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论著

调节破骨细胞分化的RANK特异性基序的再确认

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摘要 目的: 进一步确认核转录因子NF-κB受体激动剂（RANK）蛋白中调节破骨细胞(OC)分化的特异性基序(motif)，为阐明OC分化机制提供理论依据。方法: 分别突变RANK蛋白膜内部分第533-540之间的8个氨基酸（突变前氨基酸序列为DIIVVYVS，突变后氨基酸序列为ELLAAFAA），用定点突变方法构建8个由肿瘤坏死因子受体1(TNFR1)和RANK跨膜部分和膜内部分共同组成的TNFR1/RANK突变嵌合体(TNFR1/RANK-533-TNFR1/RANK-540)，每1个突变体在其RANK膜内部分含1个突变氨基酸。用包装细胞plat E分别将各突变体包装成逆转录病毒，通过逆转录病毒感染分别将这些突变体转染到TNFR1/TNFR2基因敲除小鼠的骨髓巨噬细胞(BMM)中。用TNF-α和单核细胞集落刺激因子（M-CSF）刺激、观察转染哪种突变体的BMM不能诱导OC形成，不能诱导OC形成的突变体所包含的突变前氨基酸就是RANK调节OC分化的关键氨基酸，多个关键氨基酸组成的片段就是RANK特异性motif。结果: 转染TNFR1/RANK-533、TNFR1/RANK-539和TNFR1/RANK-540的BMM均能分化成OC，表明氨基酸D533、V539、S540突变后对OC分化无影响；转染TNFR1/RANK-534的BMM有极少数能分化成OC，表明氨基酸I534突变后对OC分化有部分影响；而转染TNFR1/RANK-535、TNFR1/RANK-536、TNFR1/RANK-537和TNFR1/RANK-538的BMM均不能分化成OC，表明氨基酸I535、V536 、V537和Y538突变后对OC分化起关键影响。结论: I534、I535、V536 、V537和Y538这5个氨基酸组成的片段（534-IIVVY-538）可能就是RANK调节OC分化的特异性motif。

关键词 NF-κB受体激动剂； 破骨细胞

分类号 R363.2

Re-identification of special motif regulating osteoclast differentiation in RANK

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Abstract

<FONT face=Verdana>AIM: To re-identify the special motif regulating osteoclast(OC) differentiation in receptor activator of nuclear factor kappa B(RANK)to provide evidences for studying the mechanism of OC differentiation. METHODS: Eight amino acids were mutated(from DIIVVYVS into ELLAAFAA)in the fragment between the 533th and the 540th amino acids in RANK cytoplasmic domain. Eight mutant TNFR1/RANK chimeras, each consists of TNFR1(tumor necrosis factor receptor 1) extracellular domain linked to transmembrane domain and cytoplasmic domain of RANK with one amino acid mutated in cytoplasmic domain was constructed by point mutation method. After the eight mutant chimeras were finished, they were packed with plat E cell line to produce the retrovirus expressing mutant TNFR1/RANK. The bone marrow macrophages(BMMs), isolated from TNFR1/R2 double knockout mice, were infected with retrovirus derived from different mutants and infected BMMs which did not differentiated into OCs were inspected after stimulated by TNF-α and M-CSF. The fragment consisted of different amino acids in TNFR1/RANK chimeras, which couldn't induce OC formation after mutated, may be the special motif regulating OC differentiation. RESULTS: We found that all BMMs transfected by TNFR1/RANK-533, TNFR1/RANK-539 or TNFR1/RANK-540 differentiated into OCs, indicating that none of amino acids D533, V539 or S540 had an effect on OC differentiation. A fewer of BMMs transfected by TNFR1/RANK-534 differentiated into OCs, indicating that I534 had a partial effect on OC formation. Most importantly,

BMMs transfected TNFR1/RANK-535, TNFR1/RANK-536, TNFR1/RANK-537 or TNFR1/RANK-538 did not differentiated into OCs, indicating each of amino acids I535, V536, V537 and Y538 played a pivotal role in OC differentiation.
CONCLUSION: The amino acid fragment consists of I534, I535, V536, V537 and Y538 may be the special motif regulating OC differentiation in RANK.
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Key words Receptor activator of nuclear factor kappa B Osteoclasts

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