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論　文　内　容　要　旨

題　目

TAK1 inhibition subverts the osteoclastogenic action of TRAIL while potentiating its antimyeloma effects

（TAK1阻害はTRAILの抗骨髄腫作用を増強するともに骨吸収促進活性を抑制活性に変換する）

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内容要旨

TNF-related apoptosis-inducing ligand (TRAIL) binds to its cognate death receptors (DRs) to activate caspase-8 and induce apoptosis in cancer cells. TRAIL-mediated immunotherapy is potentially an attractive therapeutic strategy against cancers, including MM. In addition, cytotoxic T cells and NK cells, major effectors in different types of immunotherapies, highly express TRAIL to induce tumor cell death. However, little information has been available on the effects of TRAIL on the tumor microenvironment.

Receptor activator of NF-κB ligand (RANKL), a critical mediator of osteoclastogenesis, is upregulated to extensively enhance osteoclastogenesis and bone resorption in MM. Thus, activated osteoclasts (OCs) in turn enhance MM growth, thereby forming a vicious cycle between MM tumor expansion and osteoclastic bone destruction. OCs are not merely bone resorbing cells, but rather facilitators for tumor growth; therefore, OCs should be targeted to improve treatment efficacy, especially in MM expanding in the bone marrow with enhanced bone resorption. However, the effects of TRAIL on osteoclastogenesis enhanced in MM remain largely unknown. The present study was therefore undertaken to clarify the impact of TRAIL on osteoclastogenesis and the MM-OC interaction.

We used murine preosteoclastic cell line, RAW264.7, bone marrow macrophages (BMMs) from mice and peripheral blood mononuclear cells (PBMCs) from healthy human donors to analyze OC differentiation, viability and signaling pathways in OCs. Cell viability and signaling pathways were analyzed in OCs as well as human MM cell lines, RPMI8226, MM.1S, INA-6, TSPC-1, and murine MM cell line 5TGM1.

Although OCs express DRs, TRAIL did not induce apoptosis in OCs. But TRAIL rather facilitated RANKL-induced osteoclastogenesis along with upregulation of cellular FLICE inhibitory protein (c-FLIP), an endogenous inhibitor for caspase-8, in RAW264.7 cells, mouse BMMs and human PBMCs.

TRAIL preferentially formed the complex with FADD, TRAF2 and RIP (Complex II), an initiator of NF-κB activation, but not death-inducing signaling complex (DISC) in OCs, while potently inducing DISC formation in MM cells. *c-FLIP* siRNA abolished theTRAIL-induced Complex II formation and triggered apoptosis in OCs, indicating a predominant role of c-FLIP in blocking of apoptosis by TRAIL. To clarify the downstream signaling of Complex II, we looked at TGF-β-activated kinase-1 (TAK1). The treatment with TRAIL induced the phosphorylation of TAK1 in OCs but not in MM cells. The induction of TAK1 phosphorylation by TRAIL was followed by the phosphorylation of IκBα in OCs. However, the TAK1 inhibitor LLZ1640-2 abolished the TRAIL-induced phosphorylation of IκBα in OCs.

The c-FLIP upregulation in OCs by TRAIL was abolished by the NF-κB inhibitor BAY11-7085 as well as LLZ. In addition, terameprocol, an inhibitor of Sp-1, a transcription factor responsible for *c-FLIP* gene expression, also abolished c-FLIP expression in OCs. TRAIL substantially reduced Sp1 and c-FLIP protein levels in parallel with caspase-8 activation in the presence of the TAK1 inhibitor LLZ, which were restored by the caspase-8 inhibitor z-IETD-FMK, indicating enzymatic degradation of Sp1 and thereby c-FLIP reduction. In MM cells, TRAIL reduced Sp-1 and c-FLIP protein levels in a caspase-8-dependent manner, which was further enhanced by the TAK1 inhibition.

Cocultures with MM cells enhanced the formation of OCs from bone marrow cells. Treatment with TRAIL further enhanced osteoclastogenesis in the cocultures; however, LLZ abolished the enhancement of osteoclastogenesis by TRAIL in the presence of MM cells. Cocultures with OCs lowered TRAIL-induced MM cell death. However, addition of the LLZ substantially reduced MM cell viability in combination with TRAIL in cocultures with OCs. These results suggest that TAK1 inhibition may disrupt MM cell-OC interaction and potentiate TRAIL's anti-tumor effects while also converting TRAIL to an anti-bone resorptive agent.

These results collectively demonstrate that osteoclastic lineage cells tilt TRAIL-mediated apoptosis into the NF-κB survival signaling, and that TAK1 inhibition subverts TRAIL-mediated NF-κB activation in OCs to resume TRAIL-induced apoptosis in OCs while further potentiating it in MM cells.