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Inactivation of the *LnmE* Gene Revealing New Insights into the Biosynthesis of the Antibiotic Leinamycin in *Streptomyces atroolivaceus*

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Leinamycin (LNM, Fig. 1), which has been isolated from the culture broth of *Streptomyces atroolivaceus*, is a potent antitumor antibiotic (1,2). The structure of LNM was elucidated by spectroscopic analysis, X-ray crystallography, and chemical synthesis (3,4). The unique structural feature of LNM includes the unusual 1-oxo-1,2-dithiolan-3-one, which is fused in a spiro fashion to an 18-membered macrocyclic lactam with an extensively conjugated thiazole ring. To date no other natural products with such an unusual dithiolanone moiety have been reported.

LNM has a broad antimicrobial spectrum against both gram-positive and gram-negative bacteria and shows potent antitumor activity in murine tumor models in vivo. It was focused on its specific activity against tumors that are resistant to clinically important anticancer drugs, such as cisplatin, doxorubicin, mitomycin, and cyclophosphamide. LNM preferentially inhibits DNA synthesis and interacts directly with DNA to cause single-strand scission of DNA in the presence of thiol cofactors (Fig. 1)(5-7). This alkylative DNA cleavage by LNM is mediated by an episulfonium ion intermediate and the presence of the

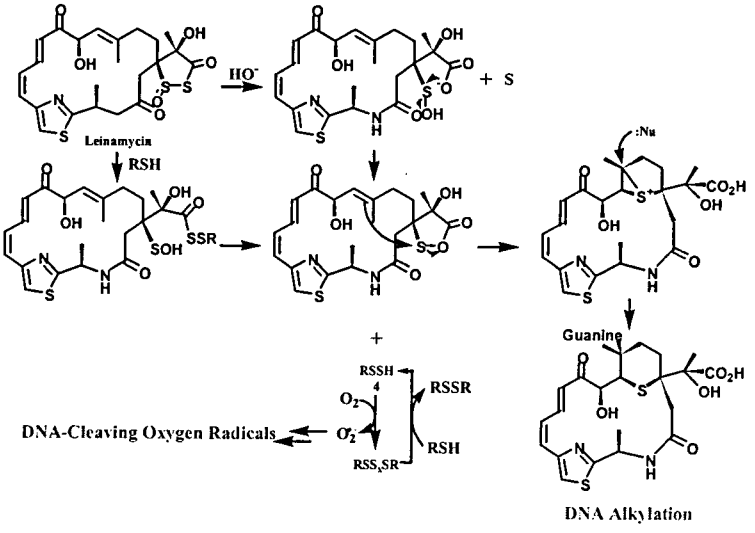

Fig. 1 Mode of action of leinamycin for anticancer activity.

dithiolanone moiety in LNM is essential for the DNA cleaving activity. LNM is also known to cause thiol-mediated oxidative DNA damage. Thiol-dependent oxidative DNA damage is triggered by release of a hydrodisulfide species that mediates the production of oxygen radicals (8-11). Such unique mechanisms provided a new class of DNA-cleaving antitumor agent.

Unique chemical structure, potent biological activities, and novel mode of action have spurred great interest in developing LNM into a clinically useful anticancer drug. To generate LNM analogs with improved antitumor activity, chemical modification of natural leinamycin and chemical synthesis have been carried out. Especially since the dithiolanone moiety of LNM plays a key role for the DNA cleaving activity, the modification without affecting this labile moiety has been focused and explored (12). These studies generated a number of LNM analogs with improved antitumor activity, supporting the wisdom of making novel anticancer drugs based on the LNM molecular scaffold.

We recently cloned and localized the LNM biosynthetic gene cluster to a 172-kb DNA region from *S. atroolivaceus* S-140 (13, 14) and sequential inactivation of ORFs from both ends of the sequenced region led to the assignment of the LNM gene cluster to consist of 27 ORFs, of which five encode nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS), and hybrid NRPS-PKS enzymes responsible for 18 membered-macrolactam ring. Six PKS modules lack their cognate AT domain that is essential for Type I PKS, and a discrete AT protein provide the AT activity (15). This finding for macrolactam ring formation in LNM gave a previously unknown PKS architecture that is characterized by a discrete, iteratively acting AT protein that loads the extender units in trans to AT-less multifunctional type I PKS proteins for polyketide biosynthesis. However, biosynthetic root of dithiolanone moiety, which is essential for biological activity, remains to be unclear.

In order to generate LNM analogs by using combinatorial biosynthesis without affecting formation of dithiolanone moiety, it is very important to characterize genes related to dithiolanone biosynthesis. Sequence analysis of the LNM gene cluster revealed several genes of unknown function, serving as candidates that could be involved in the biosynthesis of 1,3-dioxo-1,2-dithiolane moiety. To assign the functions of these genes and to delineate the pathway for 1,3-dioxo-1,2-dithiolane biosynthesis, these genes were inactivated by gene replacement, and the resultant mutants were analyzed for metabolite production. While inactivation of *lnmE* completely abolished LNM production, the lnmE mutant produced four new LNM analogs. Isolation and structural characterization of these compounds support the hypothesis of *lnmE* to be involved in 1,3-dioxo-1,2-dithiolane biosynthesis. Proposed function of LnmE, its role in LNM biosynthesis, and details of structure determination and biological activity of the new LNM analogs will be presented.

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