探讨Wnt3a对小鼠破骨细胞分化调控的影响。 方法 将细胞分为4组：阴性对照组(即空白组)、实验对照组(感染Ad-GFP组)、阳性对照组(50 ng/ml RANKL诱导组)、实验组(感染Ad-Wnt3a组)。分别给予处理因素后常规细胞培养6 d，通过酒石酸抗酸性磷酸酶(TRAP)染色观察破骨细胞的分化成熟情况；Western blot检测TRAP、Cathepsin K蛋白的表达；分别于处理因素后3、6、9 d提取细胞总RNA，荧光定量Real time(RT-PCR)检测核因子κB受体（RANK）、抗酒石酸酸性磷酸酶（TRAP）、组织蛋白酶K(Cathepsin K)、基质金属蛋白酶-9（MMP-9）基因的表达。 结果 TRAP染色显示50 ng/mL RANKL诱导组能成功诱导RAW 264.7成多核（核≥10），空白组和Ad-GFP组有少量多核细胞（3≤核≤5）,Ad-Wnt3a组为单核有个别双核；Western blot检测显示Ad-Wnt3a组TRAP、Cathepsin K蛋白表达降低，差异具有统计学意义（$P<0.05$）；RT-PCR检测Ad-Wnt3a组能下调RANK、TRAP、Cathepsin K、MMP-9基因的表达。 结论 Wnt3a能抑制RAW264.7分化成成熟的破骨细胞。

Abstract: Objective To explore the regulatory role of Wnt3a in the differentiation of mouse osteoclasts. Methods Cells were divided into a negative control group (blank), an experimental control group (Ad-GFP transfection), a positive control group (50 ng/mL RANKL induction), and an experimental group (Ad-Wnt3a transfection). All the cells were conventionally cultured for 6 d. Tartrate-resistant acid phosphatase (TRAP) staining was applied to observe osteoclast differentiation and maturation, and Western blot analysis was applied to detect the protein expression of TRAP and cathepsin K. Total cellular RNA was

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extracted after treatment for 3, 6 and 9 d, and quantitative real-time PCR was applied to determine the expression of receptor activator of nuclear factor κB (RANK), TRAP, cathepsin K and matrix metalloproteinase (MMP)-9. Results TRAP staining showed that 50 ng/ml RANKL could successfully induce RAW264.7 cells into multinucleated cells (nuclear≥10). A few multinucleated cells (3≤ nuclear≤5) were observed in the control group and the Ad-GFP group, and mononuclear cells and few dual-nuclear cells were observed in the Ad-Wnt3a group. Conclusion Wnt3a can inhibit the differentiation of RAW264.7 cells into mature osteoclasts.

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