SL02 Research on Reveromycin A Targeting Osteoclasts: Biological Activity and Biosynthesis

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Reveromycin A (RM-A) was isolated as an antitumor compound from *Streptomyces reveromyceticus* SN-593. Although the cytotoxicity of RM-A against tumor cells was weak, we found potent and specific cytotoxic activity of RM-A against osteoclasts. As osteoclasts are involved in osteoporosis and bone metastasis, RM-A was tested in animal model systems, and it showed excellent activity by inhibition of the isoleucyl-tRNA synthetase. We have expanded the *in vivo* experiments to prove the efficacy of RM-A against animal diseases.

In order to supply a large amount of RM-A for the *in vivo* experiments, we investigated the biosynthesis of RM-A. We succeeded in cloning the RM-A gene cluster consisting of 21 open reading frames spanning 90 kb. To understand the post-PKS modification pathway, all the genes found in the RM-A cluster were disrupted and the metabolites that were accumulated in each mutant strain were analyzed. We revealed the responsible genes involved in each biosynthesis process, such as spiroacetal formation (*revG, revJ*), hydroxylation (*revI*) and succinylation (*revK, revL, revM*). To clarify the enzymatic mechanism of biosynthesis, we performed the heterologous expression of the enzymes, which were purified for biochemical characterization. We carried out the *in vitro* reconstitution of the enzymes, which enabled the production of RM-A (final product) from RM-A1a (post-PKS product).