S1P MODULATOR FTY720 TARGETS OSTEOCLASTOGENESIS IN CXCR4-BASED MULTIPLE MYELOMA SYSTEMIC XENOGRAFT MODEL

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Bone disease is one of the hallmarks of multiple myeloma (MM) leading to substantial morbidity and disability. Bone lesions result from abnormally increased osteoclast (OC) formation and activation. Studying the underlying mechanism may help to develop new therapeutic targets to treat MM associated osteolytic lesions and related complications. Sphingosine-1-phosphate (S1P) was shown to play important role in osteoclast biology. We report now the effect of FTY720 on formation and activation of OCs and their functional sequel in MM model.

OCs were generated in vitro from peripheral blood mononuclear cells and expressed the genes encoding for S1P1 and S1P2 receptors and enzyme SPHK1, tested by RT-PCR. Treatment with FTY720 significantly reduced in vitro formation of TRAP+ OCs, resulting in complete abolishment of OC formation at 2.5 μM FTY720 ($p<0.0001$). Furthermore, FTY720 significantly reduced the expression of genes associated with OC activation. Thus, expression of osteoactivin, cathepsin K, NFATc1, OSCAR, RANK, RANTES, MT1-MMP and MMP9 genes were significantly down-regulated in FTY720-treated OC cultures ($p<0.001$). Mechanistically, FTY720 abrogated RANKL-induced Erk1/2 phosphorylation in OC progenitors. In addition, FTY720 targeted the microenvironment components - MM cells and BMSCs, suppressing the expression of osteoclastogenic factors. mRNA levels of MIP1a, RANTES, MCP-1 and RANKL were significantly reduced in both MM cells (RPMI8226, CAG, OPM-2) and BMSCs upon FTY720 treatment ($p<0.001$). Moreover, FTY720 altered the ability of myeloma and stroma cells to promote OC formation, and concomitantly FTY720 overcame OC-mediated support and drug resistance of MM cells. Thus, FTY720 was able to disrupt the deleterious cross-talk between the MM tumor cells and the OCs.

Next, we evaluated the effect of FTY720 on OC activation in vivo taking advantage of our novel xenograft model of CXCR4-overexpressing MM cells that results in typical BM involvement accompanying by significant increase in number of TRAP+ murine OC. Treatment of MM-bearing mice with FTY720 (10 mg/kg) effectively targeted the MM cells in the BM milieu. Correspondingly, FTY720 significantly reduced mRNA levels of murine OC differentiation marker genes in BM, including those encoding cathepsin K, integrin β3, OSCAR, RANTES and RANKL ($p<0.001$). This effect correlated with increased numbers of circulating CD11c+ and F4/80+ monocytes, with OC precursors in both cell populations. Concomitantly, significantly increased numbers of TRAP+ OCs were generated in vitro from PBMCs of FTY720-treated animals in comparison to vehicle-treated controls ($p<0.001$). To investigate whether OC precursor migration is affected by FTY720, we evaluated the in vitro migration of human monocytes toward CXCL12, well-known chemo-attractant of OC precursors. FTY720 completely blocked CXCL12-induced migration of CD14+ cells and significantly reduced their surface CXCR4 expression. These results suggest novel mechanism of action of FTY720, affecting both S1P and CXCR4 pathways, reducing the attachment of the OC precursors to the bone and thus leading to their mobilization to the blood.

Our observations uncover new roles of S1P pathway in OC formation and activation in MM, delineating a novel mechanism of FTY720 targeting OC formation and migration in vitro and in vivo and providing preclinical rationale for its therapeutic application in patients with MM bone disease.